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# Development of test methods for assessing microbial influenced degradation of cement-solidified radioactive and industrial waste

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**Abstract**

This paper reports on the development of accelerated tests for evaluating microbial influenced degradation (MID) of cement-solidified wastes. An existing U.S. Nuclear Regulatory Commission accelerated test cannot distinguish between degradation caused by biogenic acid produced under optimal conditions in a bioreactor and that caused by active biofilms formed on the waste materials. Nutrient limitations were also observed that would significantly limit the activity of any developing biofilm. Results from this work have shown that it is possible to modify this test to remove nutrient limitations and enable the effects of MID resulting from active biofilms to be examined. Aggressive MID microorganisms can form a biofilm on the surface of cement-solidified waste so that when nutrients are provided the microbes remain active. Elemental mass loss data from exposed solidified waste forms indicate the continued development and growth of microbes on the surface of samples.


*Keywords:* Cobalt; Degradation; Radioactive waste; Waste management; Solidification

## 1. Introduction

Microorganisms are responsible for processing enormous quantities of organic and inorganic materials. Examples include the purification of millions of litres of raw sewage, the production of 20 billion metric tons of the ''green house'' gas carbon dioxide per year and the extraction of nearly 25% of the copper recovered from copper ore. They are also important contributing factors to the degradation of the built environment, and in particular, the concrete-based infrastructure.

There are at least seven major chemical/physical processes commonly accepted as contributing to concrete degradation. These include sulphate and chloride attack, alkali aggregate reactions, water leaching, freeze/thaw cycling, salt crystallisation, corrosion with resulting expansion of reinforcing bars, and acid rain [1]. The adverse effects of microbial activity on the long-term performance of cementitious materials are often not considered, despite the fact that studies on the effects of microbial activity on concrete have been ongoing for many years [2–4]. The results suggest that an understanding of concrete degradation may be incomplete without including the effects of microbial influenced degradation (MID).

The microbes that cause MID of concrete are ubiquitous in the environment and they produce either organic or inorganic acids that can dissolve and disintegrate the concrete matrix [5]. Their effect is more intense than a one-time application of acid to a concrete surface because the microorganisms continuously release acid, acting as micro-point sources of continuing acid application [6].

It can be very difficult to distinguish between purely chemical and biogenic concrete deterioration as both pro-

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cesses may depend on acid attack. However, studies have clearly demonstrated the relationship between MID and degradation of concrete [7]. Concrete retrieved from various locations in the United States has been examined for MID bacteria [8]. Of the total number of samples examined, 83% were reported to be colonised by pioneering MID species or active MID organisms known to promote concrete degradation. Active MID organisms were found at 65% of the locations, with aggressive sulphur-oxidising bacteria (SOB) isolated at 22%.

Chemolithotrophic SOBs of the genus *Thiobacillus* are the most aggressive biological agents that promote MID of cementitious materials [2]. These bacteria obtain energy through the oxidation of reduced inorganic sulphur sources such as elemental sulphur, thiosulphate, and hydrogen sulphide. A cell density of $10^4$ to $10^6$ per gram of concrete of these bacteria is required before degradation is observed [9] with metabolically produced sulphuric acid causing degradation.

Most studies of MID have been concerned with the rapid degradation that can occur to concrete pipes in sewer systems [3,4] and there has been very little research on the effects of MID on cement-solidified wastes. However, microorganisms may have a significant impact on the long-term integrity and effectiveness of cementitious materials used to contain either nuclear or industrial wastes.

## 2. MID effects on cement-solidified wastes

A major benefit of using cement-based systems for the treatment of metal-containing wastes (both radionucleotides and industrial) is the alkaline buffering capacity that maintains the necessary environment for insolubility of metals. The importance of this is reflected in the number of studies that have used acidic solutions to investigate leaching. These have demonstrated the relationship between acid strength, rate of acid renewal, acid neutralisation capacity, and the extent of matrix dissolution with subsequent release of metal contaminates [10]. Therefore, the microbial production of acid in the close proximity or on the surface (micro-environment) of the waste form could have particularly deleterious effects on cement-solidified wastes, causing matrix dissolution and contaminant release.

Recent studies have direct relevance to the disposal of cement-solidified low-level wastes (LLW) [6]. Field studies have shown that MID bacteria including SOBs are present in soils at LLW disposal sites and therefore there is a need to develop testing methodologies to evaluate the effects of MID on cement-solidified wastes [6].

### 2.1. NRC test development to assess MID of cement-solidified LLW

*Thiobacillus thiooxidans* are the choice microorganism for biodegradation testing [11] and it has been shown that cement-solidified LLW can be attacked and degraded by the action of this ubiquitous microorganism. In addition, it has been conclusively demonstrated that the ability of cement-solidified or grouted LLW to retain or retard the loss of encapsulated radionucleotides can be compromised by the presence of *T. thiooxidans* [12].

An accelerated MID test protocol has been developed by INEEL at the request of the U.S. Nuclear Regulatory Commission (NRC). In this test specimens undergo an intermittent cycle of complete immersion in bioreactor propagated *T. thiooxidans* lixiviant four times every 24 h. The major criticism of this test is that the lixiviant has an inherently low pH in the range 1.5 to 2 as a consequence of the biological production of mineral acid in the bioreactor. This acidic lixiviant makes it difficult to distinguish between the effects of acid in the bulk solution and the acid generated on a micro-scale at or near the waste form surface [13]. The test cannot assess potentially higher degradation rates caused by acid production on a micro-scale [14] and it cannot assess any toxicity effects on biofilm development and activity caused by the waste components. The NRC method, therefore, needs to be refined to make the evaluation of MID more realistic [6].

### 2.2. Biofilm formation and modified NRC test

*T. thiooxidans* can form biofilms on concrete surfaces without the need for time-consuming preparation [15]. Active colonisation and biofilm formation are not adversely affected by cement chemistry, indicating that such biofilms could form under natural conditions. A more realistic method for testing cement-solidified waste forms would involve biofilm formation directly on samples rather than exposure to planktonic cells in an acid lixiviant.

This paper presents a modification to the NRC intermittent immersion protocol that has been developed to promote planktonic cell attachment to the cement-solidified waste surface and formation of a predominately *T. thiooxidans* biofilm. This modified protocol has been used to distinguish effects of MID caused by biofilm growth on sample surfaces from those caused by exposure to planktonic cells in an acid lixiviant. MID data are presented for a standardised waste form exposed to *T. thiooxidans* and sterile controls using both the original NRC and modified NRC tests. Parameters used for the comparison were pH, sulphate production, cumulative Ca leached with time, and characterisation of changes in cement microstructure.

## 3. Materials and methods

*T. thiooxidans* were used as the active microbes in these studies. The medium used to maintain growth consisted of deionized water containing (g/l): $K_2S_4O_6$ (3.0); $KH_2PO_4$ (3.0); $(NH_4)_2SO_4$ (0.5); $MgSO_4$ (0.4); $CaCl_2$ (0.1); $FeSO_4$ (0.01). This formulation is important in later calculations of

predicted versus actual concentrations of $SO_4^{-2}$. The microbial culture was propagated at 28 °C in a New Brunswick Scientific Bioflow III batch/continuous bioreactor.

A simulated aqueous waste containing $CoCl_2$ was used as a surrogate radioactive waste requiring encapsulation. Waste forms with a $CoCl_2$/Type 1 Portland cement weight ratio of 21:79 were formulated using a water/cement ratio of 0.74. The materials were thoroughly mixed and allowed to set in plastic cylindrical moulds (height 2 cm; diameter 1.5 cm) for 28 days.

