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Detoxification of PAX-21 ammunitions wastewater by zero-valent iron for microbial reduction of perchlorate

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ABSTRACT

US Army and the Department of Defense (DoD) facilities generate perchlorate (ClO_4^-) from munitions manufacturing and demilitarization processes. Ammonium perchlorate is one of the main constituents in Army's new main charge melt-pour energetic, PAX-21. In addition to ammonium perchlorate, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4-dinitroanisole (DNAN) are the major constituents of PAX-21. In order to evaluate microbial perchlorate reduction as a practical option for the treatment of perchlorate in PAX-21 wastewater, we conducted biodegradation experiments using glucose as the primary sources of electrons and carbon. Batch experiments showed that negligible perchlorate was removed in microbial reactors containing PAX-21 wastewater while control bottles containing seed bacteria and glucose rapidly and completely removed perchlorate. These results suggested that the constituents in PAX-21 wastewater may be toxic to perchlorate reducing bacteria. A series of batch toxicity test was conducted to identify the toxic constituents in PAX-21 and DNAN was identified as the primary toxicant responsible for inhibiting the activity of perchlorate reducing bacteria. It was hypothesized that pretreatment of PAX-21 by zero-valent iron granules will transform toxic constituents in PAX-21 wastewater to non-toxic products. We observed complete reduction of DNAN to 2,4-diaminoanisole (DAAN) and RDX to formaldehyde in abiotic iron reduction study. After a 3-day acclimation period, perchlorate in iron-treated PAX-21 wastewater was rapidly decreased to an undetectable level in 2 days. This result demonstrated that iron treatment not only removed energetic compounds but also eliminated the toxic constituents that inhibited the subsequent microbial process.

1. Introduction

Perchlorate has recently received a great deal of attentions due to high concentrations found in groundwater and surface waters. Perchlorate has been released to the environment primarily through the use of ammonium perchlorate as a propellant in missiles, rockets and explosives, as a pyrotechnic in fireworks, in magnesium batteries, paint and as an automobile air bag inflator [1,2]. Aqueous perchlorate is known to be highly stable and non-reactive under ambient conditions. Perchlorate is not only chemically stable in natural waters, but also extremely soluble and mobile; as a result, only a limited number of technologies are capable of removing perchlorate from contaminated water [3,4]. Recently, microbial reduction of perchlorate has been recognized as a promising technology [5–8] for the treatment of perchlorate-contaminated water. Some facultative anaerobic bacteria can utilize perchlorate as an electron acceptor and reductively transform perchlorate to environmentally benign end product, chloride (Cl^-) (Fig. 1). Many researchers successfully showed that perchlorate can be effectively removed from the biological system by supplying sufficient electron donors. A variety of different electron donors, including ethanol, fatty acids (acetate, lactate, propionate, and citrate), vegetable oils, have been shown to support the growth of perchlorate respiring bacteria [6,8]. Several perchlorate respiring bacteria have been shown to use inorganic electron donors including H_2, sulfide and Fe(II) [9]. Perchlorate respiring bacteria (PRB) are ubiquitous in natural environment such as soils, sediments, surface water, and groundwater aquifers [10]. A variety of PRB have been isolated and many of which are members of the newly identified genera *Dechloromonas*, *Dechlorospirillum*, and *Dichlorosoma* [1,6].

US Army and the Department of Defense (DoD) facilities generate perchlorate from munitions manufacturing and demilitarization processes. For example, ammonium perchlorate is one of the main constituents in Army's new main charge melt pour explosives, Picatinny Arsenal Explosive 21 (PAX-21). In addition to ammonium perchlorate, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 2,4-dinitroanisole (DNAN) are the major constituents

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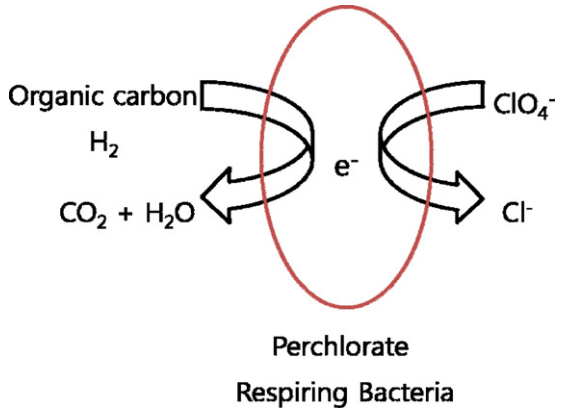

Fig. 1. Schematic diagram of microbial perchlorate reduction.

of PAX-21. Presently, most Army ammunition plants use granular activated carbon (GAC) adsorption and alkaline hydrolysis to separate and treat energetic compounds in wastewater from munitions manufacturing and demilitarization processes. The GAC process not only is expensive but generates explosive-laden spent carbon, which needs to be treated or disposed of properly to avoid secondary contamination problems. This additional treatment further increases the overall cost of wastewater treatment.

