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Evaluation of microbial stability of simulated solid and liquid waste forms using a refined biofilm formation method

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Abstract

A refined biofilm formation method was used to evaluate the stability of a simulated liquid waste form containing a simulated liquid waste (salts) and cement in three different proportions, and a simulated solid waste form containing a simulated solid waste (resin) and cement in three different proportions. The experimental samples of all the simulated liquid waste forms showed evidence of microbial growth on them after 3 days of evaluation as indicated by substantial increase in sulfate production, and exhibited considerable instability to microbial degradation as indicated by substantial leaching of calcium. The experimental samples of all the simulated solid waste forms showed evidence of inhibition of growth of *Thiobacillus thiooxidans* for about 18 days, after which the growth of the microbe became evident in two out of three. Within the growth inhibition period, the differences between experimental and control samples were minor. After the growth of *T. thiooxidans* became evident, comparatively higher degradations were observed for the experimental samples of the resin containing solid waste forms.

Keywords: Biofilm; Waste form; *Thiobacillus thiooxidans*

1. Introduction

Waste immobilization has emerged as one of the most popular disposal option for dealing with the stockpiles of the different classes of hazardous wastes. The use of cement as a binder for stabilizing and solidifying waste has been found to be especially promising for the disposal of radioactive wastes. This is because most power reactors have been shown to produce a variety of solid and liquid wastes that are compatible with cement solidification [1]. Based on an extensive analysis of various waste streams from different power reactors,

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Colombo and Neilson [2] were able to develop a simulated solid and liquid waste formula on behalf of the Nuclear Regulatory Commission (NRC). The developed simulated solid waste consists essentially of ion-exchange resins and water, while the simulated liquid waste consists of sodium sulfate, ammonium sulfate, sodium chloride and water.

The involvement of microorganisms in the degradation of ceramic materials has been well demonstrated. Since the pioneering work of Parker in 1945 [3] and Sand and Bock [4] on the involvement of thiobacilli in concrete corrosion, several other microbes have been implicated in ceramic degradation. Microorganisms like green algae, fungi, chemoorganotrophic bacteria, cyanobacteria, and lithoautotrophic bacteria have been isolated from buildings made of ceramic materials like concrete, sandstones, and bricks [5]. Even though the involvement of microbes in concrete degradation has been known since mid 1940s, it was only in the early 1990s that a comprehensive protocol for the evaluation of microbial stability of waste forms was put together for the NRC [6]. The NRC method involves exposure of experimental waste forms to bioreactor grown microorganisms (pH approximately 1.8) and control waste forms to sterile medium (pH approximately 4.0). The NRC method was used by Rogers et al. [6] to evaluate the stability of some simulated solid and liquid waste forms to *Thiobacillus thiooxidans*. Results obtained showed that experimental samples (exposed to broth containing *T. thiooxidans*, pH 1.8) of both waste forms exhibited substantially higher instability than their corresponding controls (exposed to sterile medium, pH 4.0). The simulated waste forms were based on the formula developed by Colombo and Neilson [2]. The use of a *T. thiooxidans* culture that is significantly lower in pH in comparison to the control in the NRC method has been identified as a major flaw of the NRC method [7]. A new protocol (biofilm formation method), which adequately address the limitations of the NRC protocol, and more accurately evaluate the microbial stability of waste forms, has been developed [7]. The biofilm formation method is a two-stage process. The first stage is for microbial colonization (biofilm formation) of waste forms, and involves the exposure of experimental waste forms to fermenter grown *T. thiooxidans* broth (pH 1.8) and control waste forms to chemically acidified sterile medium (pH 1.8) for 12 days. The second stage is for confirmation and evaluation of biofilm, and involves the exposure of both control and experimental waste forms to sterile medium for *T. thiooxidans* (pH 4.0). A refinement of the biofilm formation method involving substantial shortening of the duration of the first stage from 12 days to 24 h was recently reported [8]. In this study, the refined version of the biofilm formation method was used to evaluate the stability of a simulated solid and a simulated liquid waste form based on the simulated waste formulas of Colombo and Neilson [2]. This is being done in order to verify the accuracy of information obtained previously using the flawed NRC microbial stability evaluation method, as part of a broad strategy to obtain an optimized set of formulation with enhanced stability against chemical and biological degradation.

2. Materials and methods

2.1. Microorganism

T. thiooxidans was the microorganism used in this study, it was obtained from Idaho National Engineering and Environmental Laboratory (INEEL). The composition of the

T. thiooxidans growth media is as follows (g/l): $MgSO_4 \cdot 6H_2O$ (0.4), $(NH_4)_2SO_4$ (0.5), $CaCl_2$ (0.1), $FeSO_4$ (0.01), potassium tetrathionate (3.0), potassium phosphate monobasic (3.0). To avoid precipitation, the potassium phosphate monobasic was prepared, autoclaved separately, and mixed with the rest of the components prior to use. The ferrous sulfate was added to the other components using sterile disposable membrane filters to avoid oxidation to ferric during autoclaving.

2.2. Simulated waste forms

The simulated liquid/cement waste forms used in this study consist of different combinations of simulated liquid waste/cement (weight to weight) namely: 0.5:1, 1:1 and 1.5:1 combination. The composition of the simulated liquid waste is (wt.%): water 73.4, sodium sulfate 15.0, ammonium sulfate 9.6, and sodium chloride 2.0. The simulated solid waste/cement waste forms consist of different combinations of simulated solid/cement (weight to weight) namely: 1:1, 1.5:1 and 0.8:1 combination. The composition of the simulated solid waste is (wt.%): water 35, bead resin 65. The various waste forms were formulated by weighing out appropriate amounts of the components (liquid waste, solid waste, cement, and water), mixing them thoroughly, and allowing the mix to set in a 5 ml plastic vial mould. The waste forms were cylindrically shaped after setting and had the following dimensions: 2.0 cm height $\times$ 1.5 cm diameter.

2.3. Biofilm evaluation

The refined biofilm formation method of Idachaba et al. [8] was used for the evaluation of stability of the waste forms to microbial degradation. The refined biofilm formation method is a two-stage process. In the first stage (lasting 24 h), experimental waste forms were intermittently immersed in *T. thiooxidans* broth of pH approximately 2.0 which was being pumped from a continuously operated fermenter. The control waste forms were intermittently immersed in sterile medium (pH adjusted to approximately 2.0 to replicate the fermenter broth pH) which was being pumped continuously from a reservoir. Both the experimental and control samples were contained in soxhlet systems. The second stage of the process was meant for colonization of the experimental waste forms by *T. thiooxidans*. The first stage ended with shutting off supply of *T. thiooxidans* broth to the experimental waste forms and the supply of pH adjusted sterile medium to the control waste forms. The second stage began with the supply of a fresh medium for *T. thiooxidans* (pH about 4.0) to both control and experimental waste forms. This supply continued until the termination of the experiment. The supply of sterile medium (pH 2.0) and fermenter broth in stage 1, and sterile medium (pH 4.0) in stage 2 was carried out using a pump that was set to deliver the solutions to the soxhlet tubes at a rate of about 100 ml per day. The approximate time for filling and draining of the soxhlet tubes was 7 h. The effluents from the intermittent immersion process were collected in vessels and periodically removed for analysis with volume and date noted.

