Inhibition of nitrate and the accumulated denitrification intermediate (nitrite) on perchlorate bioreduction

Rui Wang, Liang Chen, Fei Liu, Hong H. Chen, Jia W. Zhang and Ming Chen

ABSTRACT

Bioreduction of perchlorate and nitrate by perchlorate-reducing microorganisms (PRMs) is an environmentally friendly, economic, and efficient technology to treat mixed plumes composed of these substances. The influence of perchlorate, nitrate, and denitrification intermediates on PRM activity is a critical factor, which may affect the efficiency of treatment technology. This study investigated the inhibition of nitrate and the intermediate (nitrite) accumulated during the denitrification process on perchlorate bioreduction via a batch-type experiment. From the experiment, it was found that perchlorate had no effect on the denitrification process and that the reduction rate of perchlorate could be improved when NO\textsubscript{3}-N/ClO\textsubscript{4}/C\textsubscript{0}/C\textsubscript{2}0 >= 1.2. However, a negative effect of nitrate on perchlorate reduction was observed when NO\textsubscript{3}-N/ClO\textsubscript{4}/C\textsubscript{0} > 1.2 with an accumulation of 18.0 mg NO\textsubscript{2}/C\textsubscript{0}-N/L. This negative effect increased with the concentration of nitrate. Moreover, nitrite from the denitrification process had a similar negative effect on perchlorate reduction. Bioreduction of perchlorate was not started until nitrite was totally reduced, and a 2–13 day lag period was observed for perchlorate reduction after nitrite depletion.

Key words | bioreduction, denitrification intermediate, negative effect, nitrate, nitrite, perchlorate

INTRODUCTION

Perchlorate contamination has been a significant concern for the last two decades (Ghosh et al. 2011). Due to the high solubility of perchlorate (ClO\textsubscript{4}, 200 g/L at 25 \degree C), pollution plumes containing ClO\textsubscript{4} are frequently detected in drinking water, surface and groundwater. In Canada, preliminary analysis of groundwater and surface water samples shows very low concentrations of perchlorate. Nevertheless, several recent studies report detectable levels of perchlorate in some surface waters and plant species, and more than 1,000 mg/kg of perchlorate has been detected in several natural minerals (Srinivasan & Viraraghavan 2009; Wang et al. 2009). The occurrence of perchlorate detected in groundwater in the United States is usually <2 mg/L (Yu et al. 2006; Kannan et al. 2009) but sometimes reaches levels >10 mg/L. In addition, high concentrations of perchlorate ranging from 160 to 3,000 mg/L are detected in groundwater or wastewater from some facilities relating to military activities, rocket-propellants manufacturing, or metal refineries (Hatzinger et al. 2002; Parker et al. 2008; Nor et al. 2011). However, a guidance value of 6 ppb has been suggested by Health Canada, while the US Environmental Protection Agency (EPA) has established the interim health advisory level of 15 \mu g/L in water (US EPA 2009). Although the perchlorate monitoring systems started later in China, widespread contamination of perchlorate in surface water, sludge rice, bottled water and milk collected from different areas has been confirmed (Wu et al. 2010). Therefore, it is urgent to develop effective technologies to decrease the pollution of perchlorate in water. With the discovery of perchlorate-reducing microorganisms (PRMs) in the contamination sites, bioreduction of perchlorate in groundwater is becoming widely investigated and applied (Waller et al. 2004; Yu et al. 2006; Gal et al. 2008; Sahu et al. 2009; Cai et al. 2010). Perchlorate is sequentially degraded to chlorate...
ever, the bioreduction of perchlorate and the denitrification process have similar Gibbs free energy (ΔG° = −801 vs. −792 kJ/mol acetate), and the utilization of perchlorate and nitrate by PRMs may vary under different conditions (Coates & Achenbach 2004; Bardiya & Bae 2011). Thus, the relationship between perchlorate bioreduction and the denitrification process must be closely considered. Although some researchers report that perchlorate can be reduced simultaneously with nitrate (Parker et al. 2008; Chung et al. 2010), the inhibition of nitrate on perchlorate reduction has been widely observed and investigated in pure and mixed PRMs (Chaudhuri et al. 2002; Coates & Achenbach 2004; Yu et al. 2006; Farhan & Hatzinger 2009; Xiao & Roberts 2013). Nozawa-Inoue et al. (2005) examined the inhibition effect of nitrate (>50 mg/L) on perchlorate reduction and found no differences in lag time (9 days) after increasing the NO3⁻/ClO₄⁻ ratio from 3.7 to 11. However, Gal et al. (2008) found that the lag time of 70 mg/L perchlorate is decreased from 7.25 to 5 days when the NO3⁻/ClO₄⁻ ratio is increased from 0.41 to 1.6.