Literature review suggested that perchlorate in PAX-21 wastewater can be removed by perchlorate respiring bacteria when it is supplied by sufficient electron donor. However, there is little information whether energetic compounds in PAX-21 wastewater can influence the rates of perchlorate biodegradation. Many nitroaromatic compounds including 2,4-dinitrotoluene (DNT) and 2,4,6-trinitrotoluene (TNT) have been shown to be toxic or mutagenic to various organisms [11]. Davies and Provatas [12] showed that DNAN was mutagen in bacteria and it exhibited larger toxicity to bacteria than munitions Composition B (60% RDX, 40% TNT). Recently, it was shown that zero-valent iron (Fe(0)) can enhance biodegradation of recalcitrant nitroaromatic compounds by removing electron-withdrawing nitro groups [13,14]. Perey et al. [13] showed that elemental iron pretreatment of azo dye-containing wastewater can reductively transform the electron-withdrawing constituents on the azoaromatic compounds and make them more amenable for aerobic biodegradation. Oh et al. [14] also showed that Fe(0) treatment transformed recalcitrant RDX to ring cleavage products, formaldehyde, that are more amenable to mineralization by aerobic bacteria. If constituents in PAX-21 wastewater have toxicity to perchlorate respiring bacteria, Fe(0) pretreatment of PAX-21 wastewater may also be considered for enhancing the biodegradability in the similar manner.

The objective of this research is to determine the toxicity of energetic compounds to perchlorate respiring bacteria and evaluate microbial perchlorate reduction as a practical option for the treatment of perchlorate in PAX-21 wastewater. Biodegradation experiments, using glucose as the primary source of electrons, were conducted to determine whether energetic compounds in PAX-21 wastewater can influence the rates of perchlorate biodegradation. We also evaluated iron pretreatment of PAX-21 wastewater to increase perchlorate removal rates, and finally we propose an integrated iron pretreatment and anaerobic biological treatment process for treatment of perchlorate containing PAX-21 wastewater.

2. Materials and methods

2.1. Chemicals

Glucose, 2,4-dinitroanisole ($C_7H_6N_2O_5$, DNAN, 98%), and 2-methoxy-5-nitroanlinine ($C_7H_8N_2O_3$, 98%) were purchased from

