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Limitations to Natural Bioremediation of Perchlorate in a Contaminated Site

Karen E. Kesterson and Penny S. Amy
Department of Biological Sciences, University of Nevada Las Vegas, Las Vegas, NV, USA

Jacimaria R. Batista
Department of Civil and Environmental Engineering, University of Nevada Las Vegas, Las Vegas, NV, USA

ABSTRACT Perchlorate (ClO_4^-) has been detected in many drinking water supplies in the United States, including the Las Vegas Wash and Lake Mead, Nevada. These locations are highly contaminated and contribute perchlorate to Lake Mead and the Colorado River system. Essential elements for perchlorate bioremediation at these locations were examined, including the presence of perchlorate-reducing bacteria (PRB), sufficient electron donors, occurrence of competing electron acceptors, and ability of PRB to utilize a variety of electron donors. Enumeration of PRB was performed anoxically using most probable number (MPN). Values ranged from $\leq$20 to 230 PRB/100 ml or $\leq$20 to $\geq 1.6 \times 10^5$ PRB/g for Lake Mead water samples and Las Vegas Wash sediments, respectively. 16S rRNA sequences revealed that isolates were γ-proteobacteria, *Aeromonas*, *Dechlorosoma*, *Rahnella* and *Shewanella*. A screening of potential electron donors using BIOLOG™ demonstrated that all isolates were capable of metabolic versatility. Measurements of total organic carbon (TOC), nitrate and dissolved oxygen (DO) indicated limited presence of electron donor at all sites, whereas the electron acceptors varied throughout the Wash and Lake Mead. The persistence of perchlorate in the sites is attributed to lack of available electron donor and/or the presence of competing electron acceptors. A location has been identified where perchlorate biodegradation could be implemented thereby halting the transport of perchlorate to Lake Mead and the Colorado River.

KEYWORDS bioremediation, contamination, electron acceptor, electron donor, Lake Mead, Las Vegas Wash, microbiology, perchlorate, perchlorate reducing bacteria

INTRODUCTION

Perchlorate contamination in the United States is associated with manufacturing for the use of solid oxidants for rocket propulsion, components of fireworks and explosives, and air bag inflators (Urbansky, 1998). Current concerns about perchlorate relate to its possible health effects on human physiology resulting in adverse low production of thyroid hormones (Urbansky et al., 2000; Wolff, 1998). The long-term, large-scale industrial manufacturing of perchlorates and the improper disposal of perchlorate-containing wastes has led to ground and surface water contamination, resulting in a national environmental concern about its presence in drinking water. There is currently no federal drinking

Address correspondence to Penny S. Amy, Director of Shadow Lane Campus, 1001 Shadow Lane, Las Vegas, NV 89106. E-mail: amy@ccmail.nevada.edu

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water standard for perchlorate (USEPA, 2005). Recently, the National Academy of Sciences (NAS) has reviewed the toxicological data on perchlorate and their recommendation has resulted in a reference dose (RfD) of 24.5 μg/L for perchlorate in drinking water (USEPA, 2005). Despite NAS recommendations, the California Department of Health Services adopted a provisional action level of 6 μg/L for perchlorate in public water supplies (CDHS, 2004). The Nevada Department of Environmental Protection has established an action level of 18 μg/L (USEPA, 1999).

Between 1945 and 1997, two perchlorate production facilities operated in Henderson, NV, approximately 13 miles southeast of Las Vegas. The facilities produced sodium chlorate and potassium, sodium, and ammonium perchlorate (KMCC, 1980; Kleinfelder Inc., 1993). These two facilities supplied the entire ammonium perchlorate demand for the USA (Committee on Science, Space and Technology, 1988). Legal perchlorate releases to the environment occurred at the plants via direct disposal of perchlorate-containing wastes into unlined ponds, and leaks from pipes and storage pads at the industrial facilities. An estimated 290,000 tons of perchlorate-containing wastes were discharged into unlined ponds built on the site (Jacobs Engineering Group, Inc. 1987). These practices resulted in contamination of the groundwater with perchlorate concentrations greater than 3,700 mg/L detected in several wells (Urbansky, 1998). The contaminated groundwater then seeped into the Las Vegas Wash (LVW), a stream that discharges into Lake Mead, an integral part of the Colorado River System. Perchlorate concentrations in the LVW range from 10–800 μg/L (Boralessa, 2001). The concentration of perchlorate in Lake Mead varies from 120 μg/L where the LVW meets Lake Mead, to 25 μg/L at the drinking water intake of the Southern Nevada Water Authority (Zhang et al., 2001). Furthermore, the USEPA (1999) reported perchlorate levels of 7–10 μg/L at the California intake point from the Colorado River. This large-scