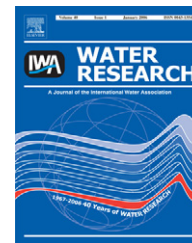


Available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/watres

Kinetics of a chlorate-accumulating, perchlorate-reducing bacterium

Margaret Dudley, Anna Salamone, Robert Nerenberg*

Department of Civil Engineering and Geological Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556, USA

ARTICLE INFO

Article history:

Received 23 October 2007

Received in revised form

8 January 2008

Accepted 9 January 2008

Available online 20 January 2008

Keywords:

Perchlorate

Chlorate

Kinetics

Competitive inhibition

S_{\min}

ABSTRACT

Kinetics parameters for perchlorate and chlorate reduction were determined for *Dechlorosoma* sp. HCAP-C, also known as *Dechlorosoma* sp. PCC, a novel perchlorate-reducing bacterium (PCRB) that accumulates significant amounts of chlorate during perchlorate reduction. This is the first report of such behavior, and we hypothesized the perchlorate reduction kinetics would be markedly different from other PCRB. In batch tests with initial perchlorate concentrations ranging from 200 to around 1400 mg/L, maximum chlorate accumulation ranged from 41 to 279 mg/L, and were consistently around 20% of the initial perchlorate concentration. For perchlorate, parameters were determined using a competitive inhibition model. The maximum specific substrate degradation rate $q_{\max P}$ was 11.5 mg $\text{ClO}_4^-/\text{mg dry weight (DW)-d}$, and the half-maximum rate constant K_P was 193 mg ClO_4^-/L . For chlorate, the $q_{\max C}$ was 8.3 mg $\text{ClO}_3^-/\text{mg DW-d}$ and the K_C was 58.3 mg ClO_3^-/L . The high K_P values relative to conventional PCRB, values suggests that HCAP-C does not play a significant role at low perchlorate concentrations. However, the relatively high $q_{\max P}$ and the potential for syntrophic relationships with chlorate-reducing bacteria that relieve the effects of chlorate inhibition, suggest that HCAP-C could play a significant role at high perchlorate concentrations.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Perchlorate contamination is widespread in the United States (Gullick et al., 2001). Due to its high stability and mobility in the environment (Urbansky, 1998; Urbansky and Schock, 1999b), perchlorate is not easily removed from contaminated waters. An effective treatment strategy for perchlorate remediation is its biological reduction to chloride by dissimilatory perchlorate-reducing bacteria (PCRB) (Hatzinger, 2005; Urbansky and Schock, 1999a; Xu et al., 2003). PCRB are ubiquitous in the environment, and are mainly facultative anaerobes and denitrifiers (Coates and Achenbach, 2004; Coates et al., 1999; Logan, 1998; Xu et al., 2003). PCRB are found in four subclasses of the Proteobacteria, but mainly in the two Beta-proteobacteria genera, *Dechloromonas* and *Azospira* (formerly *Dechlorosoma*) (Coates and Achenbach, 2004). A number of PCRB isolates have been described in the

literature (Achenbach et al., 2001; Bardiya and Bae, 2004; Bruce et al., 1999; Coates and Achenbach, 2004; Coates et al., 1999; Giblin and Frankenberger, 2001; Korenkov et al., 1976; Logan et al., 2001; Michaelidou et al., 2000; Nerenberg et al., 2006; ShROUT et al., 2005; Wallace et al., 1996; Waller et al., 2004; Wolterink et al., 2005), and several researchers have determined kinetic parameters for PCRB (Korenkov et al., 1976; Logan et al., 2001; Rikken et al., 1996; Wallace et al., 1996; Waller et al., 2004). Kinetic parameters are needed to predict the rates of perchlorate degradation in natural and engineered systems.

PCRB are thought to use a single enzyme, the (per)chlorate reductase (pcr), to reduce perchlorate (ClO_4^-) to chlorate (ClO_3^-), and chlorate to chlorite (ClO_2^-) (Bender, 2005; Kengen et al., 1999). Chlorite is then transformed by the enzyme chlorite dismutase into chloride and molecular oxygen (Bender et al., 2002; Giblin and Frankenberger, 2001; van

*Corresponding author. Tel.: +1 574 631 4098; fax: +1 574 631 9236.

E-mail address: Nerenberg.1@nd.edu (R. Nerenberg).

0043-1354/\$ - see front matter © 2008 Elsevier Ltd. All rights reserved.

doi:10.1016/j.watres.2008.01.009

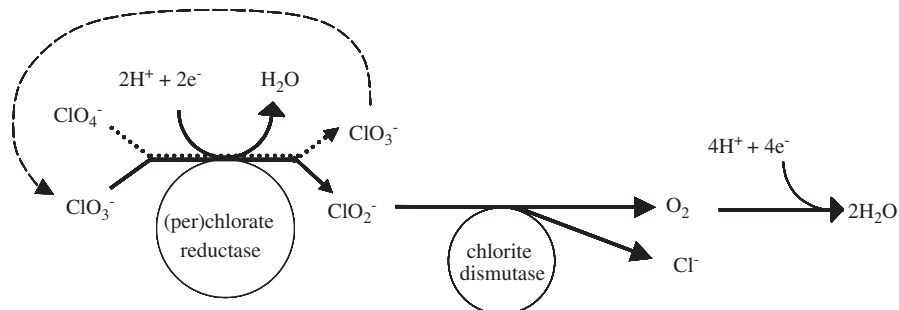


Fig. 1 – The accepted perchlorate-reduction pathway by (per)chlorate reductase and chlorite dismutase. Notice the (per)chlorate reductase reduces both perchlorate (ClO_4^-) and chlorate (ClO_3^-) (Nerenberg et al., 2006).

Ginkel et al., 1996). As shown in Fig. 1, perchlorate reduction produces chlorate, which then competes with perchlorate for the pcr enzyme. As the chlorate concentration increases, it is more likely to be reduced, thus increasing the rate of chlorate reduction and decreasing that of perchlorate.

Nerenberg and Rittmann (2002) reported 0.33 mg/L chlorate accumulation when 25 mg/L perchlorate was added to a mixed culture, perchlorate-reducing bioreactor. Also, Nerenberg et al. (2006) found that, in batch tests with *Dechloromonas* PC1, chlorate accumulated to 0.6, 2.4, and 4.3 mg/L with initial perchlorate concentrations of 100, 300, and 600 mg/L, respectively, or around 0.7% of the initial perchlorate concentrations. Most other PCRB accumulate similarly low percentages of chlorate (unpublished data), which may explain why few researchers have reported chlorate accumulation. Although published kinetic parameters have all been determined based on simple Monod kinetics, disregarding potential effects of competitive inhibition, a competitive inhibition model can more accurately assess perchlorate degradation kinetics, and also predict chlorate accumulation (Nerenberg, 2003).

We studied a novel PCRB, *Dechlorosoma* sp. HCAP-C, which accumulates high levels of chlorate during perchlorate reduction. We call such bacteria HCAP. HCAP-C is also known as *Dechlorosoma* sp. PCC. HCAP behavior has been observed in at least one other PCRB species (J.D. Coates, personal communication, 2007), but has not been reported in the literature. To better understand and quantify HCAP kinetics, we studied chlorate accumulation by HCAP-C in different growth media, and determined kinetic parameters for growth on perchlorate and chlorate. We used the competitive inhibition model to determine perchlorate kinetics, but also used simple Monod kinetics to allow comparison to parameters from the literature. We tested the competitive inhibition model for a range of initial perchlorate concentrations to determine its effectiveness in predicting perchlorate and chlorate concentrations.

2. Materials and methods

2.1. Chemicals and media

2.1.1. Growth medium

A modified minimal medium, adapted from Nerenberg and Rittmann (2002), was used for all except the Medium Tests,

described below. The modification doubled the concentration of the trace mineral solution, in order to ensure trace minerals were not limiting. One liter of medium contained 1.386 g Na_2HPO_4 , 0.849 g KH_2PO_4 , 0.1 g $(\text{NH}_4)_2\text{SO}_4$, 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 mL trace mineral solution, and 2 mL Ca-Fe solution. The Ca-Fe solution contained, per liter, 1 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 1 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. The trace mineral solution contained, per liter: 100 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 30 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 300 mg H_3BO_3 , 200 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 10 mg $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 10 mg $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 30 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, and 30 mg Na_2SeO_3 . The initial pH was around 7. Research-grade chemicals and ultra-pure water (Nanopure Diamond, Barnstead) were used for all solutions.

2.1.2. Medium tests

These tests were used to explore potential nutrient deficiencies as a cause for HCAP behavior. Five types of growth medium were tested for their effects on chlorate accumulation: (1) LB broth (Mo Bio Laboratories, Inc., Carlsbad, CA); (2) minimal medium (Nerenberg et al., 2002); (3) modified minimal medium, as described above; (4) modified minimal medium, amended with 10% LB broth; and (5) MS medium used by Logan et al. (2001). Initial perchlorate concentrations were approximately 200 mg/L.

2.2. HCAP-C Isolation

Dechlorosoma sp. HCAP-C, also known as *Dechlorosoma* sp. PCC (GenBank accession number: AY126453), was previously isolated from municipal activated sludge with no known perchlorate exposure. A 160-mL serum bottle was filled with 30 mL of growth medium containing 100 mg/L perchlorate. After autoclaving, the headspace was successively vacuum-degassed and filled with a mixture of 95% H_2 and 5% CO_2 . The bottle was then inoculated with 1 mL of activated sludge and incubated at room temperature, on its side, on a shaker table at 200 rpm. After growth was observed, the enrichment was plated on R2A agar, and colonies were re-grown in suspension and re-plated until purity was achieved. The strain was stored in 30% glycerol at -80°C . Stored isolates were reconstituted for this study in liquid medium. To confirm purity, reconstituted cultures were plated aerobically on R2A agar (Difco) and the DNA was extracted as described below.

2.3. Sequencing and phylogenetic analysis

PCR and sequencing were carried out following Nerenberg et al. (2006). Universal primers for the 16S rRNA gene were U27F and U1525R (Muyzer et al., 1993). Similar sequences were retrieved from Genbank (www.ncbi.nih.gov) using the BLAST nucleotide search, and aligned using CLC Gene Workbench version 2.2 (CLC bio A/S, Aarhus, Denmark) with an open gap cost of 10 and an extension cost of 1. The phylogenetic tree was produced using MEGA version 3.1 (Kumar et al., 2004). Bootstrap values for 100 replicates were determined.

2.4. Analytical methods

Perchlorate, chlorate, chloride, and acetate were analyzed using a Dionex ICS2500 ion chromatograph (IC, Dionex Corporation, Sunnyvale, CA) with a 4-mm Dionex AS-11 column, an AG-11 guard column, and a conductivity detector. The program consisted of a 5-min equilibration with the 4 mM sodium hydroxide eluant, injection of the sample, a 9-min isocratic run at 4 mM, and a gradient from 4 to 50 mM sodium hydroxide over 2 min. A Dionex ASRS suppressor was used in internal recycle mode. Injection was performed with a Dionex AS40 automated sampler. The injection volume was 200 μ L. The detection limit for chlorate was 5 μ g/L.