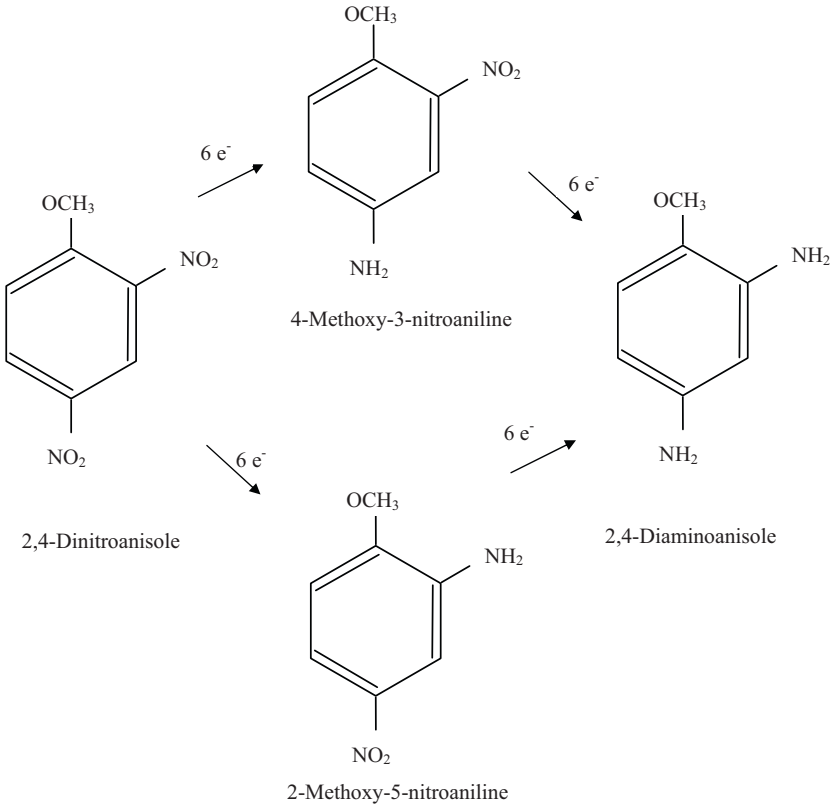

Fig. 2. The structure of DNAN and its reduction products.

Sigma (St. Louis, MO). 4-Methoxy-1,3-phenylenediamine sulfate hydrate (2,4-diaminoanisole sulfate hydrate, 99.5%) was purchased from Chem Service (West Chester, PA) and 4-methoxy-3-nitroanlinine ($C_7H_8N_2O_3$, 97.8%) was purchased from ChemPacific (Baltimore, MD). Sodium perchlorate monohydrate ($NaClO_4{\cdot}H_2O$, ~100%) was purchased from Fisher Scientific (Pittsburgh, PA). PAX-21 wastewater was obtained from Holston Army ammunition plant (Kingsport, TN). The structures of the aromatic compounds are shown in Fig. 2.

Zero-valent iron granules used in this study was purchased from Peerless Metal Powders (Detroit, MI) and was used after sieving with 10–20 mesh. The specific surface area was previously determined to be 1.67 m^2/g [15].

2.2. Microorganisms

Activated sludge cultures from an aeration basin of the Wilmington wastewater treatment plant (Wilmington, DE) were used for batch biodegradation studies without acclimation or enrichment. The culture medium contained 1.386 g/L Na_2HPO_4, 0.849/L KH_2PO_4, 0.1 g/L $(NH_4)_2SO_4$, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 1 mL trace mineral solution, and 1 mL Ca–Fe solution (adapted from Nerenberg et al. [16]). The Ca–Fe solution contained 1 g/L $CaCl_2{\cdot}2H_2O$, 1 g/L $FeSO_4{\cdot}7H_2O$. The trace mineral solution contained 100 mg/L $ZnSO_4{\cdot}7H_2O$, 30 mg/L $MnCl_2{\cdot}4H_2O$, 300 mg/L H_3BO_3, 200 mg/L $CoCl_2{\cdot}6H_2O$, 10 mg/L $CuCl_2{\cdot}2H_2O$, 10 mg/L $NiCl_2{\cdot}6H_2O$, 30 mg/L $Na_2MoO_4{\cdot}2H_2O$ and 30 mg/L Na_2SeO_3.

2.3. Batch biodegradation experiments

In order to evaluate whether microbial perchlorate reduction may be a practical option for the removal of perchlorate in PAX-21 wastewater, anaerobic batch biodegradation experiments using glucose as the primary source of electrons and carbon were conducted in 250-mL amber bottles (liquid volume = 150 mL) at room temperature ($22 \pm 2\,^{\circ}C$). Four sets of experimental reactors were prepared with the following treatments: (i) perchlorate + glucose + cells (control), (ii) perchlorate + glucose + cells + PAX-21 wastewater, (iii) perchlorate + glucose + RDX + cells, and (iv) perchlorate + glucose + DNAN + cells. The concentrations of perchlorate, RDX and DNAN were 100 mg/L, 40 mg/L and 100 mg/L, respectively. Each bottles contained 4 mM of glucose and the cell concentration of about 500 mg/L. The concentration of seed cultures were adjusted based on the total suspended solids concentrations of activated sludge. All experimental reactors were prepared in an anaerobic glove box filled with N_2 gas (Bell-Art Products, Pequannock, NJ) and they were sealed with screw-top Miniert™ caps (Alltech, Deerfield, IL) and low-permeability vinyl tape to maintain anaerobic conditions. All bottles were shaken at 150 rpm in a horizontal position on a rotary platform shaker (Lab-line, Melrose Park, IL), and at different elapsed times, about 2 mL of samples were taken from each bottle and passed through a 0.22-μm cellulose filter (Millipore, MA) for chemical analysis.

