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Measurement of chlorite dismutase activities in perchlorate respiring bacteria

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Abstract

Chlorite dismutase (CD) catalyzes the disproportionation of chlorite to chloride ($ClO_2^- \rightarrow Cl^- + O_2$) and is present in bacteria capable of cell respiration using perchlorate or chlorate. The activity of this enzyme has previously been measured by monitoring oxygen evolution using a Clark-type dissolved oxygen (DO) probe. We demonstrate here, using two other methods to measure CD activity (a chloride-specific electrode and ion chromatography (IC)) via chloride production, that the DO probe method underestimates dismutation rates. Of the three methods, the chloride probe was the easiest to use and did not require extensive sample handling or post-experimental analysis. Using the chloride electrode method, we determined whole cell rate constants ($V_{max} = 64$ U/mg DW, $K_m = 0.17$ mM) for the chlorate-grown suspensions of *Dechlorosoma* sp. strain KJ. We compared the CD activities of strain KJ at a fixed chlorite concentration (0.6 mM) to four other perchlorate respiring bacteria (PRB), and to one non-PRB (*Pseudomonas aeruginosa*). Chlorate-grown cultures of the five PRB strains had CD activities ranging from 25 to 50 U/mg of cell dry weight (DW), while aerobically grown cultures of the PRB had much lower CD activities (0.5–4 U/mg DW). To our knowledge, this is the first systematic comparison of the different methods to measure CD activities, and the first comparison of CD activities of different PRBs.

Keywords: Chlorite dismutase; Perchlorate respiring bacteria; CD

1. Introduction

Perchlorate (ClO_4^-) has been released into the environment for over half a century primarily due to the use of ammonium perchlorate as the propellant in missiles and rockets (Logan, 2001; Urbansky, 1998). Due to its limited shelf-life, ammonium perchlorate must be periodically replaced in rocket motors, and it is estimated that more than 15.9 million kg of perchlorate has been discharged into the environment since the 1950s (Motzer, 2001). It has been found that perchlorate has contaminated surface and ground waters in at least 18 states and may affect the drinking water of more than 15 million people in the US (Logan, 2001; Urbansky, 1998). Perchlorate is known to affect the function of the thyroid gland in humans by inhibiting iodide uptake (Urbansky, 1998).

Although there is no federal drinking water standard for perchlorate, it is now included in the federal

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Contaminant Candidate List and is being considered for regulation. A recent health study promoted the state of California to lower its drinking water action level from 18 to 4 ppb (μg/l), and there has been speculation that this study could lead to federal drinking water standard as low as 1 ppb (Renner, 2002). Fortunately, perchlorate respiring bacteria (PRB) have been found in many different environments making it possible to bioremediate perchlorate-contaminated environments (Attaway and Smith, 1993; Hatzinger et al., 2002; Herman and Frankenberger, 1999; Kim and Logan, 2000, 2001; Logan, 1998; Logan et al., 2001; Rikken et al., 1996; Wu et al., 2001; Xu et al., in press; Zhang et al., 2002).

Perchlorate is reduced by PRB to chloride via a three-step pathway: $ClO_4^- \rightarrow ClO_3^- \rightarrow ClO_2^- \rightarrow O_2 + Cl^-$ (Rikken et al., 1996). The first two steps are thought to be catalyzed by the same enzyme, perchlorate reductase (Kengen et al., 1999), while the last step is catalyzed by chlorite dismutase (CD) (van Ginkel et al., 1996). The disproportionation of chlorite into harmless chloride and oxygen by CD is the subject of a recent patent (van Ginkel et al., 1999). CD has been found to be highly conserved in PRB. The examination of 13 different PRB isolates demonstrated that the presence of CD in PRB was not dependent on the phylogenetic affiliation of the different isolates (Coates et al., 1999). Both an immunoprobe and a gene probe have been developed for PRB based on the presence of CD (Bender et al., 2002; O'Connor and Coates, 2002).

The only other bacteria known to express CD are bacteria capable of growth on chlorate, but not perchlorate. Denitrifiers can also reduce chlorate to chlorite using nitrate reductase, but because they lack CD for removing chlorite, the accumulation of chlorite is ultimately toxic to the cells (De Groot and Stouthamer, 1969; Oltmann et al., 1976). *Magnetospirillum magnetotacticum* has recently been found to contain a putative gene for CD, but it has neither been shown to express this enzyme in culture, nor can this microbe respire perchlorate (Bender et al., 2002). Thus, it appears CD is a central and unique enzyme only for chlorate- and perchlorate-respiring bacteria.

