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Reduction of perchlorate and nitrate by salt tolerant bacteria

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"Capsule": *Microbial reduction of perchlorate and nitrate in saline solutions.*

Abstract

Spent regenerant brine from ion-exchange technology for the removal of perchlorate and nitrate produces a high salt waste stream, which requires remediation before disposal. Bioremediation is an attractive treatment option. In this study, we enriched for salt tolerant bacteria from sediments from Cargill salt evaporation facility (California, USA), the Salton Sea (California, USA), and a high density hydrocarbon oxidizing bacterial cocktail. The bacterial cocktail enrichment culture reduced ClO_4^- from 500 to 260 mg/l in 4 weeks. Salt tolerant bacterial isolates from the enrichment cultures and two denitrifying salt tolerant bacteria, *Haloferax denitrificans* and *Paracoccus halodenitrficans*, substantially reduced perchlorate. The highest rate of perchlorate removal was recorded with the isolate, *Citrobacter* sp.: 32% reduction in 1 week. This bacterium substantially reduced perchlorate in 0–5% NaCl solutions and maximally at 30 °C and at an initial pH 7.5. In simulated brines containing 7.5% total solids, the *Citrobacter* sp. significantly reduced both perchlorate and nitrate with 34.9 and 15.6% reduction, respectively, in 1 week. Coculture of a potent perchlorate reducing, non-salt tolerant (non-saline) bacterium, perc1ace and the *Citrobacter* sp. proved most effective for perchlorate removal in the brine (46.4% in 1 week). This study demonstrates that both anions can be reduced in treatment of brines from ion exchange systems. © 2002 Published by Elsevier Science Ltd.

Keywords: Salt tolerant bacteria; *Citrobacter* sp.; Spent regenerant brine; Perchlorate; Nitrate; Bioremediation

1. Introduction

Perchlorate (ClO_4^-) is detected in ground water throughout the United States, because ammonium perchlorate was extensively used mainly for defense purposes, leading to the contamination of natural drinking water sources. In the state of California, many drinking water wells are contaminated with ClO_4^- to levels greater than the action level of 18 μg/l (CDHS, 1998). The potential health hazard of ClO_4^- is largely due to adverse effects on the thyroid gland (Capen, 1994; von Burg, 1995). Remedial strategies for removal of ClO_4^- in ground water are currently being evaluated, including membrane, ion-exchange (IX) and biological technologies. Ion-exchange, an approved water treatment technology, can effectively remove ClO_4^- from water (Gu et al., 1999; Venkatesh et al., 1999; Batista et al., 2000; Tipp and Clifford, 2000). However, such physicochemical treatment technology produces high salt waste stream, which requires remediation before disposal. Salty wastes can be remediated by physical treatment but costs are high. Bioremediation of salty IX wastes is desirable since bioremediation is considered cost effective.

In the ion exchange process, ClO_4^- and other anions such as nitrate (NO_3^-) are removed from water by adsorption to an anion exchange resin. The resin is regenerated with 3–12% NaCl (Clifford and Liu, 1993b). The spent regenerant brine contains high concentrations of salts, mainly NaCl as well as ClO_4^- (60–70 mg/l) and NO_3^- (400–4000 mg/l). Disposal of this hypersaline waste is the major drawback of this process (Venkatesh et al., 1999). Current optimization of IX, however, aims at decreasing the NaCl concentration required for regeneration to levels as low as 0.5–1% (Betts, 1998; Tripp and Clifford, 1999). If achieved, this range would permit the growth of non-saline perchlorate-reducing bacteria.

Many drinking water wells contain NO_3^- at levels above the acceptable concentration of 10 mg NO_3-N/l

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(Clifford and Liu, 1993b). Nitrate contamination of drinking water poses serious health problems. The reduced form (nitrite) can cause methemoglobinemia, particularly in infants; and nitroso compounds formed from nitrate and nitrite are potential carcinogens (Clifford and Liu 1993b). Nitrate decontamination of water using the IX processes also produces hypersaline wastes contaminated with NO_3^- and results in the same disposal problems as ClO_4^- contaminated spent regenerant brine.