Optical density (OD) at 600 nm was measured with a spectrophotometer (Spec 20, Thermo Spectronics, Rochester, NY) and converted to dry weight (DW) using an empirical conversion factor. A conversion factor for determining DW from OD was determined for HCAP-C following Nerenberg et al. (2006). The conversion factor was 575 mgDW/L per OD unit.

2.5. Scanning electron microscopy (SEM)

SEM was used to image HCAP-C. HCAP-C was grown in liquid medium to an OD at 600 nm of approximately 0.1. The sample was centrifuged at 10,000g for 10 min and washed twice with 4 mM phosphate buffer. The washed cells were then spread on sterilized aluminum sample trays and dried overnight at room temperature. Samples were sputter coated with gold using a Hummer II sputter coater (Technics) and viewed on a LEO EVO-50XVP variable-pressure/high-humidity SEM.

2.6. Batch tests

Batch tests were used to (1) assess the effect of growth medium on chlorate accumulation; (2) determine kinetic parameters; and (3) test the ability of the competitive inhibition model, with the previously determined parameters, to predict the chlorate accumulation for a variety of initial perchlorate concentrations. Batch tests were carried out in 1-L bottles with 200 mL of media. Bottles were capped with thick, butyl rubber stoppers with an aluminum crimp seal. In order to create anaerobic conditions, bottles were successively vacuum-degassed to -1.7 atm and pressurized with nitrogen, three times. The final headspace contained pure nitrogen at 1.3 atm. Bottles were shaken at 200 rpm at ambient temperature (22 °C). Bottles were inoculated with HCAP-C at an OD of around 0.005. The media was amended with acetate as an electron donor and carbon source, with an

initial acetate concentration of 600 mg/L and final concentration of around 400 mg/L. Perchlorate or chlorate were typically amended at 200 mg/L, although single tests were also carried out with perchlorate at 225, 340, 680, and 1360 mg/L. For growth on hydrogen, a mixture of 95% H₂ and 5% CO₂ was added to the headspace. For growth on oxygen, 10 mL of air was added to the headspace of a 160-mL serum bottle. For growth on nitrate or nitrite, 14 mg/L of nitrate or nitrite as N were added to the growth medium.

2.7. Estimation of kinetic and stoichiometric parameters

2.7.1. Kinetic parameters K and q_{max}

Kinetic parameters were obtained for perchlorate reduction assuming competitive inhibition, and for chlorate reduction using simple Monod kinetics, i.e., neglecting competitive inhibition. For simple Monod kinetics, the electron acceptor mass balance is as follows:

$$\frac{dS}{dt} = -q_{max} \frac{S}{S+K} X, \quad (1)$$

where S is the perchlorate (S_p) or chlorate (S_c) concentration in mg/L, K is the half-maximum rate constant for perchlorate (K_p) or chlorate (K_c) in mg/L, q_{max} is the maximum substrate utilization rate for perchlorate (q_{maxP}) or chlorate (q_{maxC}) in mgS/mgX-day, and where X is the biomass measured as DW. The biomass mass balance is as follows:

$$\frac{dX}{dt} = Yq_{max} \frac{S}{S+K} X - bX, \quad (2)$$

where b is the decay coefficient (mgX/mgX-day) and Y is the yield for perchlorate (Y_p) or chlorate (Y_c) in mgX/mgS. Using the independently determined Y and b values, Eqs. (1) and (2) were solved simultaneously and fit to results from batch tests to determine q_{max} and K . See Section 2.7.5, below.

The simple Monod expressions used in Eqs. (1) and (2) do not predict chlorate accumulation, or account for perchlorate and chlorate's effect on specific reduction rates. Competitive inhibition can be incorporated through a modifier on the K term, which increases the effective K value for the substrate when the inhibitor concentrations are high relative to the inhibitor's K (Rittmann and McCarty, 2001). The mass balances for perchlorate, chlorate, and biomass, considering competitive inhibition, are as follows:

$$\frac{dS_p}{dt} = -q_{pmax} \frac{S_p}{S_p + K_p(1 + S_c/K_c)} X, \quad (3)$$

$$\frac{dS_c}{dt} = \frac{86.5}{99.5} q_{pmax} \frac{S_p}{S_p + K_p(1 + S_c/K_c)} X - q_{maxC} \frac{S_c}{S_c + K_c(1 + S_p/K_p)} X, \quad (4)$$

$$\frac{dX}{dt} = Y_{PC} q_{pmax} \frac{S_p}{S_p + K_p(1 + S_c/K_c)} X + Y_c q_{maxC} \frac{S_c}{S_c + K_c(1 + S_p/K_p)} X - bX, \quad (5)$$

where Y_{PC} is the yield for perchlorate reduction to chlorate (mgX/mg S_p). In Eq. (4), the chlorate mass balance, the first term on the right accounts for perchlorate reduction to chlorate, with the 86.5/99.5 factor converting mg/L perchlorate to mg/L chlorate, and the second term accounts for

chlorate reduction to chloride. In a batch test with perchlorate, the high initial perchlorate concentration and low chlorate concentrations lead to net accumulation of chlorate. As the chlorate concentration increases, it lowers the first term and increases the second, slowing the net specific rate of chlorate accumulation. When the chlorate concentration is high enough, the two terms on the right of Eq. (4) become equal, and the chlorate concentration reaches its maximum value. Subsequently, as the perchlorate concentration decreases, the chlorate concentration also decreases. Interestingly, the competitive inhibition model predicts that the maximum value of chlorate accumulation is directly proportional to the initial perchlorate concentration. The proportionality constant is a function of the kinetic parameters for perchlorate and chlorate.