The release of dissolved oxygen (DO) by PRB has been shown to be useful in the degradation of some hydrocarbons. Unlike chloroperoxidase, an enzyme that converts chlorite to chloride, oxygen and chlorate, CD only produces chloride and oxygen from chlorite (Shahangian and Hager, 1981; van Ginkel et al., 1999). The addition of chlorite to suspensions of PRB has been found to release oxygen and enhance the biodegradation of both benzene and naphthalene under otherwise anaerobic conditions (Coates et al., 1998), while the addition of chlorate increased toluene degradation (Logan and Wu, 2002).

Although PRB are ubiquitous in different environments, and many strains have been isolated, CD activity has been reported with only three strains under aerobic conditions (GR1, CKB and *Ideonella dechloratans*) (Bruce et al., 1999; Stenklo et al., 2001; van Ginkel et al., 1996), and only one strain (GR1) under aerobic conditions (Rikken et al., 1996). CD activity in cell suspensions and cell extracts has been measured in these studies using a Clark-type dissolved oxygen (DO) probe by monitoring the oxygen formation rates following the addition of chlorite. While this procedure is useful to prove that oxygen is evolved by CD, there has been no critical evaluation of this method for determining CD rates. The only other method used to monitor CD activity has been a microassay using horseradish peroxidase, coupled to dianisidine as the electron donor, but details on this test have not yet been presented in the literature (Coates et al., 1999). To determine whether there was a faster or more accurate method to monitor CD activity in cell suspensions, we developed and examined two other methods based on measuring chloride production using either a chloride-specific electrode or ion chromatography (IC). We used one of these methods to compare the CD activities in five different PRB for cultures grown on either chlorate or oxygen.

2. Material and methods

2.1. Bacterial strains and culture conditions

All bacterial cultures, including *Dechlorosoma* spp. strains KJ and PDX (Logan et al., 2001), *Dechloromonas* spp. strain JM (Miller and Logan, 2000) and strain HZ (Zhang et al., 2002), strain perc1ace (Herman and Frankenberger, 1999) and *Pseudomonas aeruginosa* strain PAO1 (Hentzer et al., 2001), were kept at − 80 °C in 20% glycerol. All of these bacterial strains except PAO1 can grow using perchlorate or chlorate.

Strain PAO1 was included only as a negative control for chlorite rate measurements. Cells were grown at 29 °C on a shaker (5 ml in a 28-ml tubes or 25 ml in 125-ml serum bottles, both sealed with butyl rubber stoppers) at 150 rpm in basal medium (modified from VG medium; Logan et al., 2001; Rikken et al., 1996), containing (per liter of deionized water): 1.39 g sodium acetate, 1.55 g $K_2HPO_4 \cdot 3H_2O$, 0.85 g $NaH_2PO_4 \cdot H_2O$, 0.5 g $NH_4H_2PO_4$, 50 mg $MgSO_4 \cdot 7H_2O$, 3 mg EDTA, 2 mg $ZnSO_4 \cdot 7H_2O$, 1 mg $CaCl_2 \cdot 2H_2O$, 4 mg $FeSO_4 \cdot 7H_2O$, 0.4 mg $Na_2MoO_4 \cdot 5H_2O$, 0.2 mg $CuSO_4 \cdot 5H_2O$, 0.4 mg $CoCl_2 \cdot 6H_2O$, 1 mg $MnCl_2 \cdot 4H_2O$, 0.1 mg $NiCl_2 \cdot 6H_2O$, 0.15 mg Na_2SeO_3 and 0.6 mg H_3BO_3 and at a pH adjusted to 7.0 using NaOH. Anaerobic medium containing 0.64 g/l of sodium chlorate was prepared using basal medium degassed in an anaerobic glove box (Coy Scientific Products, Grass Lake, MI). Aerobic medium was prepared using a basal medium by shaking tubes or containers containing an air headspace. The volume of the headspace was sufficient to maintain aerobic conditions during the experiments.