Most works have focused on the inhibition effect of nitrate on perchlorate bioreduction without considering the effects of denitrification intermediates. Researchers have reported that the accumulation of nitrite-N during the denitrification process can reach 30% of the initial nitrate-N (Chaudhuri et al. 2002; Gal et al. 2008). Ghosh et al. (2011) and Ricardo et al. (2012) observed that nitrate could inhibit the reduction of perchlorate but did not analyse this effect in detail. Therefore, in this study, batch-type experiments were conducted to investigate the effect of nitrate on perchlorate bioreduction in groundwater, and the effect of the main intermediate (nitrite) accumulated during the denitrification process was also considered.

METHODS

Soil and standard basal medium

PRMs were obtained from a soil that had no history of perchlorate contamination. The percentage of organic matter in this soil was 1.18%, and the concentration of nitrate-N was 50 mg/kg. After air-drying and screening with a 200 μm sieve, the soil specimen was stored at 4°C for further tests. A standard basal medium per litre contained: 100 mg KH₂PO₄; 50 mg (NH₄)₂SO₄; 10 mg NaHCO₃; 10 mg MgSO₄·7H₂O; 10 mg Ca(H₂PO₄)₂; 5 mg H₃BO₃; 5 mg (NH₄)₆Mo₇O₂₄·4H₂O; 2 mg FeSO₄·7H₂O; 2 mg ZnSO₄·7H₂O; 2 mg CuSO₄·5H₂O; 2 mg MnSO₄·H₂O; 1 mg KAl(SO₄)₂·12H₂O; 1 mg NiSO₄·6H₂O and 1 mg CoSO₄·7H₂O. All chemicals used were of analytical grade and the majority were produced by Chemical Reagent Beijing Co., Ltd (Beijing, China). The NaClO₄ was made by Fang Shan Yi Hua Chemical Works of Beijing, China.

Enrichment, cultivation and inoculation procedures

Three hundred grams of prepared soil were added into a 1 L serum brown glass jar. Then, 800 mL of the standard basal
medium was added and treated with N₂ to reduce dissolved oxygen (DO) < 1 mg/L. This was followed by the addition of perchlorate (~10 mg/L) as electron acceptor and acetate (~300 mg/L) as electron donor. Finally, the bottles were sealed with rubber stoppers and incubated at room temperature (20 ± 2 °C) in a shaker at 150 rpm. After removal of 80% of the perchlorate, 200 mL of the solution phase was transferred to a new 1-L amber glass bottle. Fresh standard basal medium (800 mL), perchlorate (10 mg/L), and acetate (300 mg/L) were then added.

Analysis of perchlorate from the bottles that contained the soil inocula indicated that perchlorate was removed with time, suggesting that the desired bacterial population had been successfully enriched. Shrout & Parkin (2006) reported that 1 g acetate was equivalent to 1.085 g chemical oxygen demand (COD), and Nor et al. (2011) found that the optimal ratio of acetate-to-perchlorate was 1.45 mg COD/mg perchlorate. Thus, acetate added in this experiment was overdosed.

Effect of denitrification process on perchlorate bioreduction

A separate microcosm experiment was conducted to investigate how nitrate and the intermediate (nitrite) accumulated during the denitrification process affect perchlorate bioreduction. The initial concentration of perchlorate was ~10 mg/L, and the ratios of NO₃⁻-N/ClO₄⁻ were 0.7, 1.2, 4.0, and 10.5, respectively. Acetate (300 mg/L) was used as an additional electron donor here and could completely reduce all electron acceptors. Controls without nitrate were made with the same procedure.

