Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 150 (2008) 419–423
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

The effect of various reaction parameters on bioremediation of perchlorate-contaminated water

Donglei Wu, Ping He, Xinhua Xu *, Mi Zhou, Zhen Zhang, Zaidi Houda

Department of Environmental Engineering, Zhejiang University, Hangzhou 310027, PR China

Received 31 July 2006; received in revised form 11 April 2007; accepted 11 April 2007
Available online 3 May 2007

Abstract

The bioremediation was employed to treat perchlorate-contaminated water. All enrichments and growth of mixed cultures were performed in anaerobic acetate medium. Enrichment cultures were started with activated sludge obtained from a local wastewater treatment plant where it predominantly treats domestic wastewater. Several parameters affecting perchlorate removal were examined through batch experiments, these include the amount of domesticated sludge, the acetate concentration, pH, the C/N ratio and the reaction temperature. The results indicated that acetate was an effective carbon source and electron donor. Under the selected conditions, namely 1.0 g domesticated sludge, an acetate concentration of 1.2 g l^{-1}, pH 8.0, a C/N ratio of 20 at 40 °C, 50 mg l^{-1} perchlorate could be rapidly reduced to non-detectable levels within 24 h.

Keywords: Perchlorate reduction; Bioremediation; Anaerobic medium; Bacterium; Domesticated sludge

1. Introduction

Perchlorate (ClO_4^-) has become a contaminant of significant concern in surface and ground waters over the years, and it has recently been added to the drinking water Candidate Contaminant List (CCL) by the United States Environmental Protection Agency (USEPA). Perchlorate was primarily used in the manufacturing of solid rocket, fireworks, explosives, matches, batteries, and automobile air bags etc. Most of the perchlorate contaminations were resulted from historical discharge of unregulated waste effluents containing high levels of perchlorate [1]. Perchlorate has also been found in several fertilizers mined from Chilean caliche [2]. The use of perchlorate-contaminated water presumably interferes with iodine uptake and hormone production by human thyroid affecting the vital body functions [3,4]. Higher doses even result in fatal bone marrow disorders [5]. A recent survey on waterworks in Beijing has also indicated that perchlorate pollution existed to a certain extent and varied with the changing seasons, and groundwater perchlorate contamination appeared to be the main source of perchlorate pollution of drinking water [6].

The perchlorate ion is nonvolatile, highly soluble, and very stable in the aqueous phase. Traditionally, ion exchange is used as one of the effective methods for its removal, but it is also an incomplete process because it is non-selective and separates all anions besides perchlorate from the contaminated sources. Due to the high affinity of perchlorate for resins, very high salt concentrations are needed to regenerate the column and perchlorate remains in regeneration brine waste, which needs to be treated further [7]. Alternatively, bioremediation is a cost-effective treatment technology, which is capable of removing perchlorate to a safer level.

Perchlorate is used as a terminal electron acceptor by pure and mixed cultures of microorganisms during its reduction. The reductive sequence of perchlorate was suggested as: $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow Cl^- + O_2$ [8–11]. Potential ClO_4^- reducing bacteria are believed to be widespread in nature. Both pure and mixed cultures of some denitrifying bacteria were shown to utilize perchlorate as a terminal acceptor [8,9,12,13].

In present study, the optimal conditions for bacterial perchlorate reduction were preliminarily determined. Factors such as the addition of domesticated sludge, the carbon source and its addition, C/N ratio, pH and reaction temperature are known to be very important for the treatment process, so these parameters were chosen for this study.

* Corresponding author. Tel.: +86 571 87951239; fax: +86 571 87952771.
E-mail address: xhxu@hzcnc.com (X. Xu).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.04.124

2. Experiments and methods

2.1. Chemicals

All the chemicals such as sodium chlorate, sulfuric acid, sodium acetate (>99%), acetone (>99.5%), sodium phosphate dibasic dodecahydrate (>99%), sodium nitrate except sodium perchlorate (>98%, CR), were purchased as analytical reagent grade and were used as received. Iron metal was pre-treated by diluted sulfuric acid (pH 2), acetone, and subsequently rinsed with deionized water several times.