2.7.2. Yield

The yields for full reduction of chlorate (Y_C) and perchlorate (Y_P) to chloride were determined from batch experiments by dividing biomass produced by the amount of acceptor utilized. The initial chlorate or perchlorate concentrations were 200 mg/L, and the experiments were conducted at least in triplicate. The partial yield for perchlorate reduction to chlorate (Y_{PC}) was determined by difference between Y_P and Y_C on a molar basis, as one mol of perchlorate produces one mol of chlorate. The Y_{PC} was then converted back to a mass basis.

2.7.3. Endogenous-decay coefficient (b)

The decay coefficient, b (mgX/mgX-d), was determined using batch studies with no acetate. In the absence of substrate, biomass decays and the biomass mass balance (Eq. (2)) becomes $dX/dt = -bX$. For each batch test, b was determined independently by fitting the decay curve to the biomass mass balance. The tests were carried out in duplicate.

2.7.4. Growth threshold (S_{min})

The growth threshold on perchlorate, assuming simple Monod kinetics, was calculated using the following equation (Rittmann and McCarty, 2001):

$$S_{min} = q_{max} \frac{bK}{Yq_{max} - b}$$

2.7.5. Data fitting

AQUASIM version 2.1f (Reichert, 1995) was used to fit kinetic parameters. AQUASIM estimates kinetic parameters by minimizing the sum of the squares of the weighted deviations between actual data and results of the calculated model. The calculation step size was 0.01 days. The secant method was used with a maximum iteration number of 100.

3. Results and discussion

3.1. HCAP-C phylogeny and characterization

Based on 16S rRNA gene sequencing and analysis, HCAP-C belongs to the genus *Dechlorosoma*. HCAP-C is closely related to several perchlorate-reducing isolates (Fig. 2), none of which have been reported to display HCAP behavior. Based on SEM

imaging, HCAP-C is rod-shaped with average dimensions of approximately $1\mu\text{m} \times 0.3\mu\text{m}$. It grows heterotrophically with acetate and autotrophically with hydrogen as electron donors, and with molecular oxygen, nitrate, nitrite, perchlorate, and chlorate as electron acceptors. HCAP-C's 16S rDNA partial sequence was deposited with GenBank, under the name *Dechlorosoma* PCC (Accession no. AY126453).

3.2. Medium tests

In all media types tested, including minimal and complex media and PCRb growth media used by other researchers, the maximum chlorate accumulation was 20–24% of the initial perchlorate concentration, suggesting that HCAP behavior is not a result of a nutrient deficiency. A possible explanation is the existence of a distinct (per)chlorate reductase enzyme. The (per)chlorate reductase is encoded by four genes: *pcrA*, *pcrB*, *pcrC*, and *pcrD* (Bender et al., 2005). The enzyme subunit encoded by the *pcrA* gene is the predicted active site for perchlorate and chlorate reduction (Bender et al., 2005), and therefore may be responsible for chlorate accumulation among HCAP.

3.3. Kinetic parameters

The determined parameters for HCAP-C, for utilization of perchlorate and chlorate, are summarized in Table 1. The yields on perchlorate and chlorate Y_P and Y_C were 0.41 ± 0.12 mgDW/mgClO₄⁻ and 0.34 ± 0.08 mgDW/mgClO₃⁻, respectively, where the error terms indicate one standard deviation. The partial yield of perchlorate reduction to chlorate, Y_{PC} , was 0.12 mgDW/mgClO₄⁻. Fig. 3 is a typical parameter estimation batch test for chlorate. Simple Monod kinetics was used, as no perchlorate was present. The q_{maxC} was 8.3 ± 3 mgClO₃⁻/mgDW-d, which is slightly higher than the 6.3–7.48 mgClO₃⁻/mgDW-d range from the literature (Table 2). The K_C was 58.3 ± 52.7 mgClO₃⁻/L, much higher than the 0.014 mgClO₃⁻/L value from the literature. Fig. 4 is a typical batch test for growth on perchlorate, using Monod with competitive inhibition. The q_{pmax} was 11.5 ± 3 mgClO₄⁻/mgDW-d, next to the highest in the 1.68–24 mgClO₄⁻/mgDW-d range from the literature. The K_P was 193 ± 34 mgClO₄⁻/L, also substantially higher than the 0.14–33 mgClO₄⁻/L from the literature.

The simple Monod model was also used to determine kinetic parameters for perchlorate, in order to compare the results to the literature, which were determined neglecting competitive inhibition. With simple Monod, the q_{maxP} was 4.4 ± 0.7 mgClO₄⁻/mgDW-d and the K_P was 76.6 ± 11.9 mgClO₄⁻/L. This q_{maxP} is slightly below the average of the q_{maxP} values from the literature, 6.5 mgClO₄⁻/mgDW-d, while the K_P value is much higher (Table 2). The higher q_{pmax} obtained from the competitive inhibition model was expected, as competitive inhibition provides a “true” q_{maxP} that is free from the effects of inhibition, while the simple Monod equation provides an “observed” q_{maxP} that reflects inhibition from chlorate.