### 3.1. Assessment of MID using original NRC test

The cement-solidified waste forms were subjected to accelerated microbial degradation using the methodology developed for the NRC, as described by Roger et al. [6]. Fig. 1A shows the unmodified "NRC Procedure," in which the sterile growth medium, with an inherent pH of 4, is pumped into a continuous flow bioreactor for *T. thiooxidans* propagation. The lixiviant (overflow from the reactor), which contains *T. thiooxidans* biomass and spent medium with a pH < 2, was pumped at a rate of 100 ml/day from the continuously operating bioreactor to the waste forms contained in soxhlet extraction tube exposure chambers. The siphon action of the soxhlet tubes causes repeated cycling of gradual immersion and draining of the exposed waste forms once every 7 h. Effluents exiting the chambers were collected in attached flasks for analysis. Two control exposures of waste forms were carried out by substituting the *T. thiooxidans* lixiviant with either sterile growth medium (same formulation as that used for *T. thiooxidans* cultivation with a pH ~ 4.0) [6] or sterile growth media adjusted to pH 1.9 with sulphuric acid. This was done to expose the control waste forms to either the conditions of the growth medium (pH 4) used to perpetuate *T. thiooxidans* growth or to the acidity (pH 1.9) of the bioreactor lixiviant.

### 3.2. Assessment of MID using the modified NRC test

Fig. 1B shows the modified configuration used to examine effects of biofilm growth on the waste forms. Samples were exposed to either the *T. thiooxidans* lixiviant (pH < 2) (experimental) or to sterile medium (control), as in the unmodified procedure (Fig. 1A), for 12 days. After this initial inoculation period (Fig. 1A), the media flow to the waste forms was changed to sterile growth medium (pH 4) for a further 18 days (Fig. 1B). It was assumed that subsequent microbial activity on the solidified waste is due to *T. thiooxidans* established during the 12-day pretreatment phase. The length of the inoculation stage was chosen to ensure that surface pH values were suitable for *T. thiooxidans* growth and to allow biofilm development,

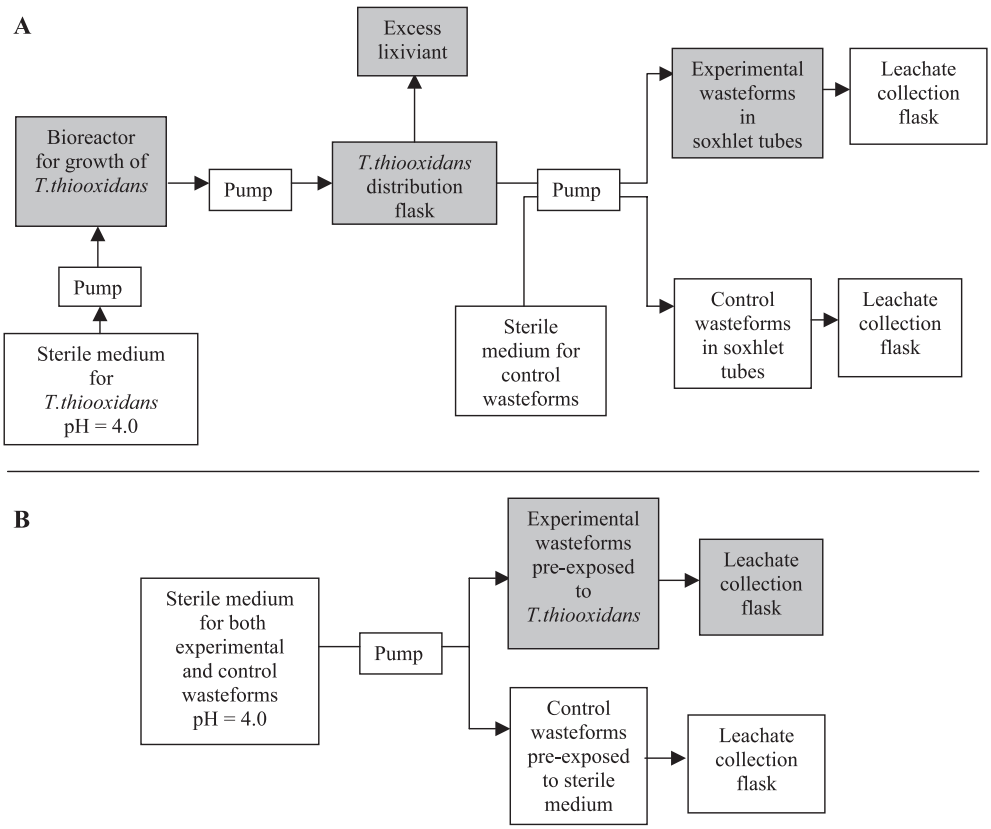


Fig. 1. Schematic material flow charts for the unmodified A and modified B (modified process uses waste forms for further testing that have been pretreated using Method A) NRC accelerated microbial exposure tests for waste form MID evaluation.

without the need for pre-inoculation with other pioneering species. Subsequent unpublished data suggest that active *T. thiooxidans* biofilms can develop in considerably less time (< 5 days), and therefore, a 12-day pretreatment stage should be considered conservative. In a control experiment, waste forms were first exposed to acidified sterile medium (pH 1.9), and after 12 days were also switched to sterile unamended growth medium (pH 4).