2.4. Analytical

The analysis of the metals in the media, fermenter broth, and the effluents were carried out using an inductively coupled plasma (ICP) Optima 3300 DV (Perkin-Elmer). The amount of each metal in the effluents was calculated as the product of the reading from ICP multiply by the volume of effluents. The amount of each metal leached from a waste form into each effluent was calculated as the difference between the total amount of the metal in the effluent and contribution from the medium or fermenter broth. The cumulative amount of metal leached for a given day was calculated as the sum total of the calculated metal leached for effluents from the beginning of evaluation to the day in question. A Mettler pH meter with combination electrodes was used to measure the pH of the media, fermenter broth, and effluents. Estimation of surface pH of waste forms before and after exposure to various solutions was carried out using universal pH indicator strips. Samples exposed to various solutions were rinsed with distilled water before pH measurements were carried out. The sulfate concentration of the media and effluents was estimated by the Turbidimetric method based on the Standard Methods for the Examination of Water and Wastewater [9]. This method involved adding a quantity of barium chloride crystals to a buffered amount of the sample and measuring the absorbance of the barium sulfate formed at 420 nm using the HacH 2010 DR spectrophotometer. The sulfate concentration was calculated using a standard calibration curve. The net sulfate balance was calculated as the difference between the sulfate concentration of the effluents and that of the control medium (for control samples) or fermenter broth (for experimental samples in the first stage). In the second stage, the net sulfate balance for experimental samples was calculated as the difference between the sulfate concentration of the effluents and that of the sterile medium.

3. Results and discussion

3.1. Evaluation of microbial stability of simulated liquid waste forms using a refined biofilm formation method

Results of evaluation of simulated liquid waste/cement waste forms are presented in Table 1 and Figs. 1–6, with Figs. 1 and 2 being results for the 0.5:1 combination, Figs. 3 and 4 for the 1:1 combination and Figs. 5 and 6 for the 1.5:1 combination. Results in Fig. 1 reveal that substantially higher levels of calcium and magnesium were leached from the experimental sample of the 0.5:1 combination as compared to the control. While a total

Table 1
Important parameters from biofilm formation evaluation of liquid waste/cement waste forms

Sample	Total Ca leached (mg)	Total Mg leached (mg)	Average pH (during second stage)	Maximum net sulfate balance (mg/l)
0.5:1 Experimental	140	12	2.5	4500
0.5:1 Control	5	3	5.5	∼0
1:1 Experimental	90	13	2.5	4300
1:1 Control	6	3	5.5	∼0
1.5:1 Experimental	180	20	2.5	6000
1.5:1 Control	10	3	5.5	∼0

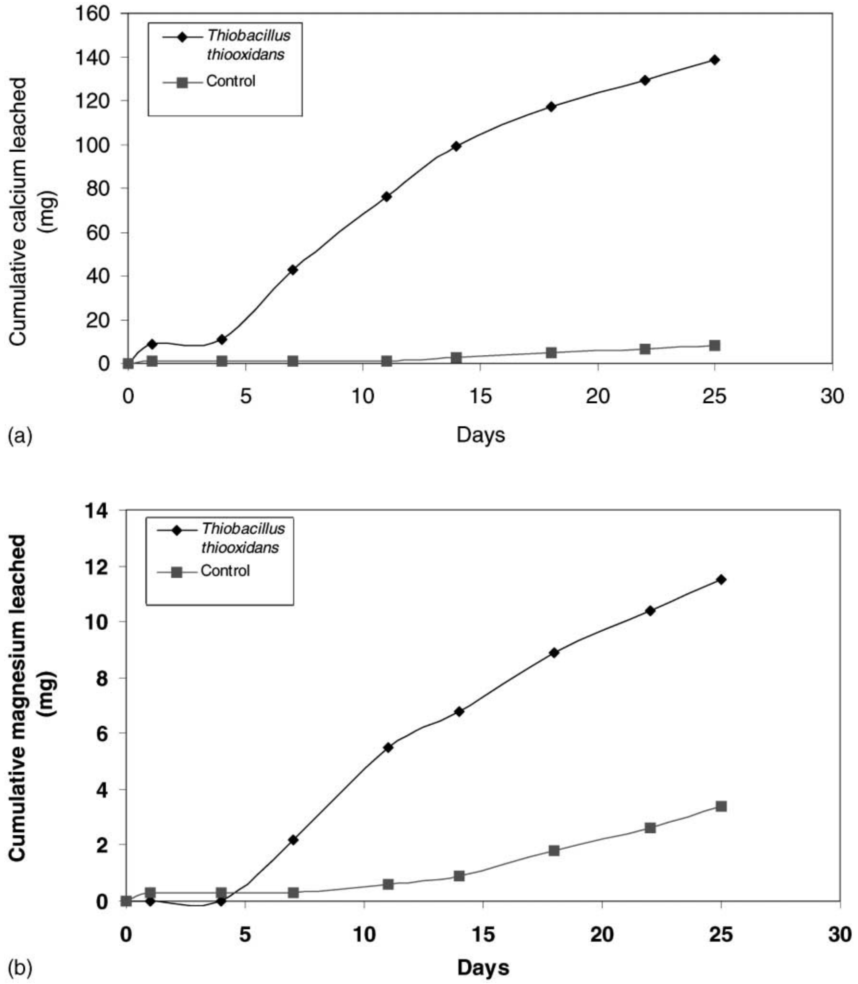


Fig. 1. The profiles of calcium (a) and magnesium (b) leached from a simulated liquid waste/cement waste form (0.5:1) using a refined biofilm formation method.

of about 140 mg of calcium and 12 mg of magnesium were leached from the experimental sample, only 5 mg of calcium and 3 mg of magnesium were leached from the control within 25 days of evaluation. It is evident from Fig. 2 that a substantially higher net sulfate balance was produced by the experimental sample of the 0.5:1 combination in comparison to the control. While a maximum net sulfate balance of about 4500 mg/l was obtained for the experimental sample at day 10 of evaluation, the maximum net sulfate balance for the control (obtained at the beginning of the evaluation when the system was just stabilizing) was about 500 mg/l. From day 6 to the end of evaluation, a net sulfate balance of approximately zero was obtained for the control. Results in Fig. 2 indicate also that significantly lower pH