2.2. Growth medium and conditions

All enrichments and growth of mixed cultures were performed in anaerobic acetate medium (AAM), which consists of the following chemicals in per litre of deionized water: 2.8 g $C_2H_3O_2Na$, 1.5 g $Na_2HPO_4{\cdot}12H_2O$, 0.95 g KH_2PO_4, 1.0 g $NaNO_3$, 0.1 g $MgSO_4$, 0.07 g Na_2SO_3, 3 mg EDTA and 10 ml trace elements solution, containing per litre: 200 mg $CaCl_2$, 50 mg $MnSO_4$, 10 mg Na_2MoO_4, 10 mg $CuSO_4{\cdot}5H_2O$ and 10 ml $CuSO_4{\cdot}5H_2O$ (10 mM). The enrichment medium was then transferred to 5-L three-necked flasks with air-tight screw caps and vitreous tubes, with the sodium acetate as the sole carbon and energy source.

2.3. Enrichment and domestication

Enrichment cultures were started with activated sludge procured from a Sibao wastewater treatment plant in Hangzhou, China, where they treat predominantly domestic wastewater. Two-litre sludge and 1-L medium were added to a 5-L three-necked flask, incubated at 30 °C in a water bath equipped with automatic temperature control. A magnetic agitator was used to stir the solution for 30 min every day, and 250 ml of fresh medium was replaced daily. For anaerobic growth, these cultures were purged with oxygen-free nitrogen gas.

In the first week of the enrichment, 250 ml fresh medium was replaced in the tank daily and only activated sludge was incubated without perchlorate during this period. Afterwards, increasing dosage (6 ml g l^{-1}) of perchlorate was added to the tank with the passage of time. After 3 weeks or so, perchlorate was reduced to chloride steadily and efficiently. The total time period required for domestication was about 1 month during which media were added to provide carbon and energy source at regular intervals.

2.4. Analysis of parameters affecting perchlorate removal

Batch experiments were conducted using the bottle-point method. Domesticated sludge harvested from three-necked flasks was centrifuged at 5000 rpm, the resulting sediment together with media containing perchlorate were added into a 100-ml serum bottle. The dissolved oxygen in the medium was not removed; however, the headspace of the serum bottle was purged with nitrogen gas for 3 min. The serum bottles were crimp-sealed with butyl-rubber stoppers and contents were mixed on a rotary shaker at a speed of 150 rpm and finally incubated at 30 °C. Several parameters affecting perchlorate removal were measured through batch experiments, including the amount of domesticated sludge, acetate concentration, pH, the C/N ratio (nitrate as N compound) and the reaction temperature. Initial pH of wastewater was adjusted by 1 M H_2SO_4 or 1 M NaOH solution.

2.5. Analytical methods

Aliquot samples in the amount of 2-ml for serum bottle tests were taken using a spindly needle and sterile syringes, and filtered through 0.45 μm membrane filters first and then through 0.22 μm membrane filters, and kept in a refrigerator at 4 °C if not being analyzed on the same day.

Perchlorate, nitrate, chloride, chlorate, chlorite, acetate were measured using a Metrohm 792 Basic Ion Chromatograph (IC) equipped with a Metrosep A Supp 4 column (250 mm × 4 mm), a Metrosep A Supp 4/5 guard column, and a conductivity detector. The detection limit for perchlorate was 0.5 ppm. Sodium carbonate (1.8 mM) and sodium bicarbonate (1.7 mM) served as the eluent, and sulfuric acid (2.0 mM) as the regenerant. Sample size was 20 μl. One hundred milligrams per litre certified perchlorate solution was confected to prepare perchlorate calibration standards. Deionized water was used for all analyses.

Solution pH was measured using a PHS-25C pH meter (Shanghai).

3. Results and discussion

3.1. Effect of the addition of domesticated sludge

After domestication, the perchlorate-reducing ability of the sludge was greatly improved, from 10 days in the beginning to 36 h for the complete reduction of 50 mg l^{-1} perchlorate. Batch experiments were conducted later to study the effect of various parameters.

The addition of different amounts of domesticated sludge to reduce 50 mg l^{-1} perchlorate is shown in Fig. 1. Nearly 100% perchlorate was removed within 2 days when the amounts of domesticated sludge were 0.8–5.0 g, but only 26.4 and 62.3%

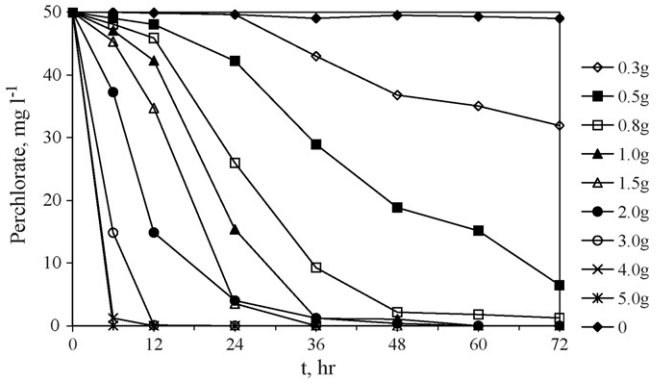

Fig. 1. Effect of addition of domesticated sludge on perchlorate reduction. Initial conditions: perchlorate, 50 mg l^{-1}; acetate, 1.2 g l^{-1}; $T = 30$ °C.

