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Bacterial reduction of highly concentrated perchlorate: Kinetics and influence of co-existing electron acceptors, temperature, pH and electron donors

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HIGHLIGHTS

- The mixed ClO_4^--acclimated culture can reduce 50–1500 mg L^{-1} ClO_4^- efficiently.
- Perchlorate reduction kinetics was well fitted by Monod equation.
- NO_3^- had a greater inhibition effect for perchlorate reduction than SO_4^{2-}.
- The optimum temperature, pH and acetate dosage were examined.
- *Dechloromonas* is the dominant bacterium in the acclimated culture.

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ABSTRACT

Perchlorate reduction kinetics and effects of various environmental conditions on removal of perchlorate from synthetic water were investigated to seek high-strength perchlorate removal using mixed perchlorate reducing bacteria. Results demonstrated that perchlorate (50–1500 mg L^{-1}) could be degraded rapidly within 28 h under the optimal conditions. The maximum specific perchlorate reduction rate (q_{max}) and half saturation constant (K_s) were 0.92 mg-perchlorate (mg-dry weight)$^{-1}$ h^{-1} and 157.7 mg L^{-1}, respectively. In the ClO_4^-–NO_3^- systems obvious but recoverable lags were caused in perchlorate reduction and the lag time increased with the ratio of nitrate to perchlorate concentration increasing from 0.5 to 3. While in the ClO_4^-–SO_4^{2-} systems inhibitions didn't occur until the ratio of sulfate to perchlorate concentration exceeded 10. The optimum temperature and pH value were 35 °C and 6.85, respectively. The optimal acetate-to-perchlorate ratio that could consume all perchlorate and acetate simultaneously was about 2. *Dechloromonas*, one of the most prominent perchlorate reducing bacteria, was identified as the dominant bacterium in the acclimated culture (69.33% of the whole clones). The study demonstrated that the perchlorate-acclimated mixed microorganisms can readily and efficiently realize reduction of highly concentrated perchlorate in wastewater.

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1. Introduction

In 1997 significant perchlorate contamination of the ground and surface water were detected by the United States Environmental Protection Agency (Calderón et al., 2014). The main source of perchlorate contamination comes from its wide use in rocket propellants, explosives, fireworks and various consumer products (Ye et al., 2012; Urbansky and Schock, 1999; Dasgupta et al., 2005; Xu et al., 2015a,b). Actually, several perchlorate-contaminated sites have been identified with extremely high concentrations between 180 and 3700 mg L^{-1} in groundwater sources (Shrout and Parkin, 2006; US EPA, 2004; DHS, 2005). Perchlorate can threat human health by interfering with the uptake of iodine into the thyroid and subsequently inhibiting the development of the skeletal system and the central nervous system of infants (Yoon et al., 2009; Renner, 1999). Since its health effects, US EPA set an interim health advisory level of perchlorate at 15 μg L^{-1} in 2009 (US EPA, 2009) and has announced a decision to regulate perchlorate under the Safe Drinking Water Act in 2011 (US EPA, 2011).

Perchlorate is non-volatile and kinetically inert due to its high

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energy of activation (Kim and Logan, 2001; Thrash et al., 2007). As a consequence, most traditional physicochemical treatment processes are not capable of removing or destroy perchlorate effectively (Logan, 2001). Of all the treatment strategies applied to perchlorate remediation, ion exchange and bacterial reduction have been proven to be the most promising technologies for treating perchlorate-contaminated water (Sharbatmaleki et al., 2015; Gingras and Batista, 2002; Lehman et al., 2008; Logan et al., 2001). Ion exchange is the most practical technology for treating trace amounts of perchlorate in drinking water, whereas the regeneration of spent resins and the disposal of concentrated brine are significant problems (Bardiya and Bae, 2011; Venkatesan et al., 2010). Currently, bacterial reduction is the preferred technology for treating waters with high concentrations of perchlorate because it can reduce perchlorate rapidly and completely (Logan et al., 2001; Cang et al., 2004). Perchlorate-reducing bacteria (PCRB) are ubiquitous in the environment and can use perchlorate as an electron acceptor for metabolism (London et al., 2011; Clesceri et al., 1998). Perchlorate is known to be reduced as follows: $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow Cl^- + O_2$, which occurs only under anaerobic conditions without accumulation of chlorate, chlorite and O_2 (Rikken et al., 1996).