### 3.3. General analysis

The pH of medium, lixiviant and leachates were determined using a pH meter (Corning model 345). Sulphate concentrations in media and leachates were determined by the barium spectrophotometer method. The barium sulphate produced was measured by UV absorbance at 420 nm (HacH 20010 DR spectrophotometer) [16]. Waste form degradation was assessed by measurement of Ca concentrations in leachates and by scanning electron microscopy (SEM) combined with energy dispersive X-ray (EDX) analysis of waste form samples. Ca analysis was performed by atomic absorption spectrophotometry (Buck Scientific model 210 GVP). SEM–EDX (JEOL JSM 35-CF scanning electron microscope with a Princeton Gamma-Tech Prism digital spectrometer and PGT software) was carried out on sectioned, polished, carbon-coated specimens after exposure to both *T. thiooxidans* and acidified sterile media. Semiquantitative spectra were collected for 10 s from a continuous series of 50 × 50 μm areas starting at the edge of the polished section and extending 3 mm into the sample.

## 4. Results and discussion

### 4.1. Microstructural changes induced by biogenic sulphuric acid degradation

The potential for degradation of cemented waste forms by biogenic acid is readily demonstrated using the original NRC test. A section of cement-solidified $CoCl_2$ synthetic waste exposed to *T. thiooxidans* lixiviant is shown as a backscattered electron (BSE) micrograph in Fig. 2. An outer, severely corroded zone (approximately 1 mm thick) is visible, which has undergone significant microstructural alteration. This has an open, porous structure and is bordered on the inner leading edge of corrosion by a narrow zone of denser precipitates. Significant cracking inside this line may be caused by expansive sulphate reactions or may result from sample drying.

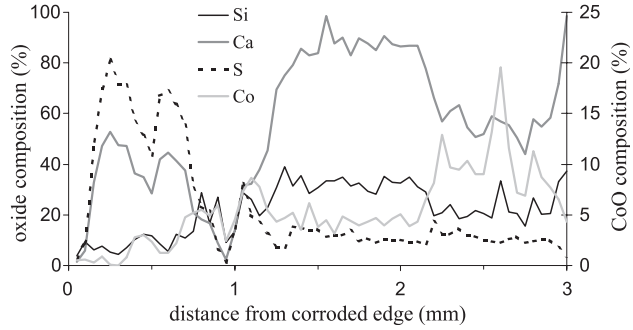


Fig. 3. Semiquantatative oxide composition along a transept from the edge of the corroded region of cement solidified $CoCl_2$ waste.

Semiquantitative oxide composition data from 50 × 50 μm areas along a transept from the outer corroded edge (Fig. 3) also show chemical differences between the corroded and uncorroded zones. Although sample heterogeneity is observed in the data at this resolution, matrix and waste leaching are evident from significantly reduced average oxide values for Si, Ca and Co in the outer 1 mm compared with the inner 1–3 mm ($P < .0005$ in all cases). In contrast, average S oxide values increased by a factor of 5 in the outer corroded zone ($P < .0005$) in part due to the formation of gypsum. To reduce interference from sample heterogeneity and show elemental associations, composition data from each EDX acquisition in both 0–1 mm ($n = 20$) and 1–3 mm zones ($n = 39$) were plotted as scatter diagrams in Figs. 4 and 5. Fig. 4 clearly shows the change in chemical association between Ca and S caused by degradation. Pearson correlation coefficients for Ca and S data were − .453 and +.996 in the uncorroded and corroded regions, respectively. This is explained by the reaction of sulphate in the lixiviant with Ca from the dissolution of portlandite and decalcification of Ca–Si hydrates to form gypsum ($CaSO_4$). The gradient of the regression line shown in Fig. 4 represents a Ca/S mole ratio of 0.999. The chemical association between Ca and Si in the hydrated cement matrix breaks down in the corroded zone as the correlation coefficients for Ca and Si change