The experiments were started by the addition of 300 mL of basal medium and 100 mL of acclimated bacteria (initial optical density at 600 nm (OD₆₀₀) was 0.025 ± 0.005) at 20 °C. Before sealing, all bottles were treated with N₂ to reduce DO < 1 mg/L. The pH value of the solutions was between 7.2 and 7.5. All experiments were performed in duplicate.

Analytical methods

The concentrations of perchlorate, chlorate, nitrate, and nitrite were measured with ICS-90 ion chromatography (Dionex, CA, USA), an Ion Pac AS20 analytical column (4 × 50 mm), an AG20 guard column (4 × 250 mm), and a suppressor (ASRS-500, Dionex, CA, USA). Sixty-five millimoles (mM) of NaOH were used as the eluent of perchlorate, while the composite solutions of NaHCO₃ (1 mM) and Na₂CO₃ (3.5 mM) were selected as the eluent of other anions. The sample injection volume was 100 μL and the flow rate was 1.0 mL/min.

Cell growth was monitored by OD₆₀₀ using a Spec 20 spectrophotometer (Thermo Spectronics, Rochester, NY, USA). Biomass concentrations were determined from the cell suspensions according to the dry weight (DW) method (Wang et al. 2008). DO was monitored by a HQ-30d dissolved oxygen meter (HACH, CO, USA).

Calculation of kinetic parameters

Ghosh et al. (2011) have reported that the reduction of perchlorate corresponds to a first-order reaction kinetics equation, the rate coefficients (k, d⁻¹) of perchlorate and nitrate in our study were calculated following the equation below:

\[ k = \frac{\ln \left( \frac{C_0}{C_t} \right)}{t} \]  \hspace{1cm} (1)

where \( t \) is the elapsed time of perchlorate or nitrate bioreduction; \( C_0 \) is the initial concentration of perchlorate or nitrate-N in solution, and \( C_t \) is the concentration at time \( t \). \( k_1 \) and \( k_{NO_3} \) are the reduction rate constants of perchlorate and nitrate during the whole reduction period.

To determine kinetic parameters for perchlorate under different conditions of nitrate and nitrite inhibition, the rate coefficients (\( k_1, k_2, k_3, \) d⁻¹) of perchlorate during different reduction periods were calculated following the equations below:

\[ k_1 = \frac{\ln \left( \frac{C_1}{C_0} \right)}{t_1} \]  \hspace{1cm} (2)

\[ k_2 = \frac{\ln \left( \frac{C_3}{C_1} \right)}{t_2} \]  \hspace{1cm} (3)

\[ k_3 = \frac{\ln \left( \frac{C_3}{C_2} \right)}{t_3} \]  \hspace{1cm} (4)

In Equations (2)–(4), \( t_1 \) is the time of perchlorate bioreduction before nitrate depletion, and \( t_2 \) and \( t_3 \) are the time of
perchlorate bioreduction after nitrate and nitrite depletion, respectively. \( C_0 \) is the initial concentration of perchlorate; \( C_1 \) is the concentration at time \( t_1 \), in other words, \( C_1 \) is initial concentration of perchlorate when nitrate was completely depleted; \( C_2 \) is initial concentration when nitrite was completely depleted; \( C_3 \) is the concentration of perchlorate at time \( t_2 \) or \( t_3 \); \( k_1 \) is the reduction rate of perchlorate before nitrate depletion; and \( k_2 \) and \( k_3 \) are the reduction rate of perchlorate after nitrate and nitrite depletion, respectively.

There was so much biological growth because of the high concentrations of nitrate and perchlorate. Therefore, in order to account for differences due to variations in bacterial activity and/or bacterial population sizes, the specific reduction rate constants (\( k_s \)) of perchlorate and nitrate were also calculated using Equation (5)

$$k_s = \ln \left( \frac{C_0/DW_0}{C_t/DW_t} \right)/t$$  \hspace{1cm} (5)

where \( t \) is the elapsed time of perchlorate or nitrate bioreduction, \( C_0 \) and \( C_t \) are the concentrations of perchlorate or nitrate-N initially and at time \( t \), and \( DW_0 \) and \( DW_t \) are the biomass concentrations initially and at time \( t \). \( k_{s1} \) and \( k_{s2} \) are the specific reduction rates of perchlorate and nitrate during the whole reduction period. Based on Equations (2)–(5), the specific reduction rates of perchlorate before nitrate depletion; \( k_{s1} \) is the specific reduction rate of perchlorate after nitrate depletion; and \( k_{s3} \) is the specific reduction rate of perchlorate after nitrite depletion.