In recent years there has been growing interest on bacteria which can remove NO_3^- from hypersaline wastes. A denitrifying moderately haloalkalophilic bacterium, *Halomonas campisalis* was isolated and characterized (Mormile et al., 1999). Liu and Clifford (1996) demonstrated a hybrid biological denitrification ion-exchange process. Clifford and Liu (1993a) reported on salt tolerant, denitrifying organisms from sewage sludge. Recently, Gevertz et al. (2000), characterized two nitrate-reducing and sulfide-oxidizing bacteria isolated from oil field brines. The transformation of uranyl nitrate and other compounds in high ionic strength brines by a *Halomomas* sp. (WIPP1A) under denitrifying conditions has also been demonstrated (Francis et al., 2000).

Although a number of studies have addressed bioremediation of ClO_4^- (Romenanko et al., 1976; Wallace et al. 1996; Rikken et al., 1996; Logan, 1998; Herman and Frankenberger, 1999; Urbansky and Schock, 1999; Frankenberger and Herman, 2000; Giblin et al., 2000a, b, 2002; Logan et al., 2000; Miller and Logan, 2000; Giblin and Frankenberger, 2001; Losi et al., 2002), growth of such monocultures in high salinity solutions typical of IX brines has not been achieved. Only recently has there been a report of biological ClO_4^- reduction in high salinity-solutions by microbial enrichment cultures (Logan et al., 2001). Perchlorate reducing bacteria such as perc1ace can also reduce NO_3^- (Herman and Frankenberger, 1999). However, there is paucity of information on the use of bacteria to remove both ClO_4^- and NO_3^- from contaminated regenerant brine. Thus, our study focused on the isolation of salt tolerant bacteria from high solid aqueous environments, for ClO_4^- and NO_3^- removal from spent regenerant brine. We also assessed co-removal of ClO_4^- and NO_3^- by a coculture of the salt sensitive bacterium, perc1ace and a salt tolerant bacterial isolate, *Citrobacter* sp.

2. Materials and methods

2.1. Enrichment culture

Sediments from the Salton Sea (California), Cargill salt evaporation facility (near San Francisco Bay, California) and a high density hydrocarbon oxidizing bacterial cocktail obtained from the Center for Environmental Microbiology (Riverside CA) were used to enrich for salt tolerant ClO_4^- reducing bacteria. The enrichment medium was 100 ml of FTW mineral salts medium (Herman and Frankenberger, 1999) consisting of the following (in g/l): K_2HPO_4, 0.225; KH_2PO_4, 0.225; $(NH_4)_2SO_4$, 0.225; $MgSO_4.7H_2O$, 0.05; $CaCO_3$, 0.005; $FeCl_2.4H_2O$, 0.005, acetate, 1.0 (as sodium acetate) and 1 ml of trace elements solution (Focht, 1994). Sodium chloride (25g/l) and resazurin 1 mg/l were also added. The medium was sparged with nitrogen gas and autoclaved (121 °C, 15 min) before aseptically adding filter sterilized ClO_4^- to a final initial concentration of 500 mg/l (as 615 mg/l $NaClO_4$). Aliquots of each sample (sediment or bacterial cocktail) were used to inoculate the medium in separate 125-ml Erlenmeyer flasks. Headspaces were flushed with nitrogen gas. Flasks were thereafter sealed with teflon-lined screw cap stoppers and incubated at 30 °C for 4 weeks (Herman and Frankenberger, 1999). Resazurin was added to the enrichment culture as a redox indicator. Resazurin is reduced from pink to clear at an E_h of −110 mV (Jacob, 1970). Perchlorate reduction has been estimated to occur below the E_h (−110 mV) necessary for resazurin color change (Attaway and Smith, 1993; Giblin et al., 2000a, b). The visible color change served as a presumptive assay of cultures reducing perchlorate. Thereafter, aliquots from each flask were transferred to a fresh enrichment culture and further incubated for 2 weeks. Potential ClO_4^- reducing salt tolerant bacteria were isolated from the enrichment cultures by plating on the enrichment medium solidified with 2% agar. Plates were incubated in an anaerobic jar (BBL GasPack) at 30 °C. Colonies were isolated, purified by repeated streaking and maintained on the same agar medium at 4 °C.