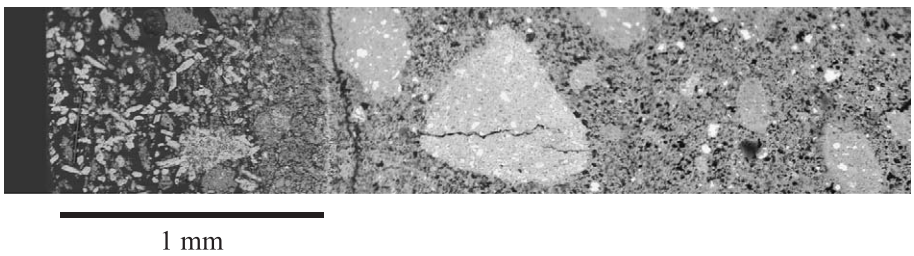


Fig. 2. BSE micrograph of sectioned waste form after exposure to *T. thiooxidans* lixiviant for 40 days. Exposed edge is on the left.

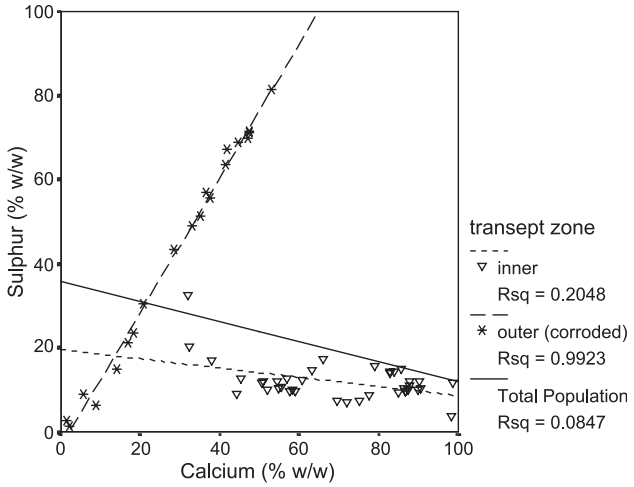


Fig. 4. Sulphur and calcium oxide compositions ($SO_3$, CaO) in outer (0–1 mm) and inner (1–3 mm) zones of waste form exposed to *T. thiooxidans* lixiviant. $R^2$ values from regression analysis of each separate zone and for the whole transept (0–3 mm) are given in key.

from +.761 for the uncorroded 1–3 mm zone to −.414 for the corroded outer 1 mm data.

The lower percentage of Ca oxide in the corroded zone shows that gypsum is also dissolved, but at a slower rate than gypsum formation. Previous studies with cement pastes [13] observed an additional layer, often lost during sample preparation, from which Ca had completely leached and only Si remained.

Fig. 5 shows the effect of acid corrosion on the association of Co with Si. In the uncorroded zone, Co was equally and negatively correlated to both Si and Ca oxide values (correlation coefficients for Si/Co = −.761, Ca/Co = −.684, both significant at the .05 level, $n = 39$), which may support observations that Co was found as coatings around cement phases. However, in the corroded zone, where Ca dissolution and re-precipitation as gypsum had occurred, no significant correlation was found between Co and Ca (correlation coefficient = −.42), and a significant positive correlation was observed between Co and Si (coefficient=+.846, significant at the .05 level, $n = 20$). This indicates that Si may have an important role in Co immobilisation.

The SEM–EDX data clearly show significant disruption of microstructure and waste leaching in solidified samples exposed to *T. thiooxidans* lixiviant in the NRC test. A major aspect of this corrosion was the dissolution of Ca from the cementitious binder. Ca leaching has been used in the following sections as an indicator for waste form degradation during the evaluation of the NRC and modified NRC tests.