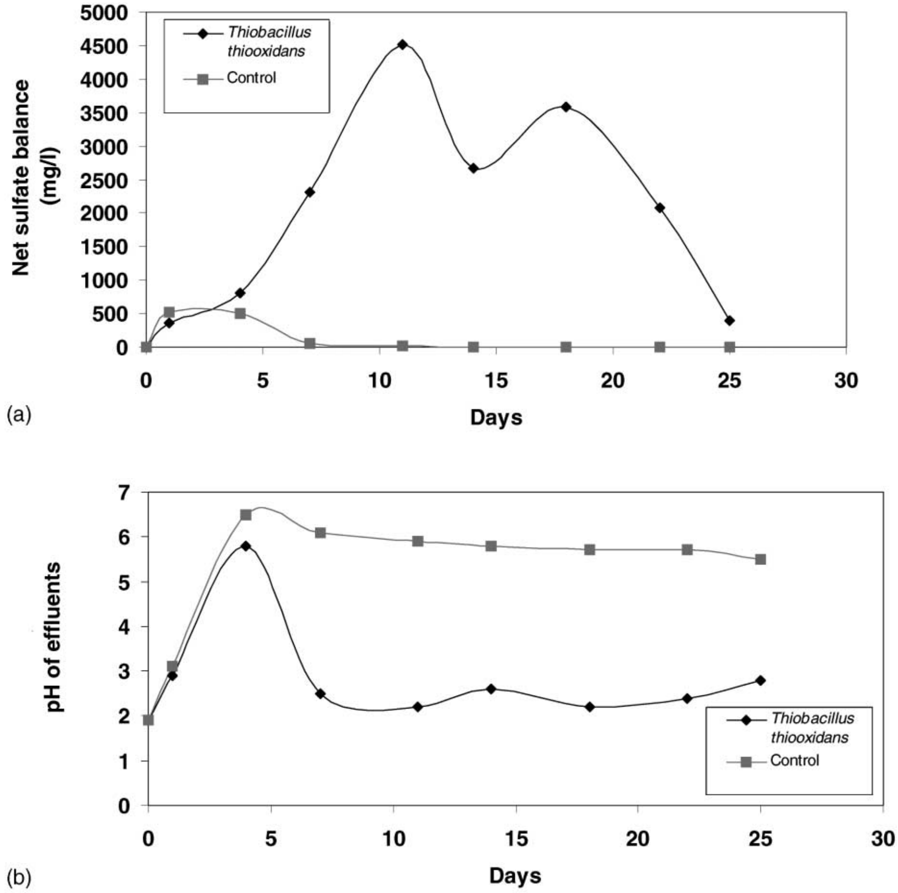


Fig. 2. The profiles of net sulfate produced (a) and pH (b) during evaluation of a simulated liquid waste/cement waste form (0.5:1) using a refined biofilm formation method.

values were obtained for the experimental sample compared to the control from day 5 to the end of the evaluation. An average pH value of about 2.5 was obtained for the experimental sample, while an average value of about 5.5 was obtained for the control sample within this period.

Results of evaluation of a simulated liquid waste/cement waste form (1:1) are presented in Figs. 3 and 4. Results in Fig. 3 indicate that substantially higher levels of both calcium and magnesium were leached from the experimental sample as compared to the control. A total of about 90 mg of calcium and 13 mg of magnesium were leached from the experimental sample, while only 6 mg of calcium and 3 mg of magnesium were leached from the control. It is evident from Fig. 4 that substantially higher amounts of sulfate were produced by the experimental sample in comparison to the control. A maximum net sulfate balance of about 4300 mg/l was obtained for the experimental sample within days 7 and 25 of evaluation,

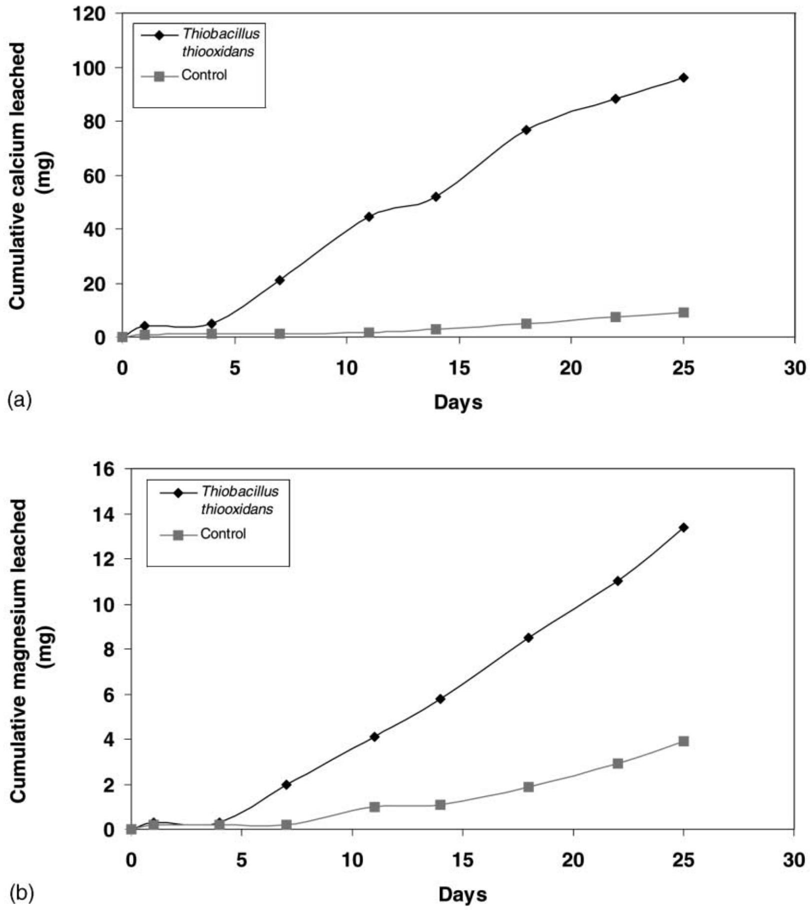


Fig. 3. The profiles of calcium (a) and magnesium (b) leached from a simulated liquid waste/cement waste form (1:1) using a refined biofilm formation method.

while an approximate net sulfate balance of zero was obtained for the control within this period. Results in Fig. 4 indicate also that substantially lower pH values were obtained for effluents from the experimental sample compared to the control, from day 5 to the end of the evaluation. An average pH value of about 2.5 was obtained for the experimental sample while an average pH value of 5.5 was obtained for the control.