2.2. Screening of isolates for perchlorate reduction

The ability of the bacterial isolates to reduce ClO_4^- was determined using 40 ml of FTW/NaCl mineral salts medium in 50-ml Erlenmeyer flasks, containing acetate (1 g/l), ClO_4^- (500 mg/l), sodium chloride (25 g/l) and yeast extract (1 g/l). Bacterial inocula were pregrown in the same medium under anaerobic condition for 5 days. Cells were recovered by centrifugation, washed with sterile FTW mineral medium and resuspended in the same medium (OD_{600} = approximately 0.36). Five-hundred microliters of each bacterial suspension was used to inoculate the 40 ml liquid culture. Headspaces were flushed with nitrogen gas and the flasks were fitted with rubber stoppers and incubated at 25, 30, and 35 °C. Other bacteria used in this study were *Paracoccus halodenitrificans* (ATCC 13511), *Haloferax denitrificans* (ATCC 35960) and perc1ace (ATCC 202172).

2.3. Identification of a salt tolerant perchlorate reducing bacterium

One bacterial isolate, tentatively designated IsoCock1 was selected for further studies. The cells were microscopically examined after Gram staining. The isolate was identified as a *Citrobacter* sp. by 16S rRNA gene sequence similarity (Midi labs, Newwark, DE, USA). Using primers corresponding to *E. coli* positions 005 and 531, the 16S rRNA gene was amplified from genomic DNA by PCR. Cycle sequencing of the PCR product was carried out using ABI Prism 377 DNA sequencer. Sequence data was analyzed using PE Applied Biosystem's Microseq™, microbial analysis software and database.

2.4. Analysis of parameters affecting perchlorate removal by Citrobacter sp.

2.4.1. Effect of carbon sources

Carbon substrate utilization for ClO_4^- reduction by the *Citrobacter* sp. was examined. Different carbon substrates (citrate, formate, fumarate, succinate, acetate, ethanol, glucose, yeast extract and molasses) were added to FTW/NaCl mineral salts medium (without a carbon source) at a final concentration of 1 g/l (w/v). Culture conditions were as described under ClO_4^- reduction by the isolates.

2.4.2. Effect of temperature and pH

The influence of temperature and pH on ClO_4^- removal were assessed using the FTW/NaCl medium and culture conditions described under ClO_4^- reduction by the isolates. Incubation temperature was varied at 20, 25, 30, 35 and 40 °C. After autoclaving, the culture medium was adjusted to pH 6.0, 7.0, 7.5, 8.0 and 10.0 using predetermined amounts of filter sterilized (0.22 μ membrane) 1M HCl or NaOH and incubated at 30 °C.

2.4.3. Effect of sodium chloride concentration

In a comparative study between *Citrobacter* sp. and perc1ace, the effect of varying concentration of NaCl (0, 2.5, 5.0, 7.5, 10%) was examined in duplicate using the FTW/NaCl medium. Culture conditions were the same as described under ClO_4^- reduction by the isolates. The initial pH was adjusted to 7.5 and incubation was at 30 °C.

2.5. Perchlorate removal from brine

FTW/NaCl mineral elements medium containing acetate (1 g/l) and yeast extract (1 g/l) as carbon sources, were used to compose two simulated spent ion-exchange regenerant brines. High sulfate and NO_3^- brine (HNS brine) was prepared by adding 11,700 mg/l NO_3^- (as 16,041 mg/l sodium nitrate) and 21,000 mg/l SO_4^{2-} (as 31,080 mg/l sodium sulfate). An alternative brine, low in SO_4^{2-} and NO_3^- (LNS brine) was prepared by adding 250 mg/l NO_3^- (as 342.75 mg/l sodium nitrate) and 2500 mg/l SO_4^{2-} (as 3700 mg/l sodium sulfate). The brines were sterilized by autoclaving (121 °C, 15 min) with the initial pH being adjusted to 7.5 before adding filter sterilized ClO_4^- to a final concentration of 100 mg/l. The HNS brine was inoculated with monocultures and cocultures of perc1ace and *Citrobacter* sp. Perc1ace was pre-cultivated in 800 ml of FTW mineral salts medium plus 100 mg/l ClO_4^- and 1 g/l acetate (30 °C, 100 rpm, 5 days). *Citrobacter* sp. was pre-grown in the same medium to which 0.1% yeast extract was added and static cultures were incubated at 30 °C, for 2 days. Cells were recovered by centrifugation, washed with FTW mineral salts medium and resuspended in FTW brine to $OD_{600} = 9.68$ and 2.57 for perc1ace and *Citrobacter* sp., respectively. Forty milliliters of FTW brine in 50-ml Erlenmeyer flasks were inoculated with 830 μl of perc1ace and 140 μl of *Citrobacter* sp. cell suspensions. Monocultures of perc1ace and *Citrobacter* sp. and uninoculated control flasks were also set up. All flasks were incubated at 30 °C, after which they were sacrificed for analysis. Perchlorate removal from LNS brine by monocultures of *Citrobacter* sp. was also studied. Anoxic conditions were maintained as described.