Syntrophic relationships may exist between HCAP and PCRb, or HCAP and chlorate-reducing bacteria (CRB). For example, *Dechloromonas* PC1 is a PCRb with a K_C of less than 0.014 mg/L. PC1 could readily reduce chlorate produced by

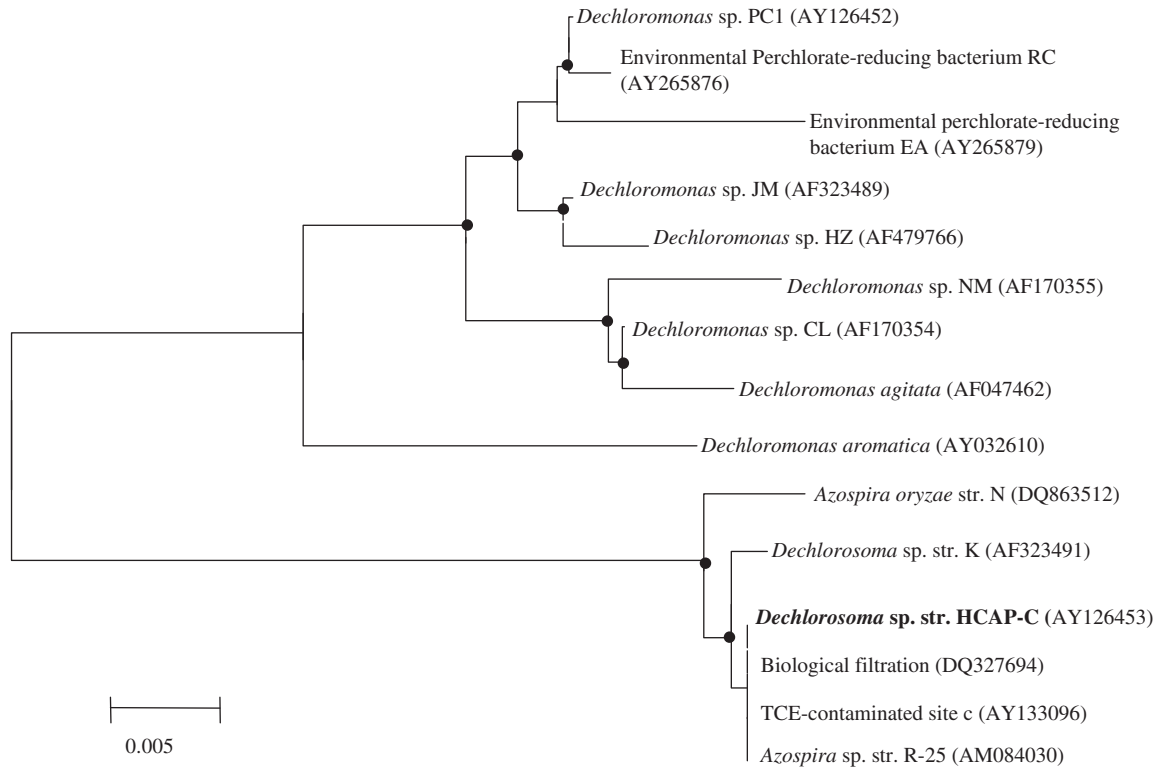


Fig. 2 – Phylogenetic position of *Dechlorosoma* sp. HCAP-C in relation to other (per)chlorate-reducing bacteria. The bootstrapped neighbor-joining tree, generated as described in the text, was based on near-full-length 16S rRNA gene sequences recovered from HCAP-C and sequences obtained from the Genbank database (Genbank accession number indicated in parenthesis). Nodes supported by the bootstrap analysis (> 70%; 1000 resamplings) are indicated by black circles at those nodes. The scale bar indicates 0.005 substitutions per nucleotide position.

Table 1 – Kinetic and stoichiometric parameters for chlorate and perchlorate

Parameter	Unit	Average	Standard deviation
Chlorate			
Y_C	mg DW/mg ClO_3^-	0.34	0.08
$q_{\max C}$	mg ClO_3^- /mg DW-d	8.3	3
K_C	mg ClO_3^- /L	58.3	53
Perchlorate			
b	1/d	0.5	0
Y_P	mg DW/mg ClO_4^-	0.41	0.12
Y_{PC}	mg DW/mg ClO_4^-	0.12	0.07
Perchlorate simple Monod			
$q_{\max P}$	mg ClO_4^- /mg DW-d	4.4	0.7
K_P	mg ClO_4^- /L	76.6	11.9
Perchlorate Monod with competitive inhibition			
$q_{\max P}$	mg ClO_4^- /mg DW-d	11.5	3
K_P	mg ClO_4^- /L	193	34.6

HCAP-C to below HCAP-C's K_C value, relieving chlorate inhibition. CRB may also be well-suited to reduce chlorate, although little is known about their kinetics. CRB reportedly are ubiquitous in the environment, perhaps more abundant than PCRB (Xu et al., 2003). If chlorate inhibition is removed, HCAP-C could operate at a specific perchlorate

reduction rate approaching the $q_{\max P}$ determined with competitive inhibition, i.e., 11.5 mg ClO_4^- /mg DW-d, instead of the observed $q_{\max P}$ value of 4.4 mg ClO_4^- /mg DW-d. In this situation, using kinetic parameters neglecting competitive inhibition could significantly underestimate perchlorate reduction kinetics.

Based on the simple Monod parameters for HCAP-C, the growth threshold concentration S_{\min} is 2.2 mg ClO_4^- /L. Since bacteria cannot grow on a sole substrate when its concentration is below its S_{\min} , the presence of HCAP-C in environments without high perchlorate concentrations, such as the activated sludge sample from which it was isolated, is probably due to HCAP-C's ability to grow on other acceptors, such as oxygen and nitrate. HCAP-C may also be able to use other acceptors, such as chromate and selenate (Chung et al., 2006).