2.4. Reduction experiment by Fe(0)

Batch iron reduction experiments were conducted with 8-mL Pyrex® vials containing 5 mL of aqueous solution and 1 g of iron. Initial concentration of DNAN solution was 100 mg/L and initial pH of the solution was 6.7. The solutions were deoxygenated by purging with N_2 gas for 30 min prior to adding iron granules. After iron was added, the vials were placed horizontally on a rotary platform shaker (Lab-line, Melrose Park, IL) and continuously stirred at 150 rpm. At selected reaction time intervals, three bottles were sacrificed and supernatants were taken from each vial and passed through a 0.22-μm cellulose filter (Millipore, MA) for chemical analysis.

Table 1
Characteristic of PAX-21 wastewater.

Parameters	
pH	7.25
TOC	87.5 mg/L
RDX	58.3 mg/L
ClO_4^-	189.7 mg/L
DNAN	198.0 mg/L

2.5. Biodegradation after iron treatment

PAX-21 wastewater was initially passed through a glass iron column (2.5 cm ID × 30 cm L, Ace Glass, Vineland, NJ) packed with Peerless iron granules (porosity = 0.72). PAX-21 wastewater was pumped into the iron column in upflow mode at a flow rate of 2.5 mL/min (a column retention time of 30 min). To examine the microbial reduction of perchlorate in PAX-21 wastewater after the iron treatment, batch biodegradation experiments described above was repeated with iron treated PAX-21 wastewater and 4 mM of glucose. The same seed cultures were used for iron-treated degradation tests. Perchlorate levels in the cell-control reactors were re-spiked to 100 mg/L when the perchlorate levels decreased to below the detection limit.

2.6. Analytical methods

Perchlorate was analyzed using a Dionex DX 500 ion chromatograph (Dionex, Sunnyvale, CA) equipped with an IonPAC AS11 column and a guard column. The detection limit was 20 μg/L. 65 mM of NaOH solution was used as the eluent and the injection volume was 25 μL. RDX, DNAN, 2,4-diaminoanisole (DAAN), 2-methoxy-5-nitroanlinine, and 4-methoxy-3-nitroanlinine were analyzed using a Dionex HPLC (Dionex, Sunnyvale, CA) equipped with a Supelguard guard column (20 mm × 4.6 mm, Supelco, Bellefonte, PA), a SUPELCO LC-18 column (250 mm × 4.6 mm, 5 μm, Supelco, Bellefonte, PA). The wavelengths of UV detector are 224 nm for RDX and 254 nm for DNAN, DAAN, 2-methoxy-5-nitroanlinine, and 4-methoxy-3-nitroanlinine, respectively. The mixture of methanol and deionized water (50:50, v/v) was used as an eluent at a flow rate of 1.0 mL/min.

3. Results and discussion

3.1. Batch biodegradation experiments

Table 1 summarizes the characteristics of the PAX-21 wastewater from the Holsten Army ammunition plant. Fig. 3 illustrates the disappearance of perchlorate in batch reactors under different conditions. After an acclimation period, perchlorate was rapidly and completely removed to an undetectable level in cell-control bottles containing only glucose and seed microorganisms (Fig. 3a). In contrast, negligible amount of perchlorate (less than 2%) was removed in batch reactors containing PAX-21 wastewater (Fig. 3a) even though glucose and seed microorganisms were present in the reactor. This result suggested that some compounds in PAX-21 wastewater were inhibiting the activity of perchlorate reducing bacteria (PRB). In order to investigate whether the known constituents in PAX-21 wastewater was toxic to the perchlorate respiring bacteria, similar anaerobic batch reduction experiments were conducted with synthetic PAX-21 wastewater containing equivalent concentrations of DNAN (100 mg/L), RDX (40 mg/L) and perchlorate (100 mg/L). Similar results were obtained with syn-