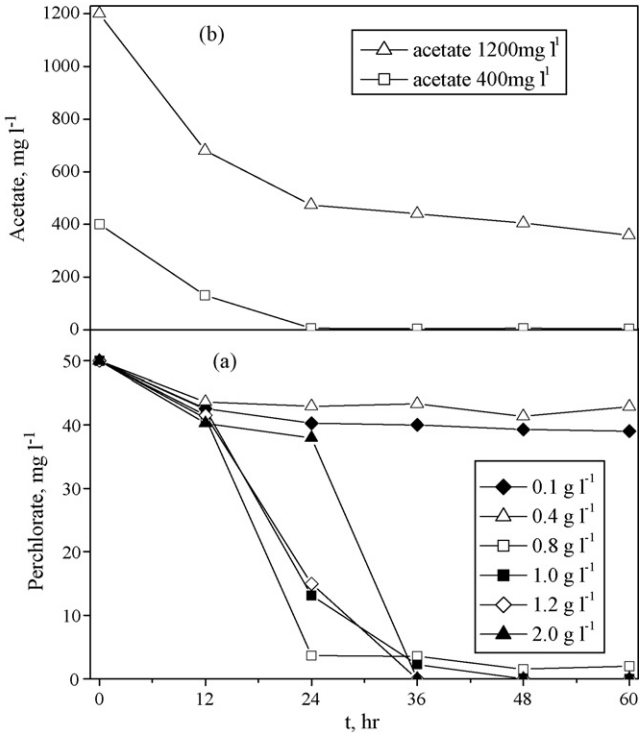

Fig. 2. Effect of acetate concentration on perchlorate reduction. Initial conditions: perchlorate, 50 mg l^{-1}; domesticated sludge, 1.0 g; $T = 30\,^{\circ}C$. (a) Perchlorate concentration, acetate 0.1–2.0 g l^{-1} and (b) acetate concentration, acetate 0.4 and 1.2 g l^{-1}.

were removed when the additions were 0.3 and 0.5 g, respectively. The results indicated that domesticated sludge had a positive effect on perchlorate reduction. The more the domesticated sludge was introduced, the faster the rate of perchlorate reduction was. Fifty milligrams per litre perchlorate was almost completely reduced within 12 h if the domesticated sludge added was more than 3.0 g, and there was no obvious difference when 4.0 and 5.0 g was used with respective 97.5 and 99.9% perchlorate removal in 6 h. Bacteria might have been competing with each other for limited substrates (i.e. perchlorate, medium and space), which might had restricted their continued growth and activity, while a small quantity of domesticated sludge might have not had such problems and the bacteria could propagate more at this circumstance. Considering better results for other parameters on perchlorate reduction, we decided to use 1.0 g domesticated sludge throughout our subsequent studies.

3.2. Effect of acetate concentration

Six initial acetate concentrations were investigated in this study. Fig. 2a shows that the rate of the perchlorate removal was similar at different acetate concentrations within 12 h, as the microorganisms might remain active even though just 0.1 and 0.4 g l^{-1} acetate were introduced in the initial 12 h. After that perchlorate was removed rapidly with acetate concentration ranging 0.8–2.0 g l^{-1}, but had no appreciable variations in the acetate concentration above 1.2 g l^{-1}, 100% perchlorate reduction took ~36 h for the acetate addition of 1.2 and 2.0 g l^{-1}.