Results of evaluation of a simulated liquid waste/cement waste form (1.5:1) are presented in Figs. 5 and 6. Results in Fig. 5 indicate that substantially higher levels of calcium and magnesium were leached from the experimental sample as compared to the control within 25 days of evaluation. While a total of about 180 mg of calcium and 20 mg of magnesium were leached from the experimental sample, only 10 mg of calcium and 3 mg of magnesium were leached from the control within this period. From Fig. 6, it is clear that the net sulfate balances produced by the experimental sample were substantially higher than the net sulfate

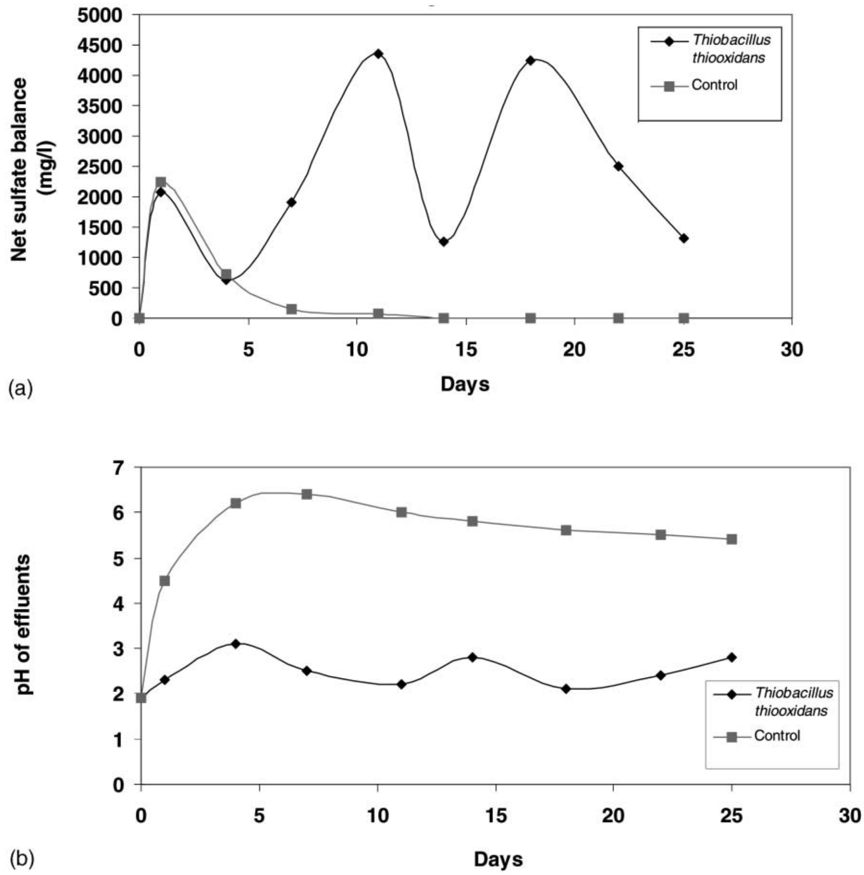


Fig. 4. The profiles of net sulfate produced (a) and pH (b) during evaluation of a simulated liquid waste/cement waste form (1:1) using a refined biofilm formation method.

balances produced by the control within days 5 and 25 of evaluation. While a maximum net sulfate balance of about 6000 mg/l was obtained for the experimental sample, the net sulfate balances for the control were generally about zero within this period. Results in Fig. 6 also indicate that the pH values of effluents from the experimental sample were substantially lower than those from the control between days 7 and 25 of evaluation. Within this period, the pH of experimental effluents averaged about 2.5 while the pH of the control effluents averaged about 5.5.

The magnitude of the difference in the amounts of metals leached from the experimental waste forms as compared to the control (e.g. 90 mg of calcium from the experimental compared to 6 mg from the control for the 1:1 combination) suggests that these waste forms are unstable to microbial degradation. The leaching of substantially higher amounts of calcium and magnesium from the experimental samples of the simulated liquid waste/cement waste forms as compared to the control was expected. This is because, in the absence of

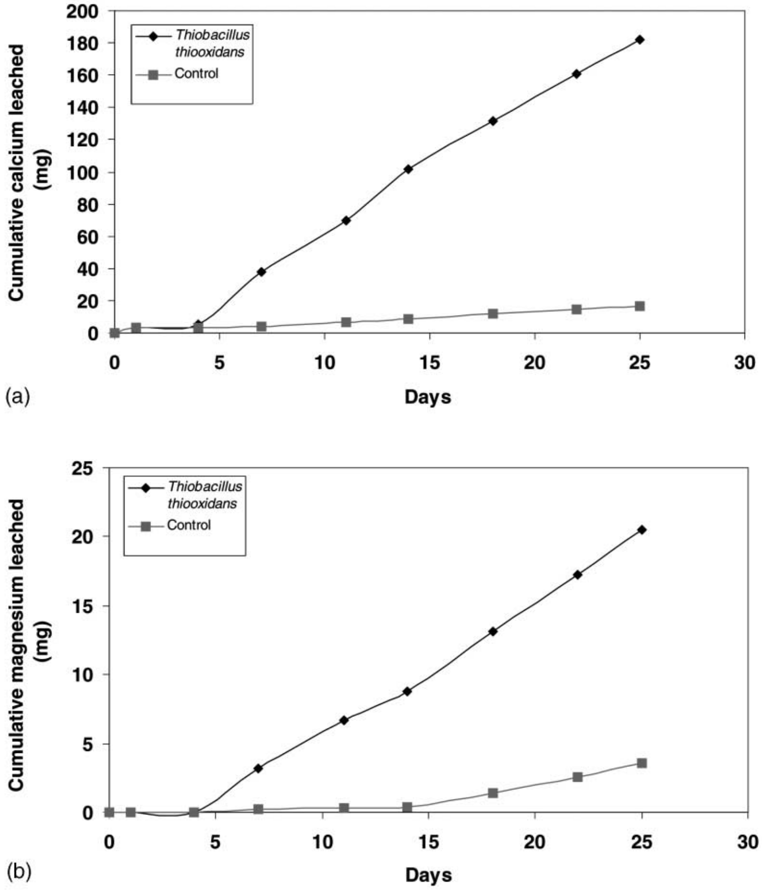


Fig. 5. The profiles of calcium (a) and magnesium (b) leached from a simulated liquid waste/cement waste form (1.5:1) using a refined biofilm formation method.

any inhibitory effects the pre-exposure of the experimental samples to *T. thiooxidans* in the first stage, and the expected propagation of biofilm in the second, should lead to the formation of more acids. Thus, the leaching of more metals from the experimental samples than from the control was expected. The production of substantially higher quantities of sulfate by the experimental samples, and the substantial lowering of the pH, all indicate extensive microbial activity that could result in the observed leaching pattern. Comparison of the leaching patterns of calcium and magnesium for the three different formulations of the liquid waste/cement waste forms in Table 1 indicates that the greatest leaching occurred from the 1.5:1 waste combination, while the least leaching occurred from the 1:1 waste combination. This suggests that the most stable combination is the 1:1 liquid waste/cement combination. It appears that an optimum ratio of the waste to cement is required to obtain the most stable combination.