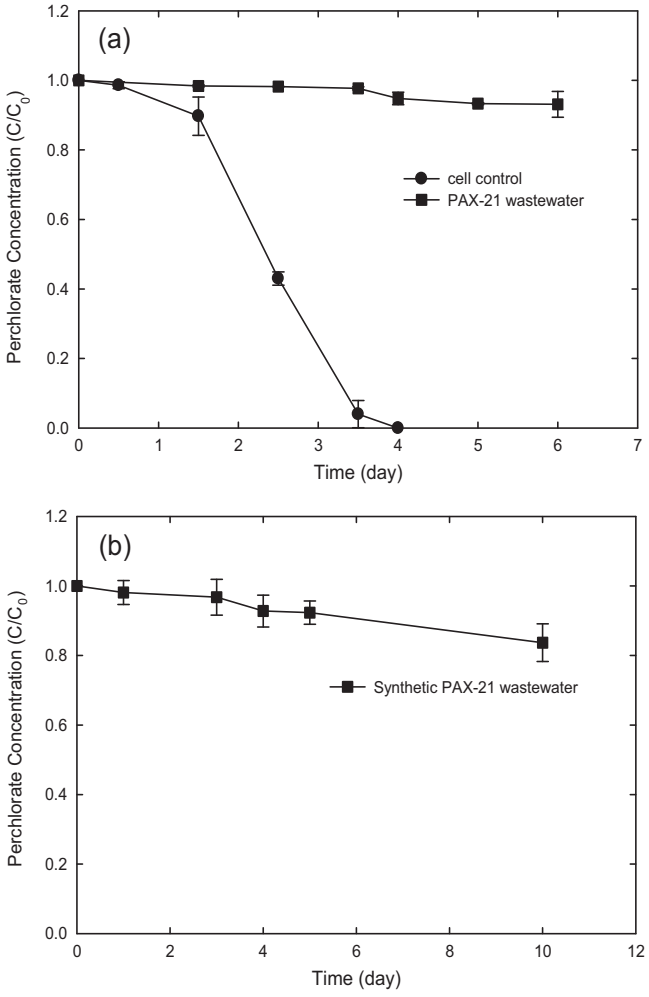

Fig. 3. Microbial perchlorate reduction in (a) PAX-21 wastewater and (b) synthesized PAX-21 wastewater. Data points are the average of replicate samples and the error bars represent standard deviation.

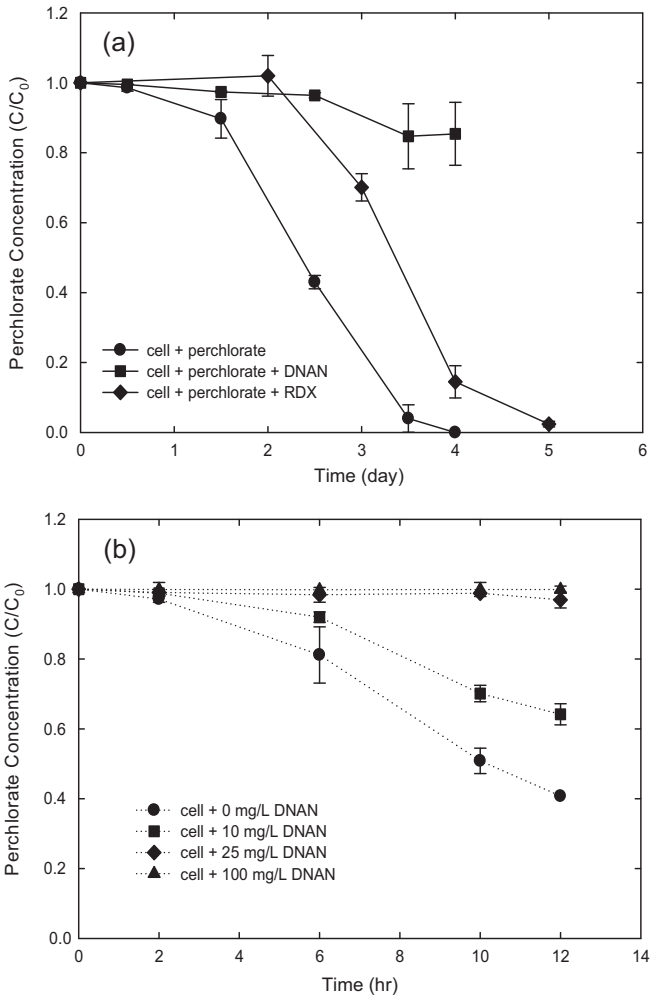

Fig. 4. Microbial perchlorate reduction in (a) reactors containing DNAN and RDX and (b) reactors containing different amount of DNAN solutions. Data points are the average of replicate samples and the error bars represent standard deviation.

thetic PAX-21 wastewater (Fig. 3b), suggesting that the primary constituents in PAX-21 wastewater may be inhibiting the activity of perchlorate reducing bacteria.