RESULTS AND DISCUSSION

Inhibition of nitrate on perchlorate reduction

The influence of nitrate on perchlorate bioreduction was evaluated in batch-type experiments. Perchlorate and nitrate reduction was investigated at \( NO_3^-/C_0^- \) ratios ranging from 0.7 to 10.5. Perchlorate, nitrate-N, and nitrite-N concentrations in the incubation bottles with time are shown in Figure 1.

As shown in Figure 1, nitrate was totally depleted in 3–7 days without any lag in all treatments, but the total

Figure 1 | Reduction of nitrate, nitrite, and perchlorate at \( NO_3^-/ClO_4^- \) ratios of 0.7 (a), 1.2 (b), 4.0 (c), and 10.5 (d). The initial concentration of \( ClO_4^- \) was ∼10 mg/L and a control sample without nitrate is shown.
reduction period of perchlorate was increased from 9 to 50 days with the increasing of NO$_3$-N/ClO$_4$ from 0.7 to 10.5. The reduction rates ($k$ values) associated with nitrate ($k$-NO$_3$-N) and perchlorate ($k_t$, $k_1$, $k_2$ and $k_3$) are listed in Tables 1 and 2.

As shown in Table 1, the reduction rates of nitrate ($k$-NO$_3$-N) were much higher than that of perchlorate ($k_t$) in all treatments during the whole reduction period. Moreover, the bacteria growth in each bottle was measured (Figure 2). The PRM population increased appreciably 2 to 6 times higher than the initial value with the increasing of NO$_3$-N/ClO$_4$ from 0 to 10.5. The same varying trend of the specific reduction rates of nitrate and perchlorate ($k_t$) during the whole bioreduction period were observed as the trend of $k$ values ($k$-NO$_3$-N vs. $k_t$; $k_t$-NO$_3$-N vs. $k_{st}$, Table 1). For example, when NO$_3$-N/ClO$_4$ ratio was ≤1.2, the $k_t$ and $k_{st}$ of perchlorate were ~8 times lower than $k$-NO$_3$-N and $k_t$-NO$_3$-N, and this trend was more obvious with the increasing NO$_3$-N/ClO$_4$ ratios. These results showed that when both perchlorate and nitrate were present in the medium, perchlorate reduction activity was much lower than that of nitrate, especially at higher initial nitrate concentrations.

The reduction rate ($k_t$) and the specific reduction rate ($k_{st}$) of perchlorate during the whole reduction period were significantly different between the various NO$_3$-N/ClO$_4$ ratios. When NO$_3$-N/ClO$_4$ ratio was ≤1.2, $k_t$ and $k_{st}$ were equal or slightly higher than that of the control without nitrate addition (as seen in Table 1). The positive effect was likely due to the high energy production associated with the increasing bacteria population sizes during denitrification (Gal et al. 2008). Since the exponential growth trend of bacteria was also observed when nitrate reduced in this study (Figure 2), the specific reduction rate of perchlorate before nitrate depletion ($k_{s1}$) was much higher than the reduction rate ($k_t$) during this period with NO$_3$-N/ClO$_4$ ratio ranging from 0.7 to 10.5 (as seen in Table 2). Meanwhile, when the microbial population reached a steady level, no obvious change was observed between the rate coefficients after nitrate depletion ($k_2$).