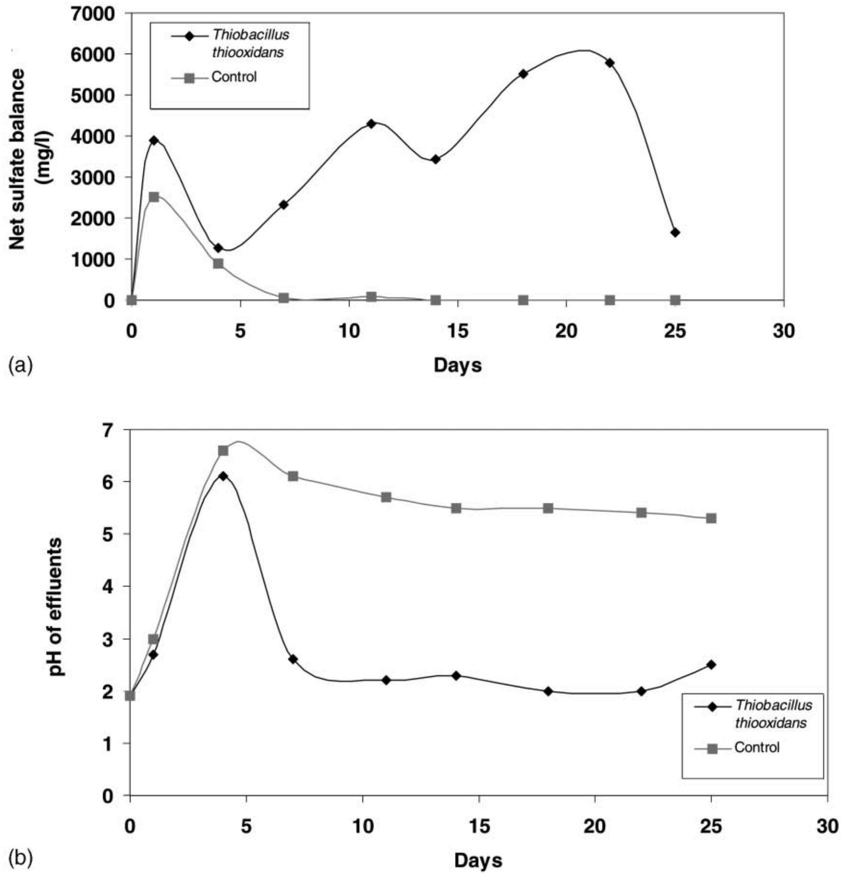


Fig. 6. The profiles of net sulfate produced (a) and pH (b) during evaluation of a simulated liquid waste/cement waste form (1.5:1) using a refined biofilm formation method.

3.2. Evaluation of simulated solid waste forms using a refined biofilm formation method

Results of evaluation of a simulated solid waste/cement waste form are presented in Table 2 and Figs. 7–12, with Figs. 7 and 8 being results for the 1:1 waste to cement combination, Figs. 9 and 10 the 1.5:1 combination, and Figs. 11 and 12 the 0.8:1 combination. Results in Fig. 7 indicate that the patterns of leaching of calcium and magnesium from experimental and control samples of the 1:1 combination between days 5 and 20 of evaluation were similar. A marginal increase in the amount of calcium leached from the experimental sample was however, observed from days 21 to 25. A total of about 45 mg of calcium and 5 mg of magnesium were leached from the experimental sample, while a total of about 35 mg of calcium and 3 mg of magnesium were leached from the control. From Fig. 8, it is evident that the net sulfate balance for both experimental and control effluents of the 1:1 combination were similar (approximately zero) much of the time. Only between days

Table 2
Important parameters from biofilm formation evaluation of solid waste/cement waste forms

Sample	Total Ca leached (mg)	Total Mg leached (mg)	Average pH (during second stage)	Maximum net sulfate balance (mg/l)
1:1 Experimental	45	5	4.5	400
1:1 Control	35	3	5.5	0
1.5:1 Experimental	45	4.5	3	500
1.5:1 Control	30	2.0	5.3	0
0.8:1 Experimental	25	4.5	5.0	0
0.8:1 Control	25	3.4	5.0	0

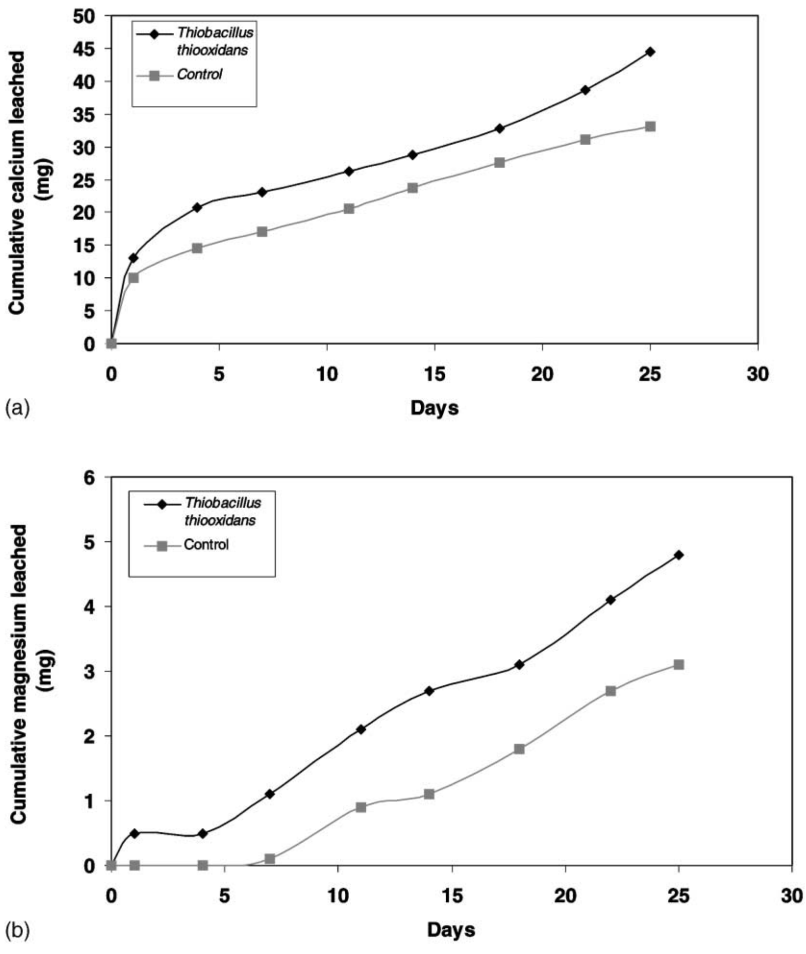


Fig. 7. The profiles of calcium (a) and magnesium (b) leached from a simulated solid waste/cement waste form (1:1) using a refined biofilm formation method.