2.2. Preparation of cell suspensions

Anaerobically grown cell suspensions of strain KJ were used in all CD measurements unless stated otherwise. Cells were harvested during late log growth by centrifugation ($10{,}000 \times g$), and pellets were washed twice with a phosphate buffer (10 mM) adjusted to pH 7.0. Cell suspensions were diluted with phosphate buffer in order to produce measurable CD activities. Cell concentrations were chosen based on OD_{600} (Shimadzu UV spectrophotometer, 1-ml cuvette) with the final cell dry weight (DW) determined using membrane filters (25 mm, 0.2-μm pore diameter; Osmonics, Minnetomka, MN) and a microbalance (± 0.1 μg; duplicate samples; Mettler Toledo UMT2, Greifensee, Switzerland).

2.3. Chloride electrode measurements of CD activities

The chloride selective electrode (P/N CL01502, pHoenix Electrode, Houston, TX) was calibrated daily with eight standards (0.1–10 mM) in 5 ml of phosphate buffer (10 mM) in a 20-ml chamber at pH 7.0. For CD activity measurements, cell suspensions were added into the phosphate buffer solution and stirred during measurements. The reaction was started by the addition of 30 μl of a freshly prepared 100-mM chlorite solution (0.6 mM final concentration) at 25 °C. Online measurements were taken every 3–5 s for 1 min. Preliminary experiments demonstrated that the pH did not change during the course of the experiment. The initial chloride formation rate was linear and was therefore used to calculate one enzyme activity unit (1 U) as 1 μmol of chloride formed (or 1 μmol of chlorite consumed) per minute. All CD activities were normalized to cell dry weight and expressed as U/mg DW. Unless stated otherwise, CD activity measurements were made under ambient air conditions.

2.4. Ion chromatographic (IC) measurements of CD activities

CD activity was also examined by measuring chloride using an IC (Dionex-500) equipped with an AS-11 column and guard column (Dionex), and an eluent of 10 mM NaOH. The instrument was calibrated each time with four standards (0.03–0.6 mM). Experiments were conducted as described above, with modifications to allow for the volume of sample needed for IC measurements. Cell suspensions were added to 100-ml bottles containing 50 ml of phosphate buffer, and after the addition of chlorite (30 μl of 1 M chlorite; final concentration: 0.6 mM), samples (2 ml) were withdrawn every 10 s for 1 min. Samples were immediately combined with 20 μl of NaOH (25% w/w) to halt enzyme reaction (Xu et al., 2000), and stored at 4 °C for less than 1 week before analysis.

2.5. Dissolved oxygen probe measurements of CD activities

A YSI Clark-type DO probe (YSI Model 5300 biological oxygen monitor, Yellow Springs Instrument, Yellow Springs, OH) used to measure dissolved oxygen concentrations during the experiments was calibrated with an air-saturated phosphate buffer solution at 25 °C before each experiment. Cell suspensions were added to a 20-ml chamber containing 3 ml of phosphate buffer, and constantly stirred. The reaction was started by adding 18 μl of 100-mM chlorite (0.6 mM final concentration). CD activities were

calculated as above, except concentrations of dissolved oxygen were used to calculate rates instead of chloride.

2.6. Statistical analyses

After comparing variances based on an *F*-test, a two-tailed student *t*-test (with equal or unequal variances as appropriate) was used to determine if differences between sample results were significant ($P<0.05$). All statistical analyses were conducted using functions in Microsoft Excel.

3. Results

3.1. Comparison of CD activities with three different methods

The rate of chloride or oxygen evolution for all three methods was linear for the first 40 s following the addition of chlorite (Fig. 1). The abiotic dismutation of chlorite was found to be negligible during the 1-min-long experiments (data not shown). Chlorite dismutation rates were calculated from the initial and linear region of the rate data as shown in Fig. 1. The CD rate measured with the chloride electrode of 0.45 ± 0.013 mM/min (triplicate tests) was not significantly different from that (0.42 ± 0.013 mM/min) measured with the IC ($P=0.15$). However, both of these rates were significantly higher ($P<0.05$) than the rate of 0.35 ± 0.022 mM/min determined by monitoring oxygen evolution using a DO probe.

