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Simultaneous bio-autotrophic reduction of perchlorate and nitrate in a sulfur packed bed reactor: Kinetics and bacterial community structure

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ABSTRACT

This study investigated the simultaneous removal of perchlorate and nitrate from aqueous solution in an up-flow sulfur autotrophic reduction reactor. A nitrate and perchlorate containing pollution solution was treated with a remarkable removal efficiency greater than 97%. The concentration of nitrate was 22.03 ± 1.07 mg-N/L coexisting with perchlorate either 21.87 ± 1.03 mg/L or 471.7 ± 50.3 μg/L, in this case the reactor could be operated at a hydraulic retention time (HRT) ranging from 12.00 h to 0.75 h. *Half-order* kinetics model fit the experimental data well; this indicates that diffusion in the biofilm was the limiting step. Perchlorate reduction required a longer reaction time than the coexisting nitrate, regardless of the perchlorate concentration. Sulfur (S) disproportionation was inhibited when nitrate was not completely removed; whereas it was accelerated when perchlorate decreased to low concentrations. This process therefore generated excessive sulfate and consumed much more alkalinity. High-throughput sequencing method was used to analyze bacterial community spatial distribution in the reactor under different operational conditions. The reduction of the two contaminants was accompanied by a decrease in biodiversity. The results indicated that *Sulfuricella*, *Sulfuritalea Thiobacillus,* and *Sulfurimonas* are effective DB (denitrification bacteria)/PRB (perchlorate reduction bacteria). The *Chlorobaculum* genus was the dominant bacteria associated with S disproportionation.

1. Introduction

Perchlorate has strong oxidizing capabilities, and is used as a propellant in solid rocket fuel, ordnance, and explosives in the defense and aerospace industries. Because of its high solubility and non-reactive properties, perchlorate contamination in surface water and groundwater has been reported throughout the United States (Tikkanen, 2006). Perchlorate poses public health risks, because it can disturb the production of metabolic hormones by the thyroid gland. This affects human development and may induce thyroid gland tumors (Charnley, 2008). In 2005, the US Environmental Protection Agency (USEPA) established a reference dose (RfD) for perchlorate of 24.5 ppb in water.

Nitrate is a common contaminant found in drinking water resources that are also contaminated with perchlorate. Perchlorate is usually present at low concentrations (in the ppb range), whereas nitrate generally exists at higher concentrations (in the ppm range) (Matos et al., 2006; Ziv-El and Rittmann, 2009). It has been reported that of 228 sites in California contaminated with 10–70 μg/L perchlorate, nitrate was found at 215 sites with concentration ranging from 5 to 27 mg-N/L (Wang et al., 2002). Nitrate may cause methemoglobinemia when ingested by infants, and may also cause carcinoma, malformations, and mutations when it is transformed into nitrosoamines.

The primary methods for removing perchlorate and nitrate from water are physical-chemical (Srinivasan and Sorial, 2009) and biological (Hatzinger, 2005; Matos et al., 2006; Lehman et al., 2008) processes. However, physicochemical technologies, such as membrane and ion exchange processes, produce waste streams with high salt levels, and further remediation is needed before disposal (Greenlee et al., 2009). Catalytic methods require high cost catalysts and restrict reaction conditions, limiting their large-scale field application (Mahmudov et al., 2008). Biological technology is the most cost-effective method to remove perchlorate and nitrate from contaminated water (Nor et al., 2011; Ricardo et al., 2012). Bacteria that already universally present in the environment can use

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perchlorate and nitrate as a terminal electron accepter under anoxic conditions. Further, many perchlorate respiring bacteria are also capable of denitrification (Bardiya and Bae, 2011).

Several biological technologies have been tested for their ability to simultaneously remove perchlorate and nitrate from drinking water sources. For heterotrophic reduction processes, organic carbon sources (such as ethanol and acetate) serve as electron donors (Srinivasan and Sorial, 2009). Consequently, the secondary pollution caused by the residual carbon source limits the application of heterotrophic technology (Srinivasan and Sorial, 2009; London et al., 2011). Groundwater and drinking water have very low concentrations of biodegradable organic materials. As such, autotrophic reduction is a more acceptable approach, as it uses inorganic elements and compounds such as hydrogen, iron, or sulfur as electron donors (Reyes et al., 2007; Ju et al., 2007; Wan et al., 2016). Previous researchers have achieved success using S^0 as an electron donor to remove nitrate from contaminated water by both batch and continuous tests (Reyes et al., 2007; Konenig and Liu, 2001). The stoichiometric equation for the reaction is (Konenig and Liu, 2001):

$$1.06NO_3^- + 1.11S + 0.3CO_2 + 0.785H_2O \rightarrow 0.06C_5H_7O_2N + 0.5N_2 + 1.11SO_4^{2-} + 1.16H^+ \quad (1)$$

However, there are only limited studies of S^0 autotrophic reduction of the perchlorate ion (ClO_4^-). The theoretical stoichiometric equation for sulfur autotrophic perchorate reduction is:

$$3ClO_4^- + 4S^0 + 4H_2O \rightarrow 8H^+ + 4SO_4^{2-} + 3Cl^- \quad (2)$$

Ju et al. explored the reduction of ClO_4^- by S^0 using batch cultures inoculated with activated sludge (Ju et al., 2007, 2008). They found that the presence of NO_3^- delayed the rate of ClO_4^- reduction. Further, S disproportionation was observed (Ju et al., 2007). In 2009, Sahu et al. investigated ClO_4^- reduction under continuous conditions, and also explored the resulting dominant microbial community. When the influent ClO_4^- concentration was 4–8 mg/L, the effluent ClO_4^- concentration was reduced to <0.5 mg/L at an empty bed contact time (EBCT) of 13 h. The presence of NO_3^- inhibited ClO_4^- reduction.