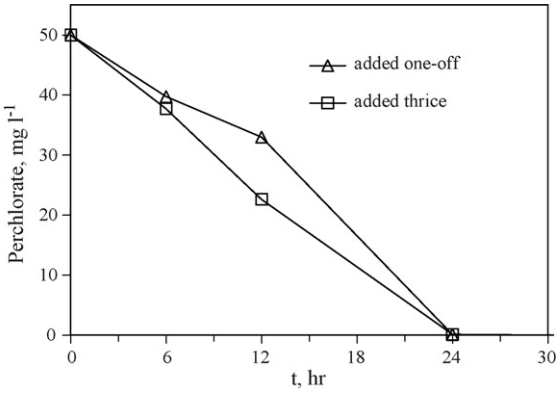

Fig. 3. Effect of time of adding acetate on perchlorate reduction. Initial conditions: perchlorate, 50 mg l^{-1}; domesticated sludge, 1.0 g; (△) acetate added one-off in the beginning, (□) acetate added thrice, respectively in 0, 12, 24 h with 0.4 g l^{-1} each time; $T = 30\,^{\circ}C$.

The results suggested that acetate concentrations of 0.1 and 0.4 g l^{-1} were not high enough to reduce 50 mg l^{-1} perchlorate. The amount of bacteria deceased due to a lack of nourishment (as observed under a microscope) which led to lower rate of the perchlorate removal. The theoretical acetate-to-perchlorate ratio based on the stoichiometry is 1 mol mol^{-1} according the total reaction: $CH_3COO^- + ClO_4^- \rightarrow 2HCO_3^- + H^+ + Cl^-$ [9], but the stoichiometric reactions of acetate can also occur with different electron acceptors, including ClO_4^-, ClO_3^-, O_2 and nitrate. In our experiments, the consumed amount of acetate is about 800 with 50 mg l^{-1} perchlorate reduction (Fig. 2b), so the approximate observed ratio of acetate to perchlorate is about 18 mol mol^{-1} perchlorate. Our experimental data indicated that the bioremediation of perchlorate could be completed under these conditions. We also investigated the effect of time on the perchlorate reduction after acetate addition, of 1.2 g l^{-1} which was added immediately in the beginning and at the other three times, i.e. in 0, 12, 24 h where 0.4 g l^{-1} acetate was added as shown in Fig. 3, no obvious difference in perchlorate removal was found in two bottles. As a result, acetate concentration of 1.2 g l^{-1} was selected as the optimal concentration for the following batch experiments.

3.3. Batch culture C/N experiments

As shown in Fig. 4a, perchlorate with different C/N ratios was almost completely removed in 2 days. In comparison, perchlorate reduction with a C/N ratio of 20 was the most rapid among the different ratios under study and the removal efficiency reached 68.2% within 24 h. Only 20.7% perchlorate was reduced in the same time frame for a C/N ratio of 5, but no substantial difference was noted for C/N ratios of 10, 20, and 30. Nitrate concentrations also decreased with the reduction of perchlorate (Fig. 4b). Perchlorate was rapidly removed and decreased from 173 to 3.763 mg l^{-1} after 1 day with the C/N ratio of 20. Rapid NO_3^- removal might have inhibited ClO_4^- removal, possibly due to diversion of electrons from ClO_4^- to NO_3^-. Such a nitrate inhibition effect on microbial perchlorate reduction was also reported in previous studies [11,14–18]. More importantly, the

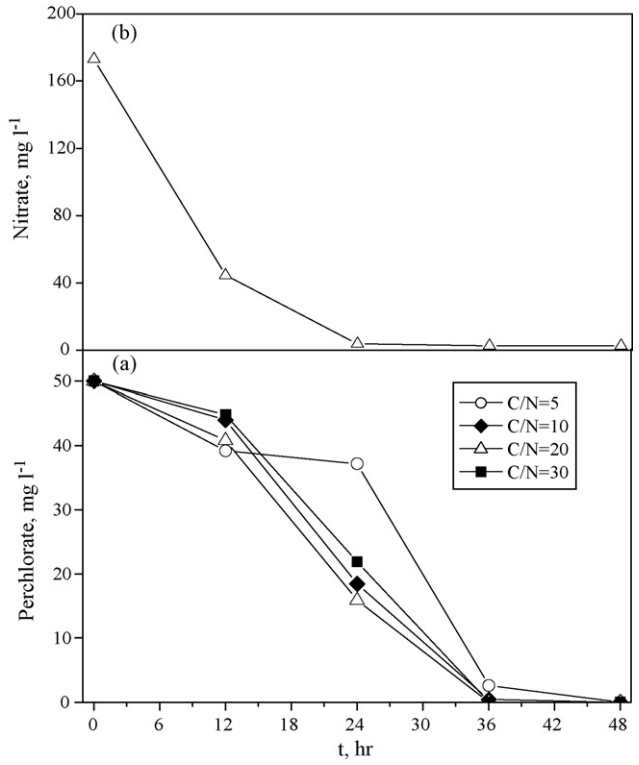

Fig. 4. Effect of initial C/N ratio on perchlorate reduction. Initial conditions: perchlorate, 50 mg l^{-1}; domesticated sludge, 1.0 g; acetate, 1.2 g l^{-1}; $T = 30\,^{\circ}C$. (a) Perchlorate concentration and (b) nitrate concentration, C/N, 20.