In order to identify the toxic constituents in PAX-21 wastewater, a series of batch biodegradation tests was conducted with DNAN (100 mg/L) and RDX (40 mg/L) solutions. Minimal perchlorate removal was observed in wastewater containing DNAN during 5 days of incubation (Fig. 4a). In order to investigate the effect of DNAN concentrations on microbial perchlorate reduction, a series of batch biodegradation experiments was conducted with the bioreactors containing different amounts of DNAN solutions and relatively low amounts of acclimated cultures (cell concentration = 150 mg/L). For reactors without DNAN, about 60% of perchlorate was removed in 12 h. The rate and extent of perchlorate reduction in reactors containing DNAN solution decreased with increasing concentrations of DNAN (Fig. 4b). With the addition of 25 mg/L of DNAN, microbial perchlorate reduction was completely inhibited during the first 12 h.

The presence of RDX (18 mM) resulted in longer acclimation period for PRB (Fig. 4a); however, the perchlorate reduction rate after the initial lag phase was similar to that of cell control. These results indicated that both DNAN and RDX in PAX-21 wastewater have detrimental effect on perchlorate respiring bacteria, but DNAN was primarily responsible for inhibiting the activity of perchlorate reducing bacteria. These results clearly suggested that DNAN and RDX need to be removed prior to subjecting PAX-21 wastewater to biological treatment processes.

3.2. Reduction experiment by Fe(0)

It was hypothesized that pretreatment of PAX-21 by zero-valent iron granules will transform toxic constituents in PAX-21 wastewater to non-toxic products. Oh et al. [14] showed that RDX was rapidly and completely removed by iron treatment and formaldehyde (HCHO) was determined as a major reduction product. Fig. 5 shows the concentrations of DNAN, reduction intermediates (total concentrations of 2-methoxy-5-nitroanlinine and 4-methoxy-3-nitroanlinine) and the end product, DAAN, during batch reduction of DNAN by zero-valent iron. This result showed that iron granule can rapidly and completely reduce DNAN to DAAN. Fig. 5 also showed that the mass recovery was low in early reaction times, but it improved to 95% mass recovery at 1 h. The lower mass balance at early reaction times may be attributed to adsorption of DNAN and intermediates to iron surface and accumulation of reduction products that was not measured.

3.3. Biodegradation experiments after iron treatment

DNAN and RDX in PAX-21 wastewater were completely removed to DAAN and formaldehyde as the solution was passed

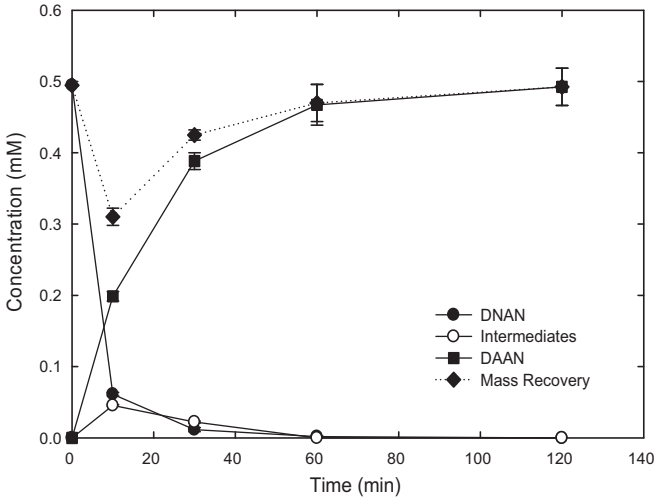

Fig. 5. Concentration of DNAN and its reduction products by zero-valent iron. Data points are the average of replicate samples and the error bars represent standard deviation.

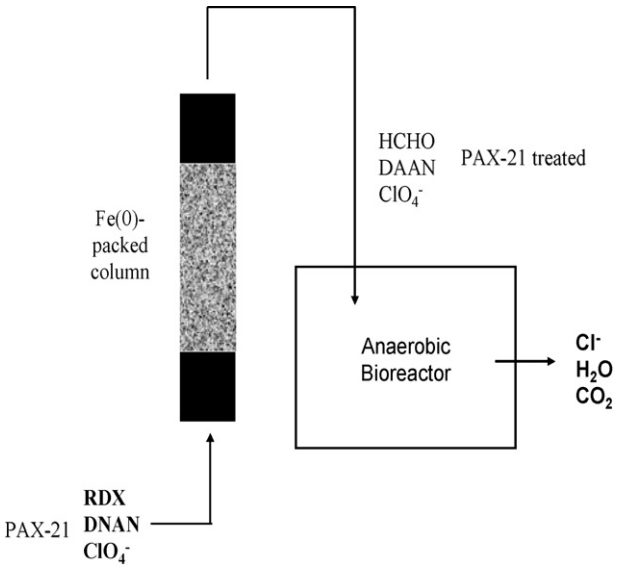

Fig. 7. Schematic diagram of integrated iron-column and anaerobic biological system for PAX-21 wastewater treatment.