While PCRB and CRB could help increase the effective specific perchlorate reduction rates for HCAP-C by consuming chlorate and reducing its inhibiting effect, they also would divert 6 of the 8 electrons available in perchlorate, decreasing the HCAP's yield from Y_P (0.41 mg DW/mg ClO_4^-) to Y_{PC} (0.12 mg DW/mg ClO_4^-). This would increase the time required for HCAP to reach a steady-state biomass concentration. However, once a stable community is reached, the rates would probably be higher.

The significant difference in HCAP-C's K values with respect to the literature suggests that HCAP-C may have a new, or

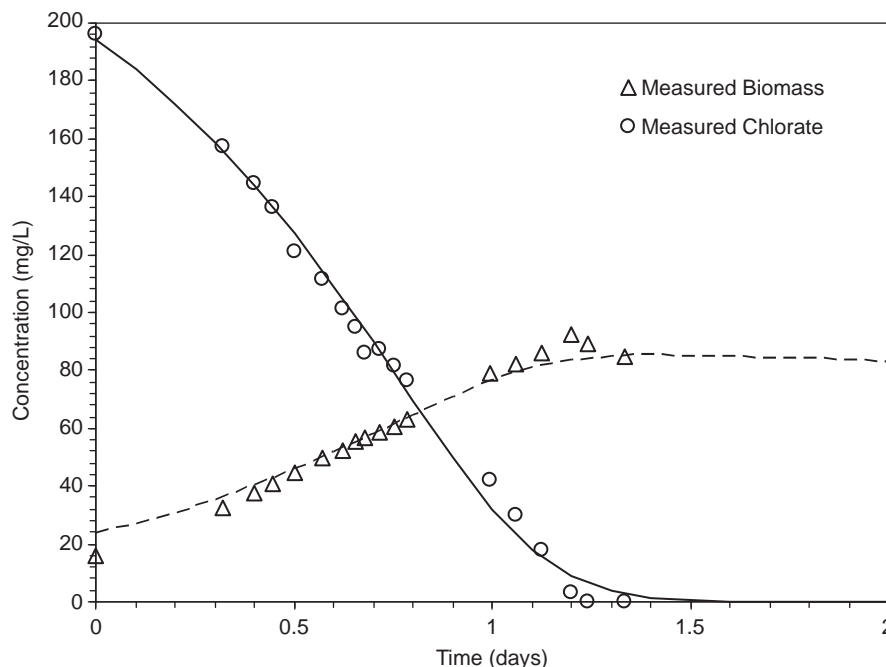


Fig. 3 – Typical experimental data and modeling of a batch chlorate-reduction test. In this test, K_C was estimated to be 45.5 mg ClO_3^-/L and $q_{\max C}$ was estimated to be 5.2 mg $\text{ClO}_3^-/\text{mg DW-d}$. The lines represent the modeled data.

Table 2 – PCRB parameters from literature and from this study^a

Isolate	Electron acceptor	q_{\max} (mg acceptor/ mg DW/day)	K (mg/L)	Reference
<i>Vibrio dechloratans</i>	Perchlorate	1.68	–	Korenkov et al. (1976)
<i>Wolinella succinogenes</i>	Perchlorate	2.57	–	Wallace et al. (1998, 1996)
HAP-1				
GR-1	Perchlorate	5.65	–	Rikken et al. (1996)
KJ	Perchlorate	24	33	Logan et al. (2001)
PDX	Perchlorate	7.5	12	Logan et al. (2001)
SN1A	Perchlorate	4.60	2.2	Waller et al. (2004)
ABL1	Perchlorate	5.43	4.8	Waller et al. (2004)
INS	Perchlorate	4.35	18	Waller et al. (2004)
RC1	Perchlorate	6.00	12	Waller et al., (2004)
PC1	Perchlorate	3.1	0.14	Nerenberg et al. (2006)
HCAP-C	Perchlorate	4.4	76.6	This study
GR-1	Chlorate	7.48	–	Rikken et al. (1996)
PC1	Chlorate	6.3	<0.014	Nerenberg et al. (2006)
HCAP-C	Chlorate	8.3	58.3	This study

^a All kinetic parameters in this table were determined based on simple Monod, i.e., neglecting competitive inhibition.

modified, pcr enzyme. Further studies on the pcr gene and on the purified enzyme are needed to confirm in what way it is different from the pcr of conventional PCRB.

3.4. Chlorate accumulation tests

Batch tests with HCAP-C at initial perchlorate concentrations from 200 to 1360 mg/L were used to determine if the competitive inhibition model was suitable for conditions other than the 200 mg/L, for which the parameters were determined.

Using the parameters described above, the maximum chlorate accumulations were predicted with surprising accuracy (Table 3). Except for Test 5, HCAP-C consistently accumulated around 20% of the initial perchlorate concentration on a weight basis. As mentioned in the Methods section, consistent percentages of chlorate accumulation, with respect to the initial perchlorate concentration, are expected with competitive inhibition. This is the first demonstration of the suitability of the competitive inhibition model for modeling perchlorate reduction and chlorate accumulation.