In 2016, Gao et al. reported the perchlorate and nitrate could be simultaneously removed in a combined reactor using sulfur and electrochemical hydrogen autotrophy. The removal efficiency of nitrate and perchlorate using the sulfur autotrophic process was controlled by hydraulic retention time (HRT) (Gao et al., 2016). Even with this studies, the kinetics of the ClO_4^- and NO_3^- reduction in a sulfur packed bed reactor, the interactions between ClO_4^- and NO_3^- degradation, the role of S disproportionation, alkalinity consumption, and pH variation still deserve further study.

Recently, high-thoughput sequencing (next generation sequencing) technology has enabled the rapid and inexpensive profiling of complex bacteria communities (Buermans and Dunnen, 2014). This new approach allows for the taxonomic identification of many species from one DNA sample. It has been used to research bacterial diversity and microbial communities in mixed cultures from sewage treatment plants (Zhang et al., 2011), membrane bioreactors (Ontiveros-Valencia et al., 2014), and drinking water distribution systems (Shaw et al., 2014). However, studies on using high-thoughput sequencing to analyze the sulfur autotrophic microbial community structure during nitrate and perchlorate reduction are very limited.

This study had three main objectives. The first was to investigate the kinetics of perchlorate and nitrate reduction in an up-flow sulfur packed bed reactor under different HRT values and pollutant concentrations. The second was to explore the regulation of sulfate generation and pH variation. The third was to use high-throughput sequencing to determine the dominant microbial community members during ClO_4^- and NO_3^- removal under different operational conditions. We also used UniFrac analysis to demonstrate the microbial community spatial distribution in the reactor under different operational conditions.

2. Materials and methods

2.1. Experimental set-up

Two Plexiglas columns were established as the up-flow fixed bed reactor packed with sulfur granules (2–3 mm diameter, YanShanpc Co. Ltd., Beijing, China). Reactor 1# had a diameter of 10 cm with a height of 50 cm, with a packed height of sulfur granules of 35 cm (Fig. S1). Reactor 2# had a diameter of 5 cm and a height of 50 cm, with a packed height of 40 cm (Fig. S1). With a porosity of 32%, the effective pore water volume was 0.880 L and 0.250 L for Reactor 1# and Reactor 2#, respectively. A peristaltic pump (Longer BT100-2J, China) was used as needed for water inflow. Three sample ports (1 cm diameter) were distributed along the columns to collect water samples to explore removal kinetics and sulfate generation. An additional three sample ports (2 cm diameter) were distributed to collect media samples for microorganism analysis.

Synthetic ClO_4^- and NO_3^- contaminated water was prepared using dechlorinated tap water with sodium perchlorate and sodium nitrate as the contaminants. Because anoxic conditions could be achieved with the aerobic S^0 oxidizing bacteria in the sulfur packed bed (Boles et al., 2012), feed water deoxygenation was not adopted in this study. The dissolved oxygen (DO) in the influent was about 3.16–4.05 mg/L. In Reactor 1#, the initial perchlorate and nitrate concentrations were close to one another. In Reactor 2#, the initial perchlorate level was approximately 413.3–540.0 μg/L, and the nitrate nitrogen level was approximately 21.34–24.23 mg/L. Reactor 2# levels are consistent with perchlorate and nitrate contaminated groundwater sites.

The nutrient such as KH_2PO_4 and Na_2CO_3 were added to the feed at level of 5 mg/L. Other influent water parameters were as follows: Total Organic Carbon (TOC) was 3.78–4.12 mg/L; Cl^- was 108.06–128.50 mg/L and SO_4^{2-} 73.35–96.81 mg/L. The two reactors were operated under a controlled temperature 27 ± 2 °C.

2.2. Experimental design

Concentrated activated sludge (Wulongkou waste water treatment plant, Zhengzhou, China) was added to both reactors. The amounts of inoculated culture were 20 g and 15 g for Reactor 1# and Reactor 2#, respectively. The two reactors operated with an HRT of 36 h for 3 days before shifting to continuous testing.

After inoculation, the removal efficiency, sulfate/sulfide generation, and pH variation were each determined as a function of HRT. For Reactor 1#, the full operational period was 141 days, with HRT durations maintained at 12, 8, 4, 2, 1.5, 1 and 0.75 h. For Reactor 2#, the full operational period was 82 days, with tested HRT durations of 4, 2, 1 and 0.75 h. At different HRTs, the reactors ran and stabilized after more than 10 days, which is when steady-state conditions were achieved. Table 1 lists the conditions applied during each experimental stage for the two reactors.

2.3. Analytical methods

Solution samples were filtered by passing through a 0.20 μm membrane. Nitrite was measured using the UV spectrophotometric (TU-1900, Persee, China) screening method. Nitrite was analyzed using the N-(1-naphthyl) ethylenediamine colorimetric method.

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