The chloride electrode method was better suited for measuring CD rates than the other two methods for several reasons. First, chloride formation could be detected immediately after the addition of chlorite. With the IC method, collection of the sample required nearly 10 s, prohibiting a shorter sampling interval. With the DO probe, there was an initial delay (5 to 10 s) in data acquisition as the probe had to be inserted into the chamber to force out air (to avoid aeration) in the headspace through a small opening. However, it was easy to insert the chloride electrode into the vial for measurement of Cl^- in the liquid, especially for large-volume samples. Second, the chloride electrode provided data immediately (as did the DO probe),

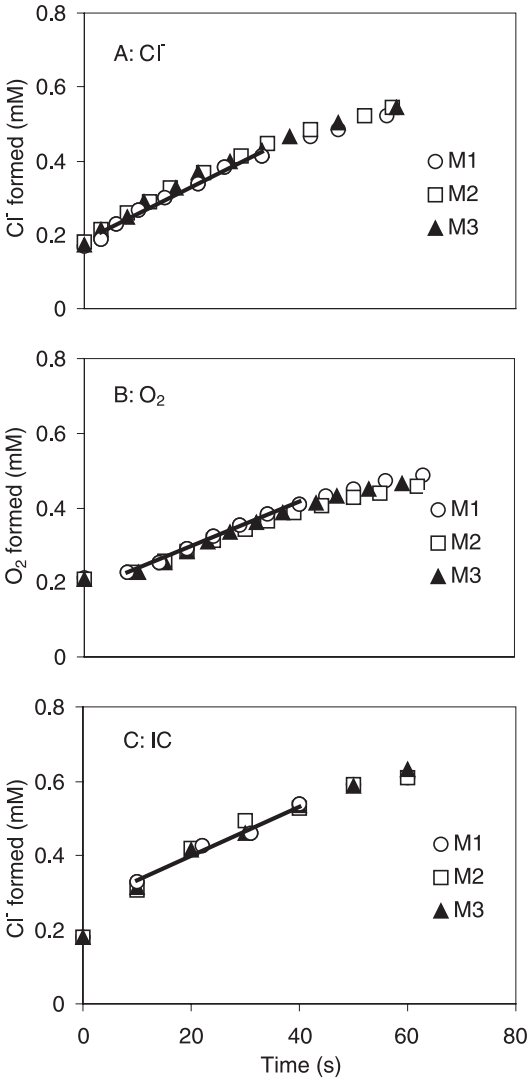

Fig. 1. CD activities (triplicate measurements, M1, M2 and M3) for chlorate-grown cell suspensions measured with: (A) chloride electrode, (B) dissolved oxygen probe and (C) ion chromatograph. The solid line in each figure is an example of a linear regression (M1 in each case) used to calculate the CD rate.

while the IC analysis method required sample pretreatment (NaOH to halt the dismutation reaction), filtration (to remove cells) and later sample analysis (approximately 15 min/sample) on the IC. Third, the DO probe indicated lower rates than the other two methods, likely as a result of a slower characteristic response time of DO probe versus that of the chloride probe.

3.2. CD kinetics

CD rates obtained with whole cells exhibited saturation kinetics with respect to chlorite concentration, as shown in Fig. 2 for anaerobically grown cell suspensions of strain KJ (~ 10 mg DW/l). The maximum CD activity (V_{max}) was 64 U/mg DW, and the half saturation constant (K_m) was 0.17 mM based on measurements with the chloride electrode. In contrast, rate constants determined with the conventional DO technique were $V_{max} = 50$ U/mg DW and $K_m = 0.15$ mM. When the chlorite concentrations were lower than 0.5 mM, CD rates were significantly affected by the chlorite concentrations (Fig. 2). At a chlorite concentration of 0.6 mM used for comparison of CD activities, rates were 80% of V_{max} for both methods.

Based on the formation rate of chloride, chlorite dismutation rates (0.05–1.2 mM/min) increased linearly with cell dry weight (1.5 to 25 mg DW/l) for anaerobically grown cell suspensions of strain KJ (Fig. 3). In contrast, CD rates measured with the DO probe were significantly (based on SE of slopes shown in Fig. 3) less than those obtained with the chloride probe. The CD rate, normalized to cell dry weight, measured with the chloride probe was 0.050 ± 0.001 mM l/min DW, versus 0.034 ± 0.003 mM l/min DW using the DO probe (Fig. 3).