### Table 1 | Experimental conditions and kinetic parameters of nitrate and perchlorate reduction

<table>
<thead>
<tr>
<th>NO$_3$-N</th>
<th>ClO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3$-N/ClO$_4$</td>
<td>$C_0$ mg/L</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>0.7</td>
<td>7.5</td>
</tr>
<tr>
<td>1.2</td>
<td>12.2</td>
</tr>
<tr>
<td>4.0</td>
<td>42.7</td>
</tr>
<tr>
<td>10.5</td>
<td>108.6</td>
</tr>
</tbody>
</table>

$k$-NO$_3$-N and $k_t$ are the reduction rates of nitrate and perchlorate during the whole reduction period, $k_t$-NO$_3$-N and $k_{st}$ are the specific reduction rates of nitrate and perchlorate during the whole reduction period. Lag periods were determined as those where >5% of perchlorate was lost (Nozawa-Inoue et al. 2005).

### Table 2 | The $k_1$ and $k_s$ value of perchlorate reduction

<table>
<thead>
<tr>
<th>NO$_3$-N/ClO$_4$</th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$k_3$</th>
<th>$k_{s1}$</th>
<th>$k_{s2}$</th>
<th>$k_{s3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>d$^{-1}$</td>
<td>L/(mg DW)$^{-1}$ d$^{-1}$</td>
<td>L/(mg DW)$^{-1}$ d$^{-1}$</td>
<td>L/(mg DW)$^{-1}$ d$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0.7</td>
<td>0.06 ± 0.04</td>
<td>0.25 ± 0.09</td>
<td>0.24 ± 0.11</td>
<td>0.32 ± 0.09</td>
<td>0.23 ± 0.08</td>
<td>0.25 ± 0.05</td>
</tr>
<tr>
<td>1.2</td>
<td>0.02 ± 0.03</td>
<td>0.53 ± 0.18</td>
<td>0.47 ± 0.11</td>
<td>0.31 ± 0.07</td>
<td>0.52 ± 0.19</td>
<td>0.49 ± 0.13</td>
</tr>
<tr>
<td>4.0</td>
<td>0.02 ± 0.02</td>
<td>0.07 ± 0.01</td>
<td>0.10 ± 0.02</td>
<td>0.45 ± 0.05</td>
<td>0.06 ± 0.02</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>10.5</td>
<td>0.01 ± 0.00</td>
<td>0.10 ± 0.06</td>
<td>0.17 ± 0.11</td>
<td>0.32 ± 0.10</td>
<td>0.10 ± 0.06</td>
<td>0.17 ± 0.11</td>
</tr>
</tbody>
</table>

$k_t$, and $k_s$ are the reduction rates of ClO$_4$ before and after nitrate depletion, respectively; $k_{s1}$ is the reduction rate of ClO$_4$ after nitrite depletion; $k_{s1}$ and $k_{s2}$ are the specific reduction rates of ClO$_4$ before and after nitrate depletion, respectively; $k_{s3}$ is the specific reduction rate of ClO$_4$ after nitrite depletion.
vs. $k_{s2}$) or nitrite depletion ($k_3$ vs. $k_{s3}$), which indicated that the population of microbes indeed have an important effect on perchlorate bioreduction. However, nitrate had a negative effect on perchlorate reduction when $\text{NO}_3^{-}/\text{ClO}_4^-$ ratio $>1.2$. This inhibitive effect displayed a positive correlation with nitrate concentration. As seen from Table 1, the lag period for perchlorate reduction was 11 and 38 days when $\text{NO}_3^{-}/\text{ClO}_4^-$ ratio increased from 4.0 to 10.5, respectively. Moreover, the reduction rate $k_1$ decreased by about 50–70% of the only perchlorate control reactor. Okeke & Frankenberger Jr (2003) and Xu et al. (2003) reported that the inhibition of nitrate on perchlorate bioreduction may be caused by a preference for nitrate over perchlorate in the production of enzymes in microorganisms.

Inhibition of denitrification intermediates on perchlorate reduction

Nerenberg et al. (2006) reported that chlorate ($\text{ClO}_3^-$) produced by perchlorate reduction could slow the reduction rate of perchlorate, since chlorate would compete for the catalytic site of the (per)chlorate-reductase enzyme with perchlorate. However, only 2.4 mg/L of chlorate accumulated during the bioreduction of 100 mg/L of perchlorate and the inhibiting effect of chlorate on perchlorate was not observed until chlorate $>50$ mg/L (Nerenberg & Rittmann 2002). Since chlorate was not detected in this study, the inhibition of chlorate on perchlorate reduction was not considered.