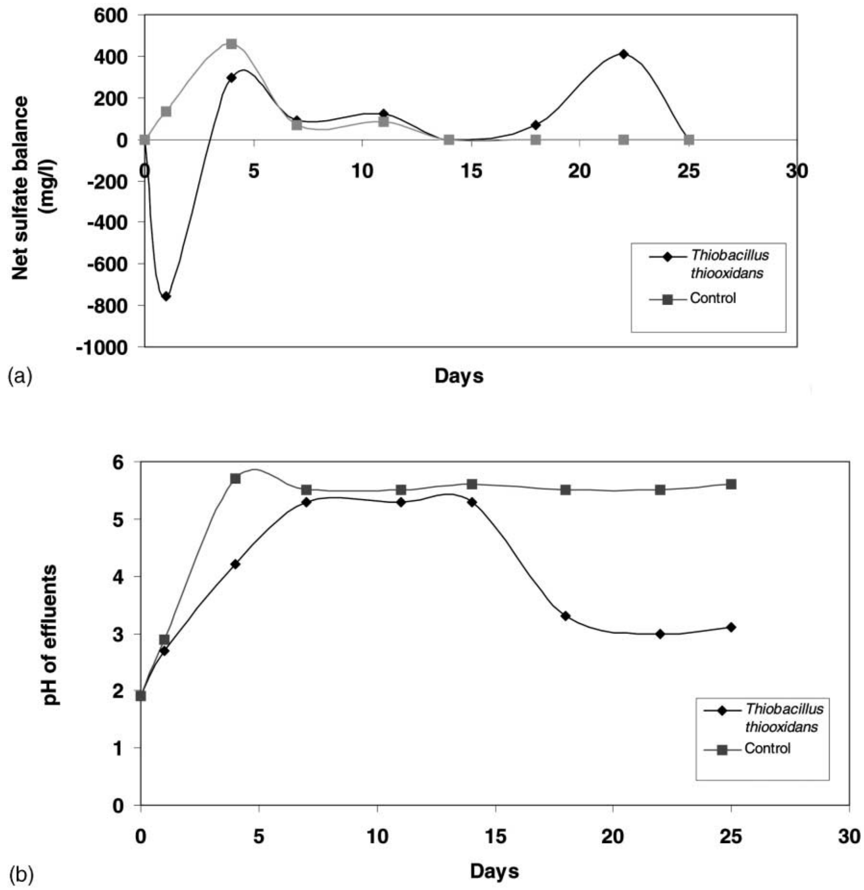


Fig. 8. The profiles of net sulfate produced (a) and pH (b) during evaluation of a simulated solid waste/cement waste form (1:1) using a refined biofilm formation method.

18 and 22 were marginal increases (to a maximum of about 400 mg/l) observed for the experimental sample. Results in Fig. 8 indicate also that the pH of effluents from control and experimental samples were similar up to day 15 of evaluation. The pH of both samples averaged 5.5 between days 5 and 15. However, the pH of the experimental effluents decreased to the lowest value of about 3 on day 18 and was maintained at this value until the termination of the evaluation.

Results in Fig. 9 indicate that the levels of calcium leached from both control and experimental samples of the 1.5:1 combination were similar up to day 15 of evaluation after which the levels of calcium leached from the experimental became moderately higher. A total of about 45 mg of calcium were leached from the experimental sample while a total of about 30 mg were leached from the control. Results in Fig. 9 indicate also that the overall level of magnesium leached from the experimental sample was comparatively higher than

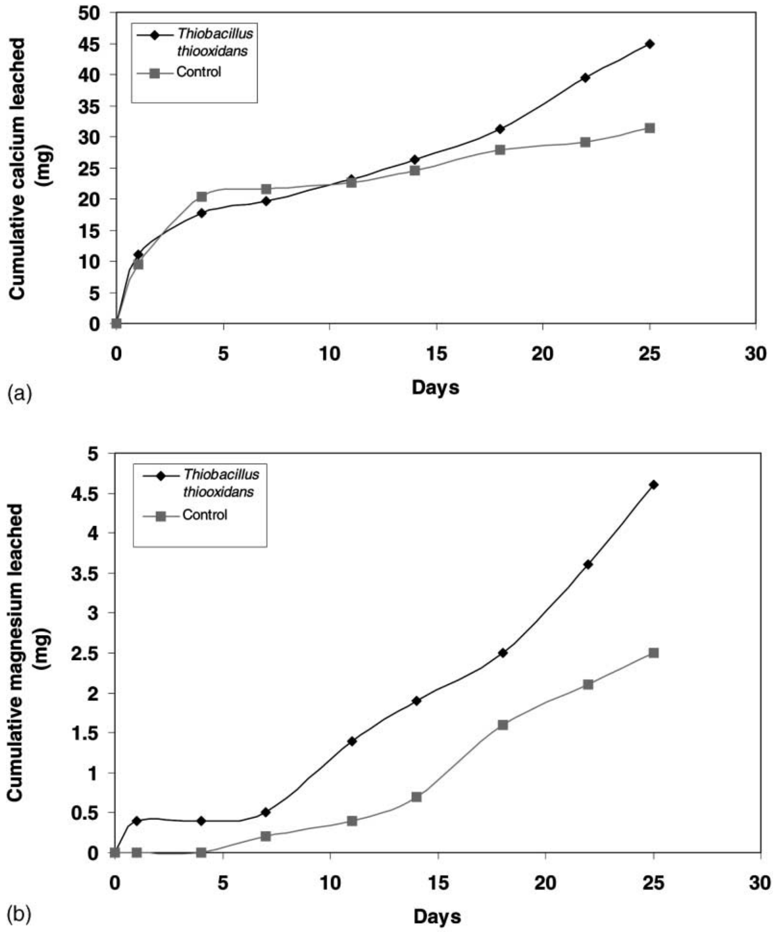
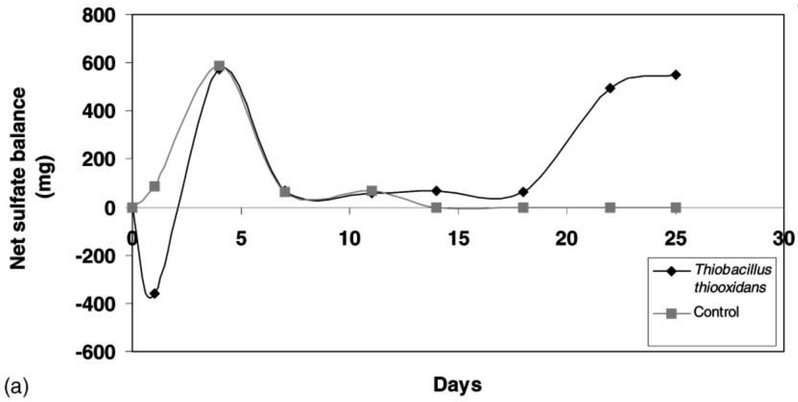