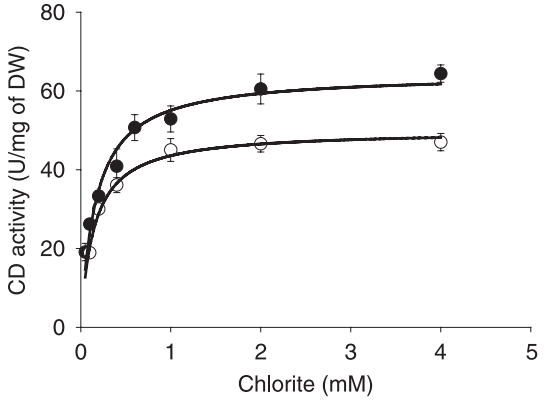

Fig. 2. Influence of chlorite concentration, C (mM), on CD rates, V (U/mg DW) using a chloride electrode (solid symbol) or a dissolved oxygen probe (open symbol) for chlorate-grown suspensions of strain KJ. The constants for a Monod-type rate equation are: $V = 64\ C/(0.17 + C)$, chloride electrode; $V = 50\ C/(0.15 + C)$, dissolved oxygen probe.

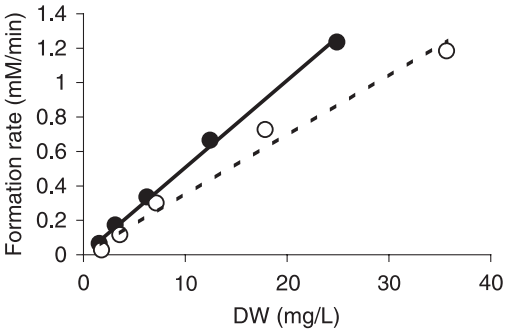

Fig. 3. CD rates measured in terms of the formation rate (F) of chloride (solid symbol) or dissolved oxygen (open symbol) as a function of cell concentration (cell dry weight; DW) for cell suspensions of strain KJ grown anaerobically on chlorate. The regression lines are: chloride (solid line), $F = 0.050 \pm 0.001$ DW + 0.017 ± 0.018 [$R^2 = 0.998$; P(slope) = 0.00005]; oxygen (dashed line), $F = 0.034 \pm 0.003$ DW + 0.023 ± 0.050 [$R^2 = 0.981$; P(slope) = 0.0011].

3.3. Influence of pH, DO and NaCl on CD activity measurements

CD rates were significantly influenced by pH (Fig. 4). The optimal pH was 6.0, with activity nearly completely reduced at a pH = 9. At a pH = 4.7, CD rates were approximately the same as those measured at pH = 7.0.

To examine if dissolved oxygen affected CD rates, we compared oxygen evolution rates measured for cell suspensions kept in the anaerobic chamber with those made with the same anaerobically grown KJ cell

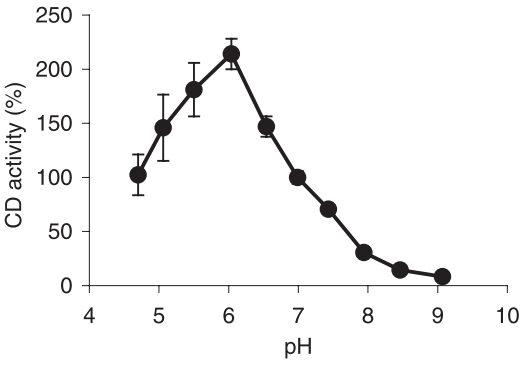

Fig. 4. Influence of pH on CD activities measured with the chloride electrode for the chlorate-grown cell suspensions of strain KJ in 10 mM of phosphate buffer. The CD activity was defined as 100% at pH 7.0.

suspension removed from the glove box and exposed to laboratory air for 1 h. The initial DO level in the suspensions exposed to air was 0.22 mM (about 90% air saturation). We found CD rates were unchanged at a higher DO concentrations (data not shown), in agreement with findings by others that DO does not affect CD rates (van Ginkel et al., 1996). Chloride produced from the dismutation of chlorite did not adversely affect CD rates over a range of 0–100 mM (NaCl) when CD rates were measured in a 10 mM phosphate buffer (data not shown).

3.4. CD activities in different PRB strains

CD activities were measured for the six different bacteria using the chloride electrode method for both anaerobically (except strain PAO1, which did not grow on chlorate) and aerobically grown cells (Table 1). Three strains (perc1ace, HZ and PDX) had very similar CD activities of 34.9–37.2 U/mg DW (mean 36.2 U/mg DW) when grown under anaerobic conditions. The other two PRB had CD rates that differed by 68% (24.5 U/mg DW, strain JM) and 144% (52.3 U/mg DW, strain KJ) from this mean rate.