For denitrification, nitrite ($\text{NO}_2^-$), NO, nitrous oxide ($\text{N}_2\text{O}$), and nitrogen gas ($\text{N}_2$) were the produced intermediates during this process (Chaudhuri et al. 2002; Gal et al. 2008; Kumar & Lin 2010; Tall et al. 2011; Xiao & Roberts 2013). The main intermediate observed here was nitrite, and its maximum concentration ranged from 1.5 to 61.1 mg NO$_2$-N/L while nitrate ranged from 7.5 to 108.6 mg NO$_3$-N/L. Most nitrite accumulated during the fast degrading period of nitrate (Figure 1). As shown in Table 2, when the $\text{NO}_3^{-}/\text{ClO}_4^-$ ratio was $\leq 1.2$, the reduction rate coefficients of perchlorate after nitrate depletion ($k_2$) increased by at least 4 fold higher than the rate constants before nitrate depletion ($k_1$). Meanwhile, no significant change was observed between the $k$ values after nitrate and nitrite depletion ($k_2$ vs. $k_3$) with the maximum nitrite-N concentrations ranging from 1.5 to 3.3 mg/L, which indicated that relatively low levels of the accumulated denitrification intermediate (nitrite) had no effect on perchlorate bioreduction. Additionally, when $\text{NO}_3^{-}/\text{ClO}_4^-$ ratios increased from 0.7 to 1.2, nitrate and nitrite did not completely inhibit perchlorate reduction; the lag time for perchlorate reduction was shorter than the nitrate or nitrite depletion time (Figure 3).

However, when $\text{NO}_3^{-}/\text{ClO}_4^-$ $>1.2$, perchlorate bioreduction did not start immediately even when nitrate was totally depleted (7–31 days later, Figure 3). Moreover, a positive correlation between the accumulation and depletion period of nitrite and the lag period of perchlorate was
found with the accumulation of nitrite-N ranging from 18.0 to 61.1 mg/L when NO3-N/ClO4 ratios increased from 4.0 to 10.5. Bioreduction of perchlorate did not start until nitrite was totally depleted and a 2–13 day lag period of perchlorate reduction was observed after nitrite depletion for the ratios of 4.0 and 10.5 (Figure 3). It could be concluded that the generation of nitrite from the denitrification process might extend the removal period of perchlorate and that nitrite and nitrate have a similar inhibitory effect on perchlorate reduction.

Nitrite is not the only intermediate from the denitrification process. Nitric oxide (NO) and nitrous oxide (N2O) are also intermediates. It could be conjectured that there are other factors (perhaps NO or nitrous oxide) that might inhibit the bioreduction of perchlorate, because a 2–13 day lag period was observed even though nitrite was totally removed.

CONCLUSIONS

This study focused on perchlorate bioreduction inhibition from nitrate and its intermediate (nitrite) during the denitrification process. It was found that nitrate was totally depleted in 3–7 days without any lag period, and the total reduction period of perchlorate was increased from 9 to 50 days when the NO3-N/ClO4 was between 0 and 10.5. Perchlorate had no effect on the denitrification process, and the reduction rate of perchlorate was unchanged or improved when NO3-N/ClO4 <1.2. However, nitrate had a negative effect on perchlorate reduction when NO3-N/ClO4 >1.2 with the accumulation of 18.0 mg/L nitrite-N. This negative effect increased with the concentration of nitrate.

The inhibition of perchlorate reduction via chlorate was not considered here since chlorate was not detected. Nitrite from the denitrification process had a significant and negative effect on perchlorate reduction. Bioreduction of perchlorate did not start until nitrite was totally reduced, and there was a 2–15 day lag period of perchlorate reduction after nitrite depletion.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 41272268), the China Geological Survey (No. 1212011121171), and the Public Science and Technology Research Funds Projects of Land and Resources (No. 201211059). We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

REFERENCES

Kumar, M. & Lin, J. G. 2010 Co-existence of ammox and 
denitrification for simultaneous nitrogen and carbon 
1–9.