2.6. Bacterial density determination

Bacterial density was determined spectrophotometrically by monitoring optical density at OD_{600}. All measurements were read with the uninoculated controls as a blank.

2.7. Analysis of perchlorate and other anions

Culture and control samples were filtered using membrane filter (0.45 μm). Perchlorate concentration in the filtrate was analyzed using a Dionex ion-chromatography system (Dionex, Sunnyvale, CA), equipped with a GP40 gradient pump, an AS40 automated sampler, 740 μl injection loop, an ionPac AS11 column and an ED40 conductivity detector. The eluent was 100 mM NaOH at 1/ml. An ASRS-II (4 mm) suppressor, operated at 300 mA was used to suppress the eluent, using water, as the regenerant. Chloride, NO_3^-, NO_2^- and SO_4^{2-} in the supernatant were analyzed using the same ion-chromatograph except that a 10 μl injection loop was used and the eluent was 21 mM NaOH. Samples were purified using an OnGuard-H catridge (Dionex, Sunnyvale, CA) according to the manufacture's instructions.

3. Results

3.1. Perchlorate removal from enrichment culture

In each enrichment culture containing ClO_4^- reducing bacteria, resazurin was reduced from pink to clear.

Analysis of relatively high concentrations of ClO_4^- in the enrichment cultures indicated ClO_4^- removal. The enrichment culture inoculated with the high density hydrocarbon-oxidizing bacterial cocktail decreased ClO_4^- from 500 to 335 mg/l and 500 to 260 mg/l in 2 and 4 weeks, respectively. In the cultures inoculated with sediments from the Salton Sea, ClO_4^- also decreased from 500 to 356 mg/l and 500 to 284 mg/l in 2 and 4 weeks, respectively.

3.2. Perchlorate removal by isolates

Five bacterial isolates (IsoA, IsoB, IsoC, IsoCock1 and IsoSol1) substantially reduced ClO_4^- concentration in the FTW/NaCl medium (Table 1). Several other isolates from the enrichment culture did not reduce ClO_4^- as monocultures. Two denitrifying bacteria, *H. denitrificans* and *P. halodenitrficans* tested, also reduced ClO_4^- in the same medium. Assessment of the degrees of ClO_4^- reduction at 25, 30 and 35 °C, showed that IsoCock1 was the best ClO_4^- reducer amongst the isolates tested and was selected for further studies. The isolate reduced ClO_4^- from 500 to 340 mg/l (32% reduction) in 1 week.

3.3. Identity of IsoCock1

The salt tolerant ClO_4^- reducing bacterial isolate (IsoCock1) is a Gram negative rod, that is facultatively anaerobic. The bacterium was identified as *Citrobacter* sp. by 16S rRNA gene sequence analysis. Based on the first 500 base pairs of the 16S rRNA gene, the isolate is most closely related to *Citrobacter farmeri* (99.3% identity) followed by *Citrobacter amalonaticus* (99.2% identity). Sequence alignment also showed 98.7 and 98.6% identity to *Citrobacter rodentium* and *Citrobacter sedlaki*, respectively.

3.4. Parameters influencing perchlorate reduction by Citrobacter *sp.*

3.4.1. Carbon substrate utilization for perchlorate breakdown

Citrobacter sp. utilized various carbon sources for growth and ClO_4^- reduction (Table 2). Although citrate and ethanol supported the growth of the salt tolerant bacterium, no ClO_4^- reduction was recorded with these carbon substrates. For ClO_4^- removal, yeast extract was the best carbon source, followed by acetate. A combination of yeast extract and sodium acetate was more effective for ClO_4^- removal, decreasing ClO_4^- from 500 to 341 mg/l (31.8% removal).