All aerobically grown PRB cultures tested had measurable CD activities (Table 1). Chloride measurements on a cell suspension of *P. aeruginosa* PAO1 produced a CD rate of less than 0.1 U/mg DW. Because this bacterium does not contain CD, this rate was used to define the lower detection limit of this procedure. The CD activities of the five PRB, averaging only 1.9 U/mg DW (range of 0.5 to 4 U/mg DW), were lower by an order-of-magnitude or more than activities measured for anaerobically grown cells. Therefore, CD expression in these PRBs was induced by anaerobic growth on chlorate.

Table 1
CD activities (U/mg DW) measured with the chloride electrode for different bacteria grown aerobically, or anaerobically on chlorate

Strain	Type	CD activity		Ratio (Anaerobic/Aerobic)
		Anaerobic	Aerobic	
Pseudomonas aeruginosa PAO1	Non-PRB	–[a]	<0.1	–[b]
Perc1ace	PRB	37.2	0.92	40
Dechloromonas sp. JM	PRB	24.5	0.51	48
Dechloromonas sp. HZ	PRB	35.6	3.03	12
Dechlorosoma sp. KJ	PRB	52.3	1.02	51
Dechlorosoma sp. PDX	PRB	34.9	3.90	9

[a] Does not grow anaerobically on chlorate.
[b] Cannot be calculated.

4. Discussion

4.1. Comparison of different methods

CD rates were more easily and accurately measured with the chloride electrode than with a DO probe or by using an IC. The DO probe technique routinely used by others (Bruce et al., 1999; Stenklo et al., 2001; van Ginkel et al., 1996) also had several limitations. First, oxygen is rapidly evolved in these tests, but data could not be collected during the first 5–10 s of the experiment when using a DO probe due to time lost during insertion of the probe into the sample apparatus. This initial time period represented a considerable loss of data on CD activity as rates were constant only during the first 40 s of the 1-min-long experiment. Second, the detection range of the DO probe was much more limited than that of a chloride electrode, particularly if samples were measured outside of an anaerobic chamber. The maximum range of measurable DO concentrations under aerobic conditions was only 0.27 mM, based on a starting concentration of 0.22 mM (90% air saturation) and a maximum DO concentration for the probe of 0.49 mM (200% air saturation). In contrast, the chloride electrode has a detection range of 0.01–100 mM of chloride. Third, the calibration and operation of chloride electrode were easier and more accurate than the dissolved oxygen probe. When compared with the IC method, the chloride electrode method provided results that were comparable to those obtained using the IC to measure chloride concentrations. However, the chloride electrode method did not require the extra steps needed for the IC method (NaOH addition, filtration and sample analysis time using the IC). The only other method that has been used to measure CD activities is based on horseradish peroxidase (Coates et al., 1999). However, we note that this test, like the IC method, also requires several additional

steps to process the sample before the results can be obtained, such as sample-heating to stop dismutation reaction, and measurement of residual chlorite by a colorimetric microassay.

Our measured CD rates for whole cells seem reasonable when compared to the results by others for whole cells and cell extracts for strains GR1 and CKB (Table 2), although a direct comparison is not possible due to all the differences between the tests including concentrations of chlorite, procedures (probes and enzymes), materials (whole cells versus extracts) and normalized masses (protein versus cell dry weight). These rate differences clearly point out the need for measurements to be made on a common basis.

The initial chlorite concentration is an important consideration in a CD rate test. High concentrations of chlorite should be avoided because chlorite can be toxic to PRB (5 mM for strains KJ and PDX; unpublished data). The chlorite concentrations used for CD measurements by others have ranged from 0.035 to 15 mM (Table 2). These values span the first and zero-order kinetic regions found here for *Dechlorosoma* sp. strain KJ (Fig. 2). We chose 0.6 mM of chlorite for our CD rate comparison of different bacteria because this concentration was 3–4 times the measured K_m for strain KJ. The half-saturation constant of 0.17 mM found here agrees well with the value previously reported for the chlorite dismutase purified from strain GR1 (K_m = 0.17 mM) (van Ginkel et al., 1996).