Speitel Jr, G. E. 2011 Autohdrogenotrophic perchlorate 
reduction kinetics of a microbial consortium in the presence 

Nerenberg, R. & Rittmann, B. E. 2002 Perchlorate as a 
secondary substrate in a denitrifying hollow-fiber 
2 (2), 259–265.

Nerenberg, R., Kawagoshi, Y. & Rittmann, B. E. 2006 Kinetics of a 
hydrogen-oxidizing, perchlorate-reducing bacterium. Water 
Res. 40 (17), 3290–3296.

W. 2011 Microbial treatment of high-strength perchlorate 

Nozawa-Inoue, M., Scow, K. M. & Rolston, D. E. 2005 Reduction of 
perchlorate and nitrate by microbial communities in 

Okeke, B. C. & Frankenberger Jr, W. T. 2003 Molecular analysis of 
a perchlorate reductase from a perchlorate-respiring 

Parker, D. R., Seyfferth, A. L. & Reese, B. K. 2008 Perchlorate in 
groundwater: A synoptic survey of ‘Pristinesites’ in the 
1465–1471.

Ricardo, A. R., Carvalho, G., Velizarov, S., Crespo, J. G. & Reis, 
M. A. M. 2012 Kinetics of nitrate and perchlorate removal and 
biofilm stratification in an ion exchange membrane 
biofilter. Water Res. 46 (14), 4556–4568.

Robertson, W. D., Piatek, C. J. & Brown, S. J. 2007 Geochemical 
and hydrogeological impacts of a wood particle barrier 
treating nitrate and perchlorate in ground water. Ground 
Water Monit. Rem. 27 (2), 85–95.

Biological perchlorate reduction in packed bed reactors using 

Shrout, J. D. & Parkin, G. F. 2006 Influence of electron donor, 
oxygen, and redox potential on bacterial perchlorate 

Srinivasan, A. & Viraraghavan, T. 2009 Perchlorate: Health effects 
and technologies for its removal from water resources. Int. J. 

Tall, L., Caraco, N. & Maranger, R. 2011 Denitrification hot spots: 
dominant role of invasive macrophyte Trapa natans in removing 
nitrogen from a tidal river. Ecol. Appl. 21 (8), 3104–3114.

US EPA 2009 Perchlorate Contamination of Drinking Water: 
Regulatory Issues and Legislative Actions. US Environmental 

Waller, A. S., Cox, E. E. & Edwards, E. A. 2004 Perchlorate- 
reducing microorganisms isolated from contaminated sites. 
Environ. Microbiol. 6 (5), 517–527.

perchlorate reduction and pH effect. J. Hazard. Mater. 153 
(1–2), 663–669.

Wang, Z. W., Forsyth, D., Lau, B. P. Y., Pelletier, L., Bronson, R. & 
Gaertner, D. 2009 Perchlorate: Estimated dietary exposure of 
Canadians to perchlorate through the consumption of fruits 
and vegetables available in Ottawa markets. J. Agric. Food 
Chem. 57 (1), 9250–9255.

Wu, J., Zhang, T., Sun, H. W. & Kannan, K. 2010 Perchlorate in tap 
water, groundwater, surface waters, and bottled water from 
China and its association with other inorganic anions 
and with disinfection byproducts. Archives Environ. 

Xiao, Y. Y. & Roberts, D. J. 2013 Kinetics analysis of a salt-tolerant 
perchlorate-reducing bacterium: effects of sodium, magnesium, 

Xu, J. L., Trimble, J. J., Steinberg, L. & Logan, B. E. 2004 Chlorate 
and nitrate reduction pathways are separately induced in the 
perchlorate-respiring bacterium Dechlorosoma sp. KJ and the 
chlorate-respiring bacterium Pseudomonas sp. PDA. Water 

Perchlorate reduction by autotrophic bacteria in the presence 

Ziv-El, M. C. & Rittmann, B. E. 2009 Systematic evaluation of 
nitrate and perchlorate bioreduction kinetics in groundwater 
using a hydrogen-based membrane biofilm reactor. Water 
Res. 43 (1), 173–181.

First received 27 March 2014; accepted in revised form 4 August 2014. Available online 27 August 2014.