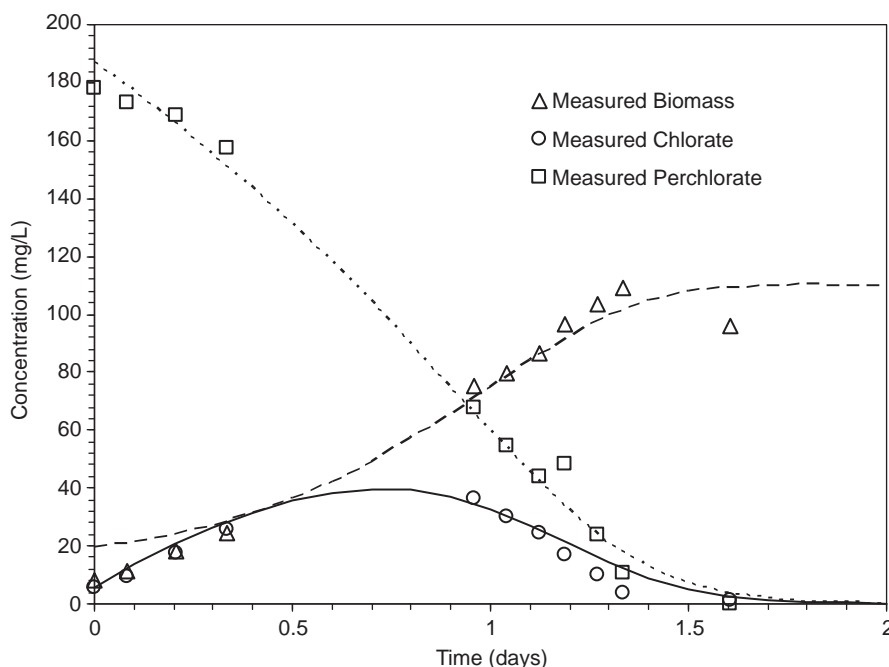


Fig. 4 – Typical experimental data and modeling of a batch perchlorate-reduction test. The model assumes competitive inhibition between perchlorate and chlorate. In this test, K was estimated to be $159 \text{ mg ClO}_4^-/\text{L}$ and q_{maxP} was estimated to be $10.3 \text{ mg ClO}_4^-/\text{mg DW-d}$.

Table 3 – Observed and model-predicted maximum chlorate accumulation for batch growth on perchlorate

Batch test	Initial perchlorate concentration (mg/L)	Maximum observed chlorate accumulation (mg/L)	Percentage of initial perchlorate, weight basis (%)	Model-predicted maximum chlorate accumulation (mg/L)
1	200	38.8	19.4	41.0
2	225	47.5	21.1	46.2
3	340	66.9	19.7	69.8
4	680	140.5	20.7	140
5	1360	283.1	20.8	279
Average			20.3	
Std. dev.			0.7	

4. Conclusions

- The competitive inhibition model accurately describes chlorate accumulation.
- While the q_{Pmax} and q_{Cmax} values are similar to those reported for PCRb, the K_{P} and K_{C} values are much higher.
- HCAP probably would not be effective for reducing perchlorate at low concentrations, e.g. below 200 mg/L , but could be effective at high perchlorate concentrations.
- There is a potential for syntrophic relationships between HCAP bacteria and conventional PCRb or CRb, where these reduce chlorate produced by HCAP. This could lead to overall higher perchlorate degradation rates.
- A modified or distinct (per)chlorate reductase enzyme is likely the cause of the different kinetic behavior. Further research is needed to confirm this.

Acknowledgement

The authors gratefully acknowledge Dr. Stefan Green of the SETI Institute, Moffet Field, California, for his insightful comments on our preliminary manuscript.

REFERENCES

- Achenbach, L.A., Michaelidou, U., Bruce, R.A., Fryman, J., Coates, J.D., 2001. *Dechloromonas agitata* gen. nov., sp. nov. and *Dechlorosoma suillum* gen. nov., sp. nov., two novel environmentally dominant (per)chlorate-reducing bacteria and their phylogenetic position. *Int. J. Syst. Evol. Microbiol.* 51, 527–533.
- Bardiya, N., Bae, J.-H., 2004. Role of *Citrobacter amalonaticus* and *Citrobacter farmeri* in dissimilatory perchlorate reduction. *J. Basic Microbiol.* 44 (2), 88–97.