### *4.2. Evaluation of NRC test for assessing MID effects on cement-solidified $CoCl_2$*

Fig. 6 shows the cumulative Ca leached by the cement-solidified waste forms during exposure to *T. thiooxidans* (pH 1.7–1.9) and sterile medium (pH 4) using the NRC test procedure. Waste forms exposed to lixiviant from the *T. thiooxidans* bioreactor showed almost a 10-fold increased loss of Ca compared to those exposed to the sterile control medium, corresponding to significant dissolution of the cementitious matrix. These data are similar to observations from other studies [6]. However, the increased rate of degradation is likely to be a function of the difference in pH between lixiviant and medium. Although this pH differential is a direct result of *T. thiooxidans* activity, and therefore indicates a potential for degradation resulting from biogenic acid, it is unclear whether any microbial activity occurs directly on sample surfaces. This is one of the criticisms leveled at the NRC test.

If Ca release was a function of acid generation only within the bioreactor, then equivalent rates of degradation would be expected from exposure to sterile medium acid-

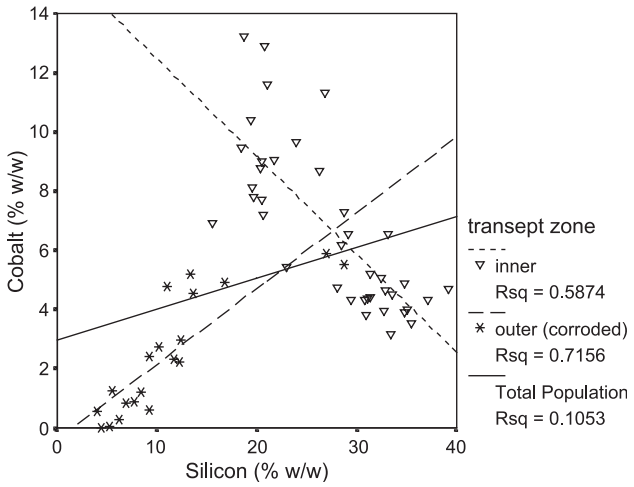


Fig. 5. Cobalt and silicon oxide compositions in outer (0–1 mm) and inner (1–3 mm) zones of waste form exposed to *T. thiooxidans* lixiviant. $R^2$ values from regression analysis of each separate zone and for the whole transept (0–3 mm) are given in key.

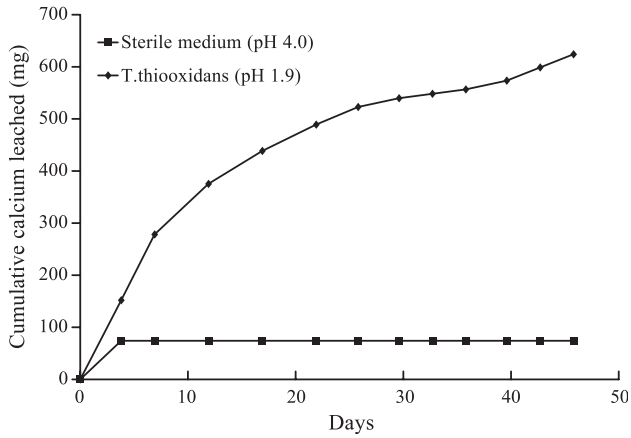


Fig. 6. Evaluation of the effect of *T. thiooxidans* on the leaching of calcium from cement/cobalt waste forms (21% cobalt chloride/79% cement) showing cumulative calcium leached versus time (days). Results were obtained using the NRC method.

ified with sulphuric acid to a similar pH. No discernible differences were observed in the appearance or phase alteration between samples exposed to *T. thiooxidans* and acidified sterile medium under SEM–EDX. Fig. 7 compares Ca release data from exposure to *T. thiooxidans* lixiviant with exposure to chemically acidified sterile medium using the NRC protocol. During the first 22 days the rate of Ca leaching was nearly the same with an average leach rate for both treatments (pH 1.9 sterile medium and for the pH 1.9 *T. thiooxidans* lixiviant) of 22 mg Ca/day. After that time the rate of Ca removal by the lixiviant decreased (5 mg Ca/day) while that of the low pH sterile medium remained nearly constant for another 11 days (Day 33). By Day 33, the leach rate of the sterile medium had decreased to 8 mg Ca/day, while that of the lixiviant increased to 6 mg Ca/day. The cause of the apparent extended activity of the sterile acid medium compared to the lixiviant (33 vs. 22 days) may be related to the development of a protective biofilm in the *T. thiooxidans* exposed samples and to nutrient limitation.