3.4.2. Temperature and pH

Temperature and pH profiles of ClO_4^- removal by *Citrobacter* sp. are illustrated in Fig. 1. The bacterium substantially reduced ClO_4^- in the temperature range of 20–35 °C with considerably less reduction at 40 °C (Fig. 1). Maximal reduction of ClO_4^- was observed at 30 °C. Perchlorate reduction was observed at the initial pH range 6.0–9.0. Optimal pH for ClO_4^- reduction was observed at an initial pH of 7.5 (Fig. 1). With growth of *Citrobacter* sp. in the FTW/NaCl medium, changes in pH as a result of bacterial growth were noted.

3.4.3. NaCl concentration

Citrobacter sp. is much more tolerant of NaCl than perc1ace (Fig. 2). With the *Citrobacter* sp., approximately 84±7, 105±5 and 88.3±6 mg of ClO_4^- per liter were removed in 1 week from a starting concentration of 500 mg/l at 0%, 2.5% and 5% NaCl, respectively. The growth of perc1ace, was drastically inhibited with the addition of 2.5% sodium chloride. With perc1ace, ClO_4^- was rapidly reduced to non-detectable levels within 1 week at 0% NaCl, but its

Table 1
Preliminary screening of salt tolerant bacteria for ClO_4^- reduction

Isolate	Source	$C1O_4^-$ removal (mg/l)[a]		
		25 °C	30 °C	35 °C
IsoCock1	HDBC	147	160	151
IsoA	SCSEF	28.2	38.6	17.7
IsoB	SCSEF	21.3	58.9	18.9
IsoC	SCSEF	28.4	56.8	20.6
IsoSol1	SS	34.7	58.3	39.9
Paracoccus halodenitrificans	ATCC	30.0	63.2	49.9
Haloferax denitrificans	ATCC	13.8	72.9	54.3

[a] Calculated as ΔClO_4^- of uninoculated control and inoculated test cultures. Values are means of duplicate independent determinations. Pooled sample standard deviations were, ±7.4 mg/l (25 °C), ±8.5 mg/l (30 °C) and ±8.0 mg/l (35 °C). High density hydrocarbon oxidizing bacterial cocktail (HDBC), sediments from Cargill salt evaporation facility (SCSEF), and sediments from Salton Sea (SS). Acetate and yeast extract were used as carbon sources (see Section 2).

Table 2
Effect of carbon sources on ClO_4^- and final bacterial optical density

Carbon substrate (1 g/l)	ΔClO_4^- (mg/l)	ClO_4^- removal[a] (%)	OD_{600}
Acetate	54.9±1.8	11.1	0.05
Citrate	0	0	0.07
Ethanol	0	0	0.04
Formate	26.9±2.1	5.41	0.03
Fumerate	9.4±0.7	1.92	0.04
Glucose	18.9±2.1	3.81	0.09
Molasses	19.3±1.8	3.90	0.09
Succinate	31.8±1.6	6.42	0.03
Yeast extract	78.1±4.5	15.8	0.11

[a] Calculated relative to the initial ClO_4^- concentration (500 mg/l) ΔClO_4^- was calculated relative to residual ClO_4^- in control. Grandma's molasses (density = 1.23, Stamford, CT USA) was used. ΔClO_4^- are means of triplicate independent determinations ±S.D. ($n-1$).