One difference between our findings with whole cells and previous measurements with extracted enzyme is the response of CD rates to pH. We found the highest CD rate was measured for cell suspensions at a pH = 6.0, which is the same as that for the purified CD from strain GR1 (van Ginkel et al., 1996). However, at a pH of 4.7, we found the same CD rate as at pH = 7.0 (Fig. 4), while van Ginkel et al. (1996) reported that the purified CD activity was near zero at pH 5.0. The maintenance of CD activity at pH = 5.0 may reflect the ability of the cell to buffer external pH changes inside the cell versus those in solution. Because CD activity is a function of pH, and it is very sensitive to pH values below pH = 6.0, we recommend that the measurements be performed in a buffer at neutral pH. In our tests, 10 mM of phosphate buffer was sufficient to maintain a pH at 7.0 during 1-min CD rate measurements.

4.2. CD activities of different PRB

CD activities have previously been reported for only three strains (GR1, CKB and *I. dechloratans*) under anaerobic conditions, and only one strain (GR1) under anaerobic conditions (Table 2). In our study, there appeared to be a relatively small range of CD rates for genus *Dechlorosoma* or *Dechloromonas*, in which most of PRB strains are located (Achenbach et al., 2001). CD rates measured for bacteria grown under anaerobic conditions ranged from 24.5 (strain JM) to 52.3 U/mg (strain KJ), while the remaining PRB had activities in the relatively narrow range of 34.9–37.2 U/mg (Table 1). Although the CD activ-

Table 2
CD activities reported in other studies using a dissolved oxygen probe (unless indicated otherwise)

Strain	Sample fraction	Chlorite (mM)	CD activity (U/mg protein)	Reference
GR1	Purified CD	15	2200	(van Ginkel et al., 1996)
	Cell extract	15	145	(van Ginkel et al., 1996)
	Anaerobically grown cells	0.035	10	(van Ginkel et al., 1999)
	Anaerobically grown cells	0.035	10000[a]	(Rikken et al., 1996)
	Aerobically grown cells	0.035	1000[a]	(Rikken et al., 1996)
CKB	Supernatant of cell lysate	0.1	14130[a]	(Bruce et al., 1999)
	Membrane fraction	0.1	12080[a]	(Bruce et al., 1999)
	Purified CD	0.1	1928[b]	(Coates et al., 1999)
	Cell extract	0.1	35[b]	(Coates et al., 1999)
Ideonella dechloratans	Purified CD	0.25	2800	(Stenklo et al., 2001)
	Periplasmic extract	0.25	350	(Stenklo et al., 2001)

[a] This is believed to be a typographic error, and the value should actually be 1000 times lower.
[b] In this study, the CD activity was measured with horseradish peroxidase.

ities in aerobic PRB cultures cannot be detected using an immunoprobe or a gene probe (Bender et al., 2002; O'Connor and Coates, 2002), we found that CD activities in all aerobic PRB cultures were detectable using a chloride electrode. Nitrate-grown KJ and PDX also have similar basal CD activities (Unpublished data). These basal CD activities, present in all PRB tested, may be important for PRB survival, particularly under nitrate-reducing conditions. If chlorate is present in the environment, it will react with nitrate reductase and produce chlorite that can be toxic to the cells in the absence of chlorite dismutase. When PRB were grown aerobically, CD rates dropped by an order-of-magnitude or more for the *Dechloromonas*, *Dechlorosoma* and perc1ace strains.

These findings demonstrate that the production of CD is inducible in PRB bacteria, which is in agreement with other reports for strain GR1 and strain CKB (O'Connor and Coates, 2002; Rikken et al., 1996). Other non-PRB such as *P. aeruginosa* PAO1 did not have CD activity (Coates et al., 1999; van Ginkel et al., 1996) but were useful in defining a minimum detectable CD rate of 0.1 U/mg DW. To our knowledge, this is the first systematic comparison of the CD activities of five different PRB grown on different conditions.

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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.

Attaway, H., Smith, M., 1993. Reduction of perchlorate by an anaerobic enrichment culture. J. Ind. Microbiol. 12, 408–412.

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, 4820–4826.

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, 319–329.

Coates, J.D., Bruce, R.A., Haddock, J.D., 1998. Anoxic bioremediation of hydrocarbons. Nature 396, 730.

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, 5234–5241.

De Groot, G.N., Stouthamer, A.H., 1969. Regulation of reductase formation in *Proteus mirabilis*: 1. Formation of reductases and enzymes of the formic hydrogenlyase complex in the wild type and in chlorate-resistant mutants. Arch. Microbiol. 66, 220–233.