accumulation of nitrite, a toxic intermediate, was not detected in the solution, which might be a proof of denitrifying ability of the domesticated sludge.

3.4. Effect of pH

Initial pH of wastewater was adjusted using 1 M H_2SO_4 or NaOH solution, perchlorate reduction was observed at the initial pH range 6.0–10.0 (Fig. 5). The perchlorate-reducing culture substantially reduced perchlorate in the pH range of 7.0–9.0 while the reaction was considerably slower at pH 6 and 10. The maximum reduction of ClO_4^- was observed at pH 8, in which perchlorate decreased substantially from an initial concentration of 50 to 1.736 mg l^{-1} (96.5% reduction) with 12 h, and beyond detection limit (about 100% reduction) within 36 h. The bacterial growth was drastically inhibited at the pH 6 and 10 as observed microscopically, only 25.3 and 21.4%, respectively, of perchlorate was removed. The pH of solutions in the batch experiments were always near 8 in the range of 7.5–8.5.

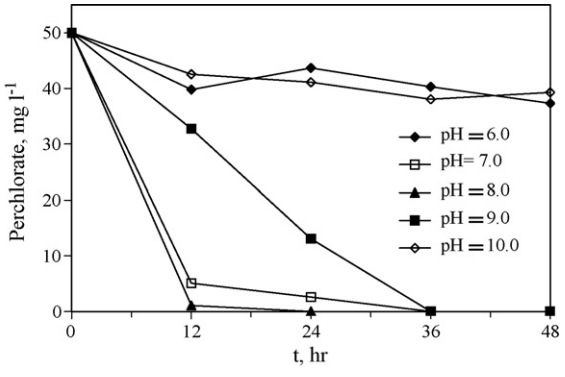

Fig. 5. Effect of pH on perchlorate reduction. Initial conditions: perchlorate, 50 mg l^{-1}; domesticated sludge, 1.0 g; acetate, 1.2 g l^{-1}; C/N, 20; $T = 30\,^{\circ}C$.

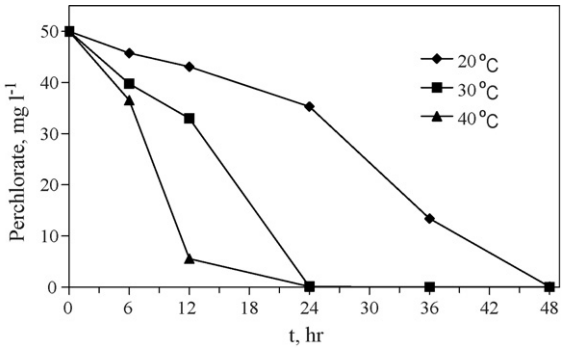

Fig. 6. Effect of temperature on perchlorate reduction. Initial conditions: perchlorate, 50 mg l^{-1}; domesticated sludge, 1.0 g; acetate, 1.2 g l^{-1}; C/N, 20.

3.5. Effect of temperature

Perchlorate was quickly reduced at both temperatures of 30 and 40 °C, as shown in Fig. 6, but the removal rate at 40 °C was much more rapid than that at 30 °C. About 41 mg l^{-1} perchlorate was reduced (82%) within 12 h at 40 °C, while only 32.8% perchlorate was removed in the same time frame at 30 °C. One hundred percent perchlorate reduction needed 24 h at 40 °C and 48 h at 30 °C. The results indicated that a slight increase in the reaction temperature had a stimulatory effect on perchlorate reduction by bacterial perchlorate reduction on a large scale.

4. Conclusions

Recently, remediation of perchlorate-contaminated drinking water supplies and underground waters emerged as a pressing issue in environmental protection. Among others, bioremediation is proven to be an attractive option for removing low level of perchlorate from contaminated water because of its high efficiency and minimal impact on water quality. While cultures inoculated with activated sludge from a plant treating predominantly domestic wastewater are shown to be capable of perchlorate reduction.