The supposition of a nutrient starved biofilm can be supported by data from initial tests showing that there was no oxidisable sulphur available in the lixiviant for continued metabolic activity. Apparently, all of the tetrathionate had been oxidised to $SO_4$ during residence in the bioreactor. Sterile medium, being used for bioreactor feed and control medium fed directly to the exposure chambers, theoretically contained 550 mg $SO_4$/l. Applying the same calculations, the amount of $SO_4$/l produced by 100% microbial oxidation of the available tetrathionate was estimated to be 4310 mg $SO_4$/l. Analysis of the medium and reactor lixiviant leaving the bioreactor showed that the actual concentrations were 574 and 5194 mg $SO_4$/l, respectively. It therefore appears that the tetrathionate-exhausted lixiviant could not support continued microbial activity (sulphuric acid production) in the exposure chambers. If no additional sulphuric acid was produced and if the waste forms were coated with an acid-

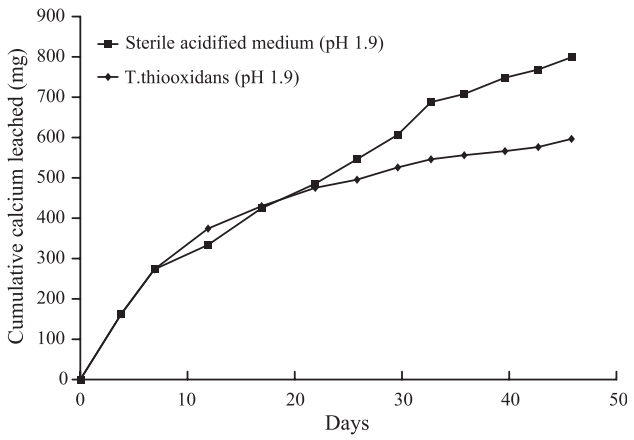


Fig. 7. Comparative evaluation of leaching of calcium from cement/cobalt based waste forms (21% cobalt chloride/79% cement) exposed to low pH sterile medium (1.9) and *T. thiooxidans* (pH 1.88). Results were obtained using the NRC method.

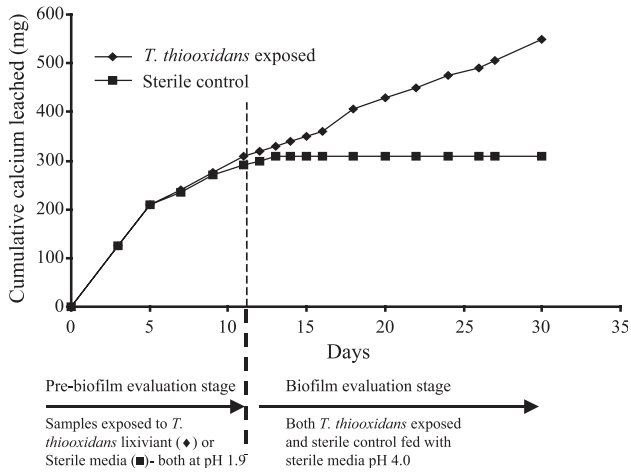


Fig. 8. Evaluation of biofilm formation on cement/cobalt based waste forms (21% cobalt and 79% cement) showing cumulative calcium leached.

resistant biofilm, then it is possible that Ca leaching was prohibited or even sorbed.

Other testing [13] also showed that the activity of *T. thiooxidans* mimicked the physical effects seen when cementitious materials were simultaneously exposed to sterile medium containing similar quantities of chemical derived sulphuric acid. At question with this work was whether acid generation by a biofilm growing on a waste form would be affected by potential toxicity of waste components, and whether biofilm derived sulphuric acid production could have a more significant effect on cementitious waste forms because of more intimate surface contact than that afforded by acid in the bulk solution regardless of its origin (biologically or chemically derived). Interest in the consequence of biofilm amplification of acid activity comes from the observation that attachment of *T. thiooxidans* biofilm can result in as much as a 20-fold increase in concrete degradation [14]. The results presented so far demonstrate that the existing NRC protocol is not able to address this issue without modification.