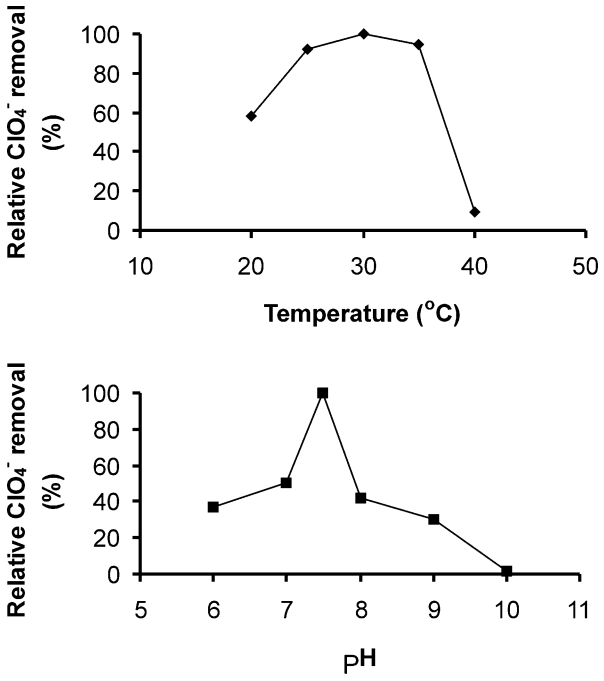

Fig. 1. Temperature and pH profiles of ClO_4^- removal by *Citrobacter* sp. Results are normalized with respect to maximal ClO_4^- removal of 160±3 at 30 °C, as 100% and with respect to maximal ClO_4^- removal of 92±13 at pH 7.5, as 100%.

capacity to efficiently reduce ClO_4^- declined with the addition of 2.5% NaCl.

3.5. Perchlorate and nitrate removal from brine

With cocultures of *Citrobacter*/perc1ace in the HNS brine, ClO_4^- decreased substantially from an initial concentration of 100 to 70.8±4.4 mg/l (29.2% reduction) and 53.6±2.8 mg/l (46.4% reduction) within 2 and 7 days, respectively (Table 3). Substantial decreases in ClO_4^- concentration were also noted with monocultures of *Citrobacter* sp., i.e. 34.9% reduction in 7 days. Chloride concentration increased in both coculture and monocultures indicating ClO_4^- breakdown to chloride and oxygen. Nitrate concentrations also decreased with 16.4% reduction for the *Citrobacter*/perc1ace coculture and 15.6% for the *Citrobacter* monoculture, in 7 days (Table 4). Nitrite accumulation was not detected. Sulfate levels were generally stable in the cultures and controls. In experiments conducted with the low nitrate and sulfate (LNS) brine, using the *Citrobacter* monoculture, NO_3^- concentrations decreased substantially. Nitrate was rapidly removed and decreased from 250 to 179.1±7.9 mg/l after 1 week. After 2 weeks of incubation, NO_3^- was not detectable. No appreciable variation in sulfate concentrations in the cultures and controls was observed in 4 weeks. Average OD_{600} readings were 0.194, 0.174, 0.165 and 0.152 after 1, 2, 3 and 4 weeks, respectively.

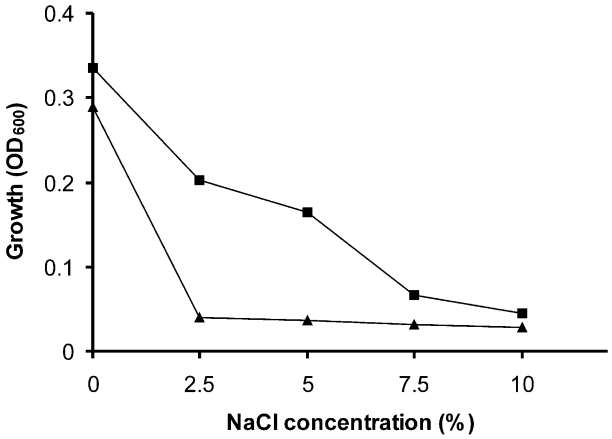

Fig. 2. Effect of sodium chloride on the growth of *Citrobacter* sp. (■) and perc1ace (▲). Initial OD values were approximately 0.01 and 0.04 for *Citrobacter* sp. and perc1ace, respectively.

4. Discussion

Most brine wastes do not meet discharge standards because they contain high concentrations of dissolved inorganic solids including potentially toxic oxyanions such as ClO_4^- and NO_3^-. Consequently, remediation of contaminated natural water sources and hypersaline wastes is an urgent issue. Bioremediation is an attractive option because the technology is environmentally compatible and considered cost-effective. Potential ClO_4^- reducing bacteria are believed to be widespread in nature (Logan, 1998; Coates et al., 1999). We have enriched for ClO_4^- degrading salt tolerant bacteria from diverse environments. In all the enrichment cultures with ClO_4^- reducing bacteria, resazurin was reduced from pink to clear. This served as a useful indicator of enrichment cultures reducing ClO_4^-. A similar observation was noted for other ClO_4^- reducing cultures (Attaway and Smith, 1993; Giblin et al., 2000a, b). Reduction of resazurin from pink to clear occurs at an E_h below −110 mV (Jacob, 1970).