The perchlorate reduction was significantly affected by various reaction parameters including the amount of domesticated sludge, the concentration of acetate, pH value, the C/N ratio and the reaction temperature. The results of our batch experiments suggested that acetate is a very effective carbon source and electron donor for perchlorate reduction. We have determined that 50 mg l^{-1} perchlorate could be rapidly reduced to a non-detectable level within 24 h, using a domesticated sludge of 1.0 g, an acetate concentration of 1.2 g l^{-1}, a pH of 8.0, and a C/N ratio of 20 at 40 °C.

Acknowledgements

This research was supported by the Natural Science Foundation of Zhejiang Province (No. Y505057), the National Natural Science Foundation of China (No. 20407015) and the Program for New Century Excellent Talents in University (No. NCET-06-0525).

References

[1] P. Damian, F.W. Pontius, From rockets to remediation: the perchlorate problem, Environ. Protect. (1999) 24–31.
[2] E.T. Urbansky, S.K. Brown, M.L. Magnuson, C.A. Kelty, Perchlorate levels in samples of sodium nitrate fertilizer derived from Chilean caliche, Environ. Pollution 112 (2001) 299–302.
[3] J.C. Siglin, D.R. Mattie, D.E. Dodd, P.K. Hildebrandt, W.H. Baker, A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate, Toxicol. Sci. 57 (2000) 61–74.
[4] E.A. Merrill, R.A. Clewell, J.M. Gearhart, P.J. Robinson, T.R. Sterner, K.O. Yu, D.R. Mattie, J.W. Fisher, PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat, Toxicol. Sci. 73 (2003) 256–269.
[5] E.T. Urbansky, M.R. Schock, Issues in managing the risks associated with perchlorate in drinking water, J. Environ. Manage. 56 (1999) 79–95.
[6] Y.J. Liu, S.F. Mou, A.W. Liu, B. Du, Investigation of bromate, haloacetic acids and perchlorate in Beijing's drinking water, Environ. Sci. 25 (2004) 51–55.
[7] J.R. Batista, F.X. McGarvey, A.R. Vieira, The removal of perchlorate from waters using ion exchange resins, in: E.T. Urbansky (Ed.), Perchlorate in the Environment, Plenum, New York, 2000, p. 135.
[8] H. Attaway, M. Smith, Reduction of perchlorate by an anaerobic enrichment culture, J. Ind. Microbiol. 12 (1993) 408–412.
[9] G.B. Rikken, G.M. Kroon, C.G. Ginkel, Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation, Appl. Microbial. Biotechnol. 45 (1996) 420–426.
[10] B.E. Logan, A review of chlorate and perchlorate respiring microorganisms, Bioremediat. J. 2 (1998) 69–79.
[11] D.C. Herman, W.T. Frankenberger, Microbial-mediated reduction of perchlorate in ground-water, J. Environ. Qual. 27 (1998) 750–754.
[12] N. Bardiya, J.H. Bae, Bioremediation potential of a perchlorate-enriched sewage sludge consortium, Chemosphere 58 (2005) 83–90.
[13] J.D. Shrout, A.G.B. Williams, M.M. Scherer, G.F. Parkin, Inhibition of bacterial perchlorate reduction by zero-valent iron, Biodegradation 16 (2005) 23–32.
[14] D.C. Herman, W.T. Frankenberger, Bacterial reduction of perchlorate and nitrate in water, J. Environ. Qual. 28 (1999) 1018–1024.
[15] S.K. Chaudhuri, S.M. O'Connor, R.L. Gustavson, L.A. Achenbach, J.D. Coates, Environmental factors that control microbial perchlorate reduction, Appl. Environ. Microbiol. 68 (2002) 4425–4430.
[16] M.E. Losi, T. Giblin, V. Hosangadi, W.T. Frankenberger, Bioremediation of perchlorate-contaminated groundwater using a packed bed biological reactor, Bioremediat. J. 6 (2002) 97–104.
[17] K. Tan, T.A. Anderson, W.A. Jackson, Degradation kinetics of perchlorate in sediments and soils, Water Air Soil Pollut. 151 (2004) 245–259.
[18] M. Nozawa-Inoue, K.M. Scow, D.E. Rolston, Reduction of perchlorate and nitrate by microbial communities in vadose soil, Appl. Microbiol. Biotechnol. 7 (2005) 3928–3934.