Hatzinger, P.B., Whittier, M.C., Arkins, M.D., Bryan, C.W., Guarini, W.J., 2002. In-situ and ex-situ bioremediation options for treating perchlorate in grougwater. Remediation J. 12 (2), 69–86.

Hentzer, M., Teitzel, G.M., Balzer, G.J., Heydorn, A., Molin, S., Givskov, M., Parsek, M.R., 2001. Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. J. Bacteriol. 183, 5395–5401.

Herman, D.C., Frankenberger, W.T., 1999. Bacterial reduction of perchlorate and nitrate in water. J. Environ. Qual. 28, 1018–1024.

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, 6706–6711.

Kim, K., Logan, B.E., 2000. Fixed-bed bioreactor treating perchlorate-contaminated waters. Environ. Eng. Sci. 17, 257–265.

Kim, K., Logan, B.E., 2001. Microbial reduction of perchlorate in pure and mixed culture packed-bed bioreactors. Water Res. 35, 3071–3076.

Logan, B.E., 1998. A review of chlorate and perchlorate respiring microorganisms. Remediation J. 2, 69–79.

Logan, B.E., 2001. Assessing the outlook for perchlorate remediation. Environ. Sci. Technol. 35, 482A–487A.

Logan, B.E., Wu, J., 2002. Enhanced toluene degradation under chlorate-reducing conditions by bioaugmentation of sand columns with chlorate- and toluene-degrading enrichments. Remediation J. 6, 87–95.

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, 2499–2506.

Miller, J.P., Logan, B.E., 2000. Sustained perchlorate degradation in an autotrophic, gas-phase, packed-bed bioreactor. Environ. Sci. Technol. 34, 3018–3022.

Motzer, W.E., 2001. Perchlorate: problems, detection, and solutions. Environ. Forensics 2, 301–311.

O'Connor, S.M., Coates, J.D., 2002. Universal immunoprobe for (per)chlorate-reducing bacteria. Appl. Environ. Microbiol. 68, 3108–3113.

Oltmann, L.F., Reijnders, W.N.M., Stouthamer, A.H., 1976. Characterisation of purified nitrate reductase A and chlorate reductase C from *Proteus mirabilis*. Arch. Microbiol. 111, 25–35.

Renner, R., 2002. Perchlorate drinking water recommendation drops. Environ. Sci. Technol., Online News (http://pubs.acs.org/subscribe/journals/esthag-w/2002/jan/policy/rr_perchlorate.html).

Rikken, G.B., Kroon, A.G.M., vanGinkel, C.G., 1996. Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation. Appl. Microbiol. Biotechnol. 45, 420–426.

Shahangian, S., Hager, L.P., 1981. The reaction of chloroperoxidase with chlorite and chlorine dioxide. J. Biol. Chem. 256, 6034–6040.

Stenklo, K., Thorell, H.D., Bergius, H., Aasa, R., Nilsson, T., 2001. Chlorite dismutase from *Ideonella dechloratans*. J. Biol. Inorg. Chem. 6, 601–607.

Urbansky, E.T., 1998. Perchlorate chemistry: implications for analysis and remediation. Remediation J. 2, 81–95.

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, 321–326.

van Ginkel, C.G., Kroon, A.G.M., van Wijk, R.J., 1999. Process for the degradation of chlorite. US Patent 5,891,339.

Wu, J., Unz, R.F., Zhang, H.S., Logan, B.E., 2001. Persistence of perchlorate and the relative numbers of perchlorate- and chlorate-respiring microorganisms in natural waters, soils, and wastewater. Remediation J. 5, 119–130.

Xu, J., Xu, X., Verstraete, W., 2000. Adaptation of *E. coli* cell method for micro-scale nitrate measurement with the Griess reaction in culture media. J. Microbiol. Methods 41, 23–33.

Xu, J., Song, Y., Min, B., Sternberg, L., Logan, B.E., 2003. Microbial degradation of perchlorate: principles and applications. Environ. Eng. Sci. 20 (In press).

Zhang, H., Bruns, M.A., Logan, B.E., 2002. Perchlorate reduction by a novel chemolithoautotrophic hydrogen-oxidizing bacterium. Environ. Microbiol. 4, 570–576.