Fig. 9. The profiles of calcium (a) and magnesium (b) leached from a simulated solid waste/cement waste form (1.5:1) using a refined biofilm formation method.

the level leached from the control. A total of about 4.5 mg of magnesium were leached from the experimental sample as compared to 2.0 mg from the control. From Fig. 10, it is evident that the net sulfate balance for effluents from both control and experimental samples of the 1.5:1 combination were generally similar (approximately zero) between days 4 and 18 of evaluation. After day 18, marginal increases, up to a maximum of about 500 mg/l were observed for the experimental sample. Results in Fig. 10 indicate also that the pH of effluents of the experimental sample was comparatively lower than the pH of effluents of the control between days 14 and 25 of evaluation. Within this period, the average pH of the experimental effluents was about 3.0, while the average pH of the control effluents was about 5.3. Between days 4 and 10 of evaluation, the pH of both control and experimental effluents were similar.



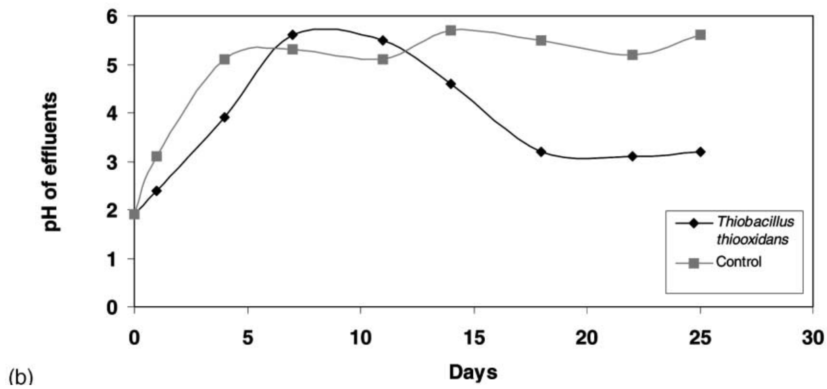
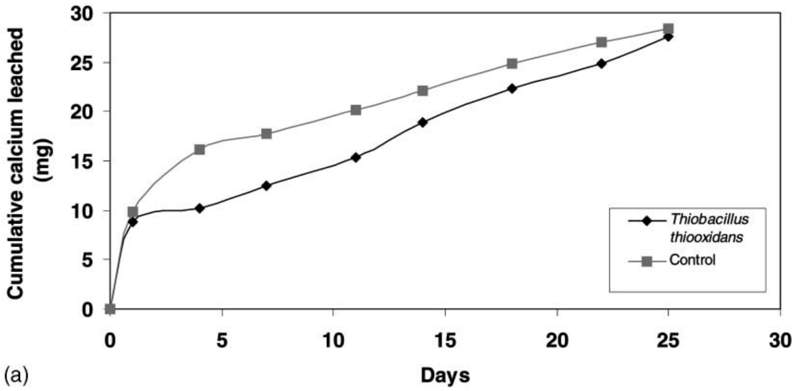


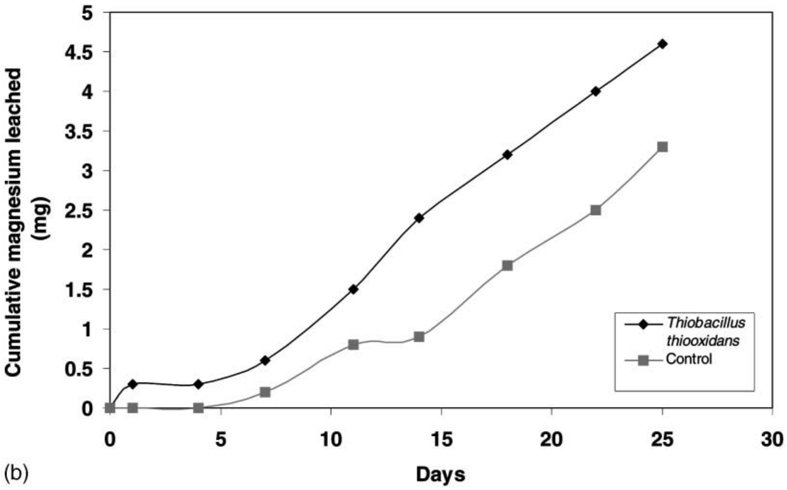
Fig. 10. The profiles of net sulfate produced (a) and pH (b) during evaluation of a simulated solid waste/cement waste form (1.5:1) using a refined biofilm formation method.

Results in Fig. 11 indicate that the overall levels of calcium leached from both control and experimental samples of the 0.8:1 combination were similar, but the overall level of magnesium leached from the experimental sample was slightly higher. While a total of 25 mg of calcium were leached from both control and experimental samples, a total of about 4.5 mg of magnesium were leached from the experimental sample and 3.4 mg from the control. It is obvious from Fig. 12 that net sulfate balances of approximately zero were obtained for both experimental and control samples in the last half of the evaluation period. Results in Fig. 12 indicate also that the pH of both control and experimental effluents were generally similar from day 7 to the end of evaluation. The pH for both samples within this period averaged 5.0.