### *4.3. Evaluation of modified NRC test for assessing MID effects on cement-solidified $CoCl_2$*

The significance of nutrient limitation in the NRC protocol, and uncertainty over possible waste/bioactivity interactions, led to the development of a modified two-stage protocol aimed at establishing a biofilm on waste samples and the provision of nutrients to maintain biofilm activity (see Fig. 1). In the first stage (first 12 days) (Fig. 8), samples were exposed to bioreactor lixiviant or to sterile acidified media to allow build up of biomass on the experimental sample, while ensuring control samples were exposed to solutions of similar acid strength. After this 12-day inoculation period, nutrient limitation in the soxhlet tubes was removed by the provision of unamended sterile media (pH 4) to both sets of samples. In this stage, evidence for the

development of an active biofilm on samples pre-exposed to *T. thiooxidans* was a drop in culture medium pH and an increase in sulphate concentrations. Similar results were also seen by Libert et al. [17]. The pH of leachates from samples pre-exposed to *T. thiooxidans* decreased from 4 to an average of 2.2. By contrast, control leachate pH was elevated from 4 to 5.5 as a result of contact with the waste forms. Sulphate concentration increased from 574 mg/l in sterile media to 3261 mg/l in the leachate from the pre-exposed waste form leachates and decreased to 555 mg/l in the leachate from the control. This demonstrates that *T. thiooxidans* activity can be maintained in the exposure chamber after a period of initial inoculation followed by switching to fresh sterile medium.

The consequence for matrix dissolution of biofilm development and continued activity is illustrated by the cumulative Ca release data in Fig. 8. During the 12-day inoculation period, the average rate of Ca removal for both treatments was 27 mg Ca/day. After the 12-day inoculation period, microbial activity increased the Ca leach rate to 13 mg Ca/day and was comparable to the Ca leaching rate of 16 mg Ca/day for both the lixiviant and acid-fortified sterile medium between Days 5 and 12. No active removal of Ca was measured in the control sterile samples over the same period.

This clearly demonstrates that an active biofilm of *T. thiooxidans* developed on the waste form and any waste/biofilm interactions did not prevent significant matrix dissolution. Although specific effects of waste components on biofilm activity/acid generation were not examined in this study, the modified protocol would enable such studies to be carried out. Comparison of data from the NRC and the modified NRC procedures shows that the development of an active biofilm may amplify degradation rates compared to continued exposure to acid in bulk solutions. In the NRC test, 600 mg of Ca were released over a period of 46 days, whereas in the modified test the same amount of calcium was leached in 30 days.

## 5. Conclusions

- Mineral acid production by *T. thiooxidans* can promote cemented waste form degradation comparable to that of media amended with sulphuric acid.
- The existing U.S. Nuclear Regulation Commission (NRC) accelerated microbial degradation test cannot distinguish between degradation caused by biogenic acid produced under optimal conditions in a bioreactor and that caused by active biofilms formed on the waste materials.
- Nutrient limitations in the NRC test would significantly limit the activity of any developing biofilm and the effects of waste components on biological acid generation cannot be examined with this test. It is necessary to establish free growing biofilm on the waste forms before the effects of MID can be determined.
- A more realistic modified NRC test was proposed with separate biofilm formation and biofilm evaluation stages. The test removed nutrient limitations on biofilm growth and enabled the effects of MID resulting from active biofilms growing on waste forms to be examined.
- Aggressive MID microorganisms (*T. thiooxidans*) can form a biofilm on the surface of cement-solidified waste so that when nutrients are provided the microbes remain active.
- Elemental leaching data from exposed solidified waste forms show that development and growth of microbes on the surface of samples cause significant matrix dissolution even in the presence of waste components (Co). Data also indicate that the development of an active biofilm may amplify degradation rates compared to continued exposure to acid in bulk solutions.

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