Denitrifying bacteria are often inhibited in growth above 1% (10 g/l) NaCl (van der Hoek et al., 1987; Clifford and Liu, 1993a). NaCl inhibited the growth of perc1ace, a prolific ClO_4^- reducer (Herman and Frankenberger, 1999). Both growth and its capacity to reduce ClO_4^- was inhibited at 2.5% NaCl. However, *Citrobacter* sp. could grow and sustain ClO_4^- removal at 5% NaCl, and apparently up to about 7.5% total solids in the HNS brine.

As ClO_4^- concentrations vary in the environment, high concentrations of ClO_4^- were used for the initial studies to ensure that the isolate would be active at both high and low concentrations of ClO_4^-. The salt tolerant *Citrobacter* sp. can reduce ClO_4^- at high and low concentrations.

In the LNS brine, ClO_4^- removal significantly slowed down after 1 week of incubation. However, NO_3^- was

Table 3
Removal of ClO_4^- from HNS brine using monocultures and cocultures of *Citrobacter* sp. and perc1ace[a]

Treatment	2 Days		7 Days	
	ΔClO_4^- (mg/l)	OD_{600}	ΔClO_4^- (mg/l)	OD_{600}
Control (uninoculated)	11.6±3.2	0	15.7±2.4	0
Perc1ace (monoculture)	18.4±3.5	0.08±0.01	28.2±3.4	0.09±0.01
Citrobacter (monoculture)	12.3±3.1	0.35±0.03	34.9±4.9	0.28±0.01
Citrobacter/perc1ace (coculture)	29.2±4.4	0.47±0.05	46.4±2.8	0.35±0.01

[a] Values represent means±S.D. ($n-1$) of triplicate determinations. ΔClO_4^- and were calculated relative to initial ClO_4^- concentration of 100 mg/l. Initial OD_{600} readings were 0.09, 0.01, 0.11 for perc1ace, *Citrobacter* and *Citrobacter*/perc1ace, respectively. Acetate and yeast extract were used as carbon sources (see Section 2).

Table 4
Nitrate concentration (mg/l) in HNS brine inoculated with monocultures and cocultures of *Citrobacter* sp. and perc1ace[a]

	2 Days	7 Days
Control (uninoculated)	11,907±293	11,516±201
Perc1ace (monoculture)	11,566±156	11,370±320
Citrobacter (monoculture)	10,669±269	9693±66
Citrobacter/perclace	9971±336	9593±228

[a] Values represent means ±S.D. ($n-1$) of triplicate determinations. The initial concentration of NO_3^- was 11,700 mg/l.

rapidly removed from the brine. Rapid NO_3^- removal may have inhibited ClO_4^- removal, possibly due to diversion of electrons from ClO_4^- to NO_3^-. Nitrate inhibition of ClO_4^- reduction has been reported by Herman and Frankenberger (1998, 1999). Nitrate reductase also catalyze ClO_4^- reduction (Romanenko et al., 1976). Similarly, two denitrifying halophilic bacteria, *P. halodenitrificans* and *H. denitrificans* tested in this study reduce ClO_4^-. More importantly, accumulation of nitrite, a toxic intermediate was not found in the *Citrobacter* sp. cultures.

Sulfate concentrations in brine remained approximately the same during incubation with the *Citrobacter* sp. Attaway and Smith (1993) had demonstrated that SO_4^{2-} reduction is not related to ClO_4^- reduction. The inability of *Citrobacter* sp. to reduce SO_4^{2-} is an important advantage since electrons generated from carbon substrate metabolism would be conserved for ClO_4^- and NO_3^- reduction.

5. Conclusion

We have reported bacterial removal of ClO_4^- and NO_3^- from saline solutions. Results indicate that the *Citrobacter* sp. could be useful for the removal of ClO_4^- and NO_3^- in saline ground water and brine. A coculture of the salt tolerant *Citrobacter* sp. with the potent ClO_4^- reducing but salt sensitive bacterium, perc1ace, displayed the most substantial rate of ClO_4^- removal in brine.

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