Recent studies have reported the effectiveness of perchlorate reduction by microbial cultures or biofilms. PCRB could accomplish perchlorate reduction using both organic (including lactate, pyruvate, methanol, ethanol, acetate and so on) and inorganic electron donors (including H_2, Fe^0, S^0, $S_2O_3^{2-}$, etc.) (Shrout and Parkin, 2006; Ju et al., 2008; Yu et al., 2006; Nerenberg and Rittmann, 2004), among which acetate was most commonly used (Coates et al., 1999), though inorganic electron donors could eliminate secondary contamination caused by organic residual (Ju et al., 2008). Perchlorate reduction kinetics for both pure and mixed PCRB have also been evaluated (London et al., 2011; Xu et al., 2015a,b). Meanwhile, water quality characters including electron donor addition, temperature, pH value and coexisting electron acceptors (e.g., nitrate and sulfate) have been shown to affect perchlorate reduction rates significantly (Shrout and Parkin, 2006; Min et al., 2004; Dugan et al., 2009; Brown et al., 2003; Matos et al., 2006; Chaudhuri et al., 2002). Nitrate was found as an alternative electron acceptor for most of the PCRB (Xiao and Roberts, 2013; Nozawa-Inoue et al., 2011) and the modified competitive inhibition model has been proposed in a study for a hydrogen-oxidizing, perchlorate-reducing microbial consortium (London et al., 2011). While sulfate competition has been evaluated to overcome through a hydrogen-fed two-stage membrane biofilm reactor (Ontiveros-Valencia et al., 2014). Besides, prior research has shown that perchlorate remediation can be achieved efficiently through integration of physicochemical and biological processes (Xie et al., 2014). In all, most studies were targeted on perchlorate bioreduction for drinking water and groundwater in relatively low concentrations (less than 50 mg L^{-1}), while studies on highly concentrated perchlorate (especially higher than 1000 mg L^{-1}) bioremediation were limited. And compared with biological reduction of perchlorate in low concentration, there may have problems of low degradation rates and incomplete removals for highly concentrated perchlorate reduction. Actually, there existed multiple high strength perchlorate wastes generated from both the spent perchlorate brine during the regeneration of the ion exchange resins (Lehman et al., 2008) and the wastewater from some facilities relating to military service or metal refineries (Hatzinger et al., 2002; Atikovic et al., 2008). Direct bio-regeneration of perchlorate laden anion-exchange resin has been found feasible by integrating ion-exchange technology and biological perchlorate reduction (Venkatesan et al., 2010; Wang et al., 2008a,b), and yet required an inefficient regeneration cycle (more than 10 d), and simultaneously the regenerated resins existed the risk of biocontamination. Microbial reduction of high strength perchlorate without dilution have also been explored for both pure and mixed culture (Song and Logan, 2004; Wang et al., 2008a,b; Nor et al., 2011). However, studies on highly concentrated perchlorate bioreduction seemed to be scattered, and rare literature was reported on systematically evaluating the efficiency and sensitivity of biologically perchlorate reduction in concentrations higher than 1000 mg L^{-1} for mixed microorganisms.

The specific objectives of this study were (1) to investigate the biological reduction kinetics of high strength perchlorate; (2) to systematically assess the effects of several different environmental factors, including co-existing electron acceptors (sulfate and nitrate), temperature, pH values and electron donors, and (3) to analyze the microbial community of the mixed biomass acclimated to perchlorate.

2. Materials and methods

2.1. Culture medium and enrichment of perchlorate-reducing mixed microorganisms

The basal medium contained (per liter): 1.55 g $K_2HPO_4 \cdot 3H_2O$, 0.85 g $NaH_2PO_4 \cdot H_2O$, 0.5 g $NH_4H_2PO_4$ and 10 mL trace mineral solution (TMS). The composition of TMS (per liter) was as follows: 5 g $MgSO_4 \cdot 7H_2O$, 0.3 g Na_2EDTA, 0.4 g $FeSO_4 \cdot 7H_2O$, 0.2 g $ZnSO_4 \cdot 7H_2O$, 0.02 g $CuSO_4 \cdot 5H_2O$, 0.1 g $MnCl_2 \cdot 4H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.04 g $Na_2MoO_4 \cdot 2H_2O$, 0.04 g $CoCl_2 \cdot 6H_2O$, 0.01 g $NiCl_2 \cdot 6H_2O$ and 0.06 g H_3BO_3 (Logan et al., 2001; Song and Logan, 2004). The pH value was maintained at 6.85 using phosphate buffer included in the media. Sodium perchlorate and sodium acetate were used as the electron acceptor and donor respectively in study tests, if not otherwise stated. All these chemical reagents were analytical pure and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) unless otherwise noted.

Originally, the seed culture was obtained from anaerobic digester sludge (ADS) taken from a local municipal wastewater treatment plant (Quyang, Shanghai, China). The seed was then acclimated and enriched in autoclaved 250-mL serum bottles capped with butyl rubber stoppers. During each cycle of batch cultivation, the seed was mixed with fresh deaerated culture medium, which was spiked with 1500 mg L^{-1} perchlorate (sodium salt) and 5000 mg L^{-1} acetate (sodium salt). Bottles were monitored at 150 rpm and 35 °C in the thermostat orbital shaker. Periodically, about two thirds of the supernatant was decanted and replaced with the same volume of fresh medium containing perchlorate and acetate. After two months of acclimation, 1500 mg L^{-1} perchlorate can be rapidly reduced into chloride within 2 h.

2.2. Experimental set-up

2.2.1. Temperature experiments

A set of experiments were conducted in serum bottles each containing 0.1 g-dry weight (DW) L^{-1} of biomass and 200 mL of fresh media that spiked with perchlorate and acetate. Initial perchlorate and acetate concentrations were 1300 mg L^{-1} and 6.5 g L^{-1}, respectively. Alternate environmental temperatures (15, 20, 25, 30, 35 and 40 °C) were employed to investigate the effects of temperature on perchlorate reduction. All tests were performed in a thermostat orbital shaker at 150 rpm. The pH of the culture was remained near neutral (6.9 ± 0.1) without any adjustments. All bottles were sparged with pure N_2 (g) to maintain anaerobic conditions (dissolved oxygen $\leq$ 0.3 mg L^{-1}) prior to the start of experiments. Tests lasted for 28 h and about 1 mL samples were taken

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