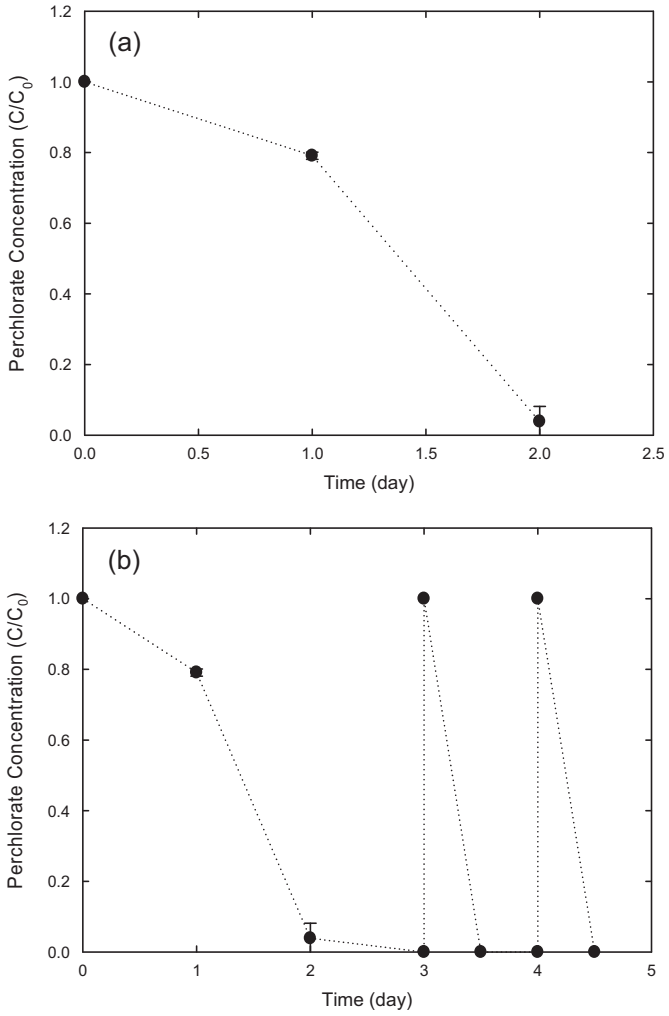

Fig. 6. Microbial perchlorate reduction in (a) iron treated PAX-21 wastewater and (b) after respiking of perchlorate when complete reduction of perchlorate occurred. Data points are the average of replicate samples and the error bars represent standard deviation.

through the iron column (data not shown). Fig. 6 showed the results of perchlorate reduction after iron treatment. After a 3-day acclimation period, perchlorate in iron-treated PAX-21 wastewater was rapidly decreased to an undetectable level in 2 days (Fig. 6a). Perchlorate stock solution was spiked into the reactor after the disappearance of the initial perchlorate in order to assess whether microbial perchlorate reduction can be sustained (Fig. 6b). Biodegradation of perchlorate was sustained over multiple respiking of perchlorate and the complete removal of perchlorate was achieved in 12 h.

Our results showed DNAN and RDX in PAX-21 wastewater are toxic to perchlorate-reducing bacteria and DNAN was identified as the primary toxic compound. The inhibitory compounds can be easily and completely transformed to DAAN and formaldehyde with iron treatment. Perchlorate in Fe(0)-treated PAX-21 wastewater was completely removed in 12 h after acclimation. In summary, iron treatment not only removed energetic compounds but also eliminated the toxic effect on perchlorate reducing bacteria.

Based on the results, we proposed an integrated Fe(0)-biological process for simultaneous removal of perchlorate and energetic compounds (Fig. 7). The integrated process consists of (1) a Fe(0) process for the reduction of toxic constituents in PAX-21 wastewater to non-toxic products and (2) an anaerobic biological treatment process using perchlorate-respiring bacteria. The combined process will result in complete removal of energetic compounds and transformation of perchlorate to innocuous chloride ions.

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