The leaching of essentially similar amounts of calcium and magnesium from both the control and experimental samples of the solid waste/cement waste forms was contrary to expectation, and in sharp contrast to the results reported by Rogers et al. [6] using the NRC method. In an evaluation on waste forms of similar formulation to the ones used in



(a)



(b)

Fig. 11. The profiles of calcium (a) and magnesium (b) leached from a simulated solid waste/cement waste form (0.8:1) using a refined biofilm formation method.

this study, Rogers et al. [6] reported a substantially higher degradation in the experimental samples than in the controls. This is undoubtedly due to the substantial pH difference between the control medium (4.0) and the fermenter broth (1.8) than to the activities of the microbes in contact with the waste forms. This is supported by previous study [7] in which fermenter grown broths using the NRC method were shown to be substrate limited, making it impossible for the microbes to grow on the waste forms and cause direct degradation. However, the reasons for the absence of any substantial microbial activity on the simulated solid waste/cement waste forms used in this study are not clear. Extremely alkaline pH of waste form surfaces appears not to be a factor in the inhibition of growth. This is because, a rough measurement of the overall surface pH of both liquid and solid waste forms before and after immersion in broth containing

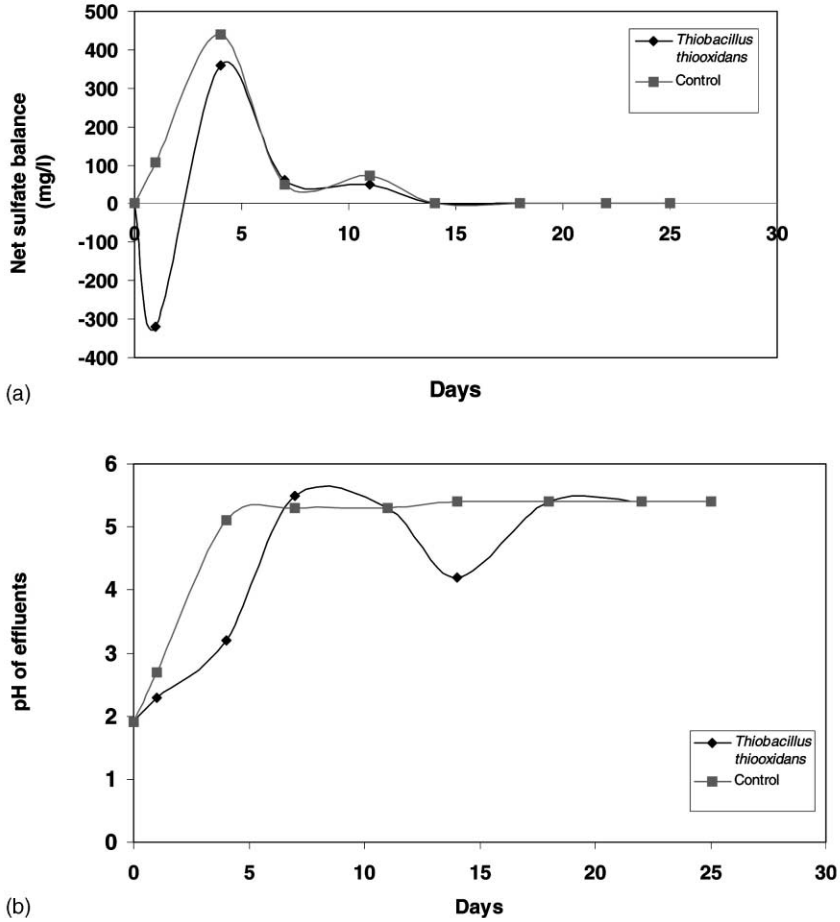


Fig. 12. The profiles of net sulfate produced (a) and pH (b) during evaluation of a simulated solid waste/cement waste form (0.8:1) using a refined biofilm formation method.

T. thiooxidans indicates that all surfaces were highly alkaline, with surface pH of about 10 obtained for all samples. Thus, the inhibition of growth in only the resin containing waste forms appears to have no bearing with the surface pH. This does not in no way rule out the effect of local pH differences, which were not evaluated by this study. It is possible that the observed microbial growth inhibition is due to the presence of inhibitory components in the resins. Most resins contain organic compounds such as phenol and formaldehyde, which are known to be toxic to a wide range of microbes. However, there are a few groups of microbes that have been shown to exhibit significant tolerance to resins. In fact a group of microbes has been reportedly isolated from areas around plants involved in the production of phenol-based resins in Germany, that exhibit capability of biodegrading resins [10].

4. Conclusions

The simulated liquid waste/cement waste forms showed evidence of high susceptibility to microbial degradation. The 1:1 combination was most stable while the 1.5:1 combination was least stable. The simulated solid waste/cement waste forms showed evidence of higher stability to microbial degradation than the simulated liquid waste/cement waste forms. Resins, which were added to the simulated solid waste/cement waste forms, may have contained compounds inhibitory to the growth of *T. thiooxidans*.

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References

[1] R.D. Rogers, M.A. Hamilton, R.H. Veeh, J.W. McConnell Jr., Final Report on Microbial Degradation of Low-Level Radioactive Waste, prepared for Division of Regulatory Applications, Office of Nuclear Regulatory Research, US Nuclear Regulatory Commission, Washington, DC, NRC Job code L1807, 1996.
[2] P. Colombo, R.M. Neilson Jr., NUREG/CR-0619, BNL-NUREG-50591, Brookhaven National Laboratory, 1979.
[3] C.D. Parker, Austral. J. Exp. Biol. Med. Sci. 23 (1945) 81–90.
[4] W. Sand, E. Bock, in: D.R. Houghton, R.N. Smith, H.O.W. Eggins (Eds.), Biodeterioration, Vol. 7, Elsevier, New York, 1988, pp. 113–117.
[5] M. Diercks, W. Sand, E. Bock, Experientia 47 (1991) 514–516.
[6] R.D. Rogers, M.A. Hamilton, R.H. Veeh, J.W. McConnell Jr., in: T.M. Gilliam, C.C. Wiles (Eds.), Stabilization and Solidification of Hazardous, Radioactive, and Mixed Wastes, Vol. 3, ASTM STP 1240, American Society for Testing and Materials, 1996, p. 116.
[7] M.A. Idachaba, K. Nyavor, N.O. Egiebor, R.D. Rogers, J. Hazard. Mater. 77 (1–3) (2000) 133–147.
[8] M.A. Idachaba, K. Nyavor, N.O. Egiebor, R.D. Rogers, J. Hazard. Mater. 84 (1) (2001) 95–106.
[9] A.D. Eaton, L.S. Clesceri, A.E. Greenberg (Eds.), Standard Methods for the Examination of Water and Wastewater, 19th Edition, American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, 1995, pp. 4–136.
[10] M. Balter, Science 252 (1991) 205.