- Bender, K., 2005. Identification, characterization, and classification of genes encoding perchlorate reductase. *J. Bacteriol.* 187 (15), 5090–5096.
- Bender, K.S., O'Connor, S.M., Chakraborty, R., Coates, J.D., Achenbach, L.A., 2002. Sequencing and transcriptional analysis of the chlorite dismutase gene of *dechloromonas agitata* and its use as a metabolic probe. *Appl. Environ. Microbiol.* 68 (10), 4820–4826.
- Bender, K.S., Shang, C., Chakraborty, R., Belchik, S.M., Coates, J.D., Achenbach, L.A., 2005. Identification, characterization, and classification of genes encoding perchlorate reductase. *J. Bacteriol.* 187 (15), 5090–5096.
- Bruce, R.A., Achenbach, L.A., Coates, J.D., 1999. Reduction of (per)chlorate by a novel organism isolated from paper mill waste. *Environ. Microbiol.* 1 (4), 319–329.
- Chung, J., Nerenberg, R., Rittmann, B., 2006. Bio-reduction of soluble chromate using a hydrogen-based membrane biofilm reactor. *Water Res.* 40 (8), 1634–1642.
- Coates, J.D., Achenbach, L.A., 2004. Microbial perchlorate reduction: rocket-fuelled metabolism. *Nat. Rev. Microbiol.* 2 (7), 569–580.
- Coates, J.D., Michaelidou, U., Bruce, R.A., O'Connor, S.M., Crespi, J.N., Achenbach, L.A., 1999. Ubiquity and diversity of dissimilatory (per)chlorate-reducing bacteria. *Appl. Environ. Microbiol.* 65 (12), 5234–5241.
- Giblin, T., Frankenberger, W.T., 2001. Perchlorate and nitrate reductase activity in the perchlorate-respiring bacterium *perclace*. *Microbiol. Res.* 156 (4), 311–315.
- Gullick, R.W., LeChevallier, M.W., Barhorst, T.S., 2001. Occurrence of perchlorate in drinking water sources. *J. AWWA* 93 (1), 66–77.
- Hatzinger, P.B., 2005. Perchlorate biodegradation for water treatment. *Environ. Sci. Technol.* 39 (11), 239A–247A.
- Kengen, S.W.M., Rikken, G.B., Hagen, W.R., van Ginkel, C.G., Stams, A.J.M., 1999. Purification and characterization of (per)chlorate reductase from the chlorate-respiring strain GR-1. *J. Bacteriol.* 181 (21), 6706–6711.
- Korenkov, V., Romanenko, V., Kuznetsov, S., Voronov, J., 1976. Process for purification of industrial waste waters from perchlorates and chlorates. US Patents.
- Kumar, S., Tamura, K., Nei, M., 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* 5, 150–163.
- Logan, B.E., 1998. A review of chlorate- and perchlorate-respiring microorganisms. *Bioremediat. J.* 2 (2), 69–79.
- Logan, B.E., Zhang, H.S., Mulvaney, P., Milner, M.G., Head, I.M., Unz, R.F., 2001. Kinetics of perchlorate- and chlorate-respiring bacteria. *Appl. Environ. Microbiol.* 67 (6), 2499–2506.
- Michaelidou, U., Achenbach, L.A., Laurie, Coates, J., 2000. Isolation and characterization of two novel perchlorate-reducing bacteria from swine waste lagoons. *Perchlorate Environ.*, 271–281.
- Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial-populations by denaturing gradient gel-electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16s ribosomal-Rna. *Appl. Environ. Microbiol.* 59 (3), 695–700.
- Nerenberg, R., 2003. Perchlorate Removal From Drinking Water With a Hydrogen-Based, Hollow-Fiber Membrane Biofilm Reactor. Northwestern University, Evanston.
- Nerenberg, R., Rittmann, B.E., 2002. Perchlorate as a secondary substrate in a denitrifying hollow-fiber membrane biofilm reactor. *Water Sci. Technol.: Water Supply* 2 (2), 259–265.
- Nerenberg, R., Rittmann, B.E., Najm, I., 2002. Perchlorate reduction in a hydrogen-based membrane-biofilm reactor. *J. AWWA* 94 (11), 103–114.
- Nerenberg, R., Kawagoshi, Y., Rittmann, B.E., 2006. Kinetics of a hydrogen-oxidizing, perchlorate-reducing bacterium. *Water Res.* 40 (17), 3290–3296.
- Reichert, P., 1995. A tool for simulation and data analysis of aquatic systems. *Water Sci. Technol.* 30 (2), 21–30.
- Rikken, G.B., Kroon, A.G.M., van Ginkel, C.G., 1996. Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation. *Appl. Microbiol. Biotechnol.* 45, 420–426.
- Rittmann, B.E., McCarty, P.L., 2001. *Environmental Biotechnology: Principles and Applications*. McGraw-Hill, New York, NY.
- Shrout, J.D., Scheetz, T.E., Casavant, T.L., Parkin, G.F., 2005. Isolation and characterization of autotrophic, hydrogen-utilizing, perchlorate-reducing bacteria. *Appl. Microbiol. Biotechnol.* 67 (2), 261–268.
- Urbansky, E.T., 1998. Perchlorate chemistry: implications of analysis and remediation. *Bioremediat. J.* 2 (2), 81–95.
- Urbansky, E.T., Schock, M.R., 1999a. Issues in managing the risks associated with perchlorate in drinking water. *J. Environ. Manage.* 56 (2), 79–95.
- Urbansky, E.T., Schock, M.R., 1999b. Issues in managing the risks associated with perchlorate in drinking water. *J. Environ. Manage.* 56, 79–95.
- van Ginkel, C.G., Rikken, G.B., Kroon, A.G.M., Kengen, S.W.M., 1996. Purification and characterization of chlorite dismutase: a novel oxygen-generating enzyme. *Arch. Microbiol.* 166 (5), 321–326.
- Wallace, W., Ward, T., Breen, A., Attaway, H., 1996. Identification of an anaerobic bacterium which reduces perchlorate and chlorate as *Wolinella succinogenes*. *J. Ind. Microbiol.* 16, 68–72.
- Wallace, W., Beshear, S., Williams, D., Hospadar, S., Owens, M., 1998. Perchlorate reduction in a mixed culture in an up-flow anaerobic fixed bed reactor. *J. Ind. Microbiol. Biot.* 20, 126–131.
- Waller, A.S., Cox, E.E., Edwards, E.A., 2004. Perchlorate-reducing microorganisms isolated from contaminated sites. *Environ. Microbiol.* 6 (5), 517–527.
- Wolterink, A., Kim, S., Muusse, M., Kim, I.S., Roholl, P.J.M., van Ginkel, C.G., Stams, A.J.M., Kengen, S.W.M., 2005. *Dechloromonas hortensis* sp. nov and strain ASK-1, two novel (per)chlorate-reducing bacteria, and taxonomic description of strain GR-1. *Int. J. Syst. Evol. Microbiol.* 55, 2063–2068.
- Xu, J.L., Song, Y.U., Min, B.K., Steinberg, L., Logan, B.E., 2003. Microbial degradation of perchlorate: principles and applications. *Environ. Eng. Sci.* 20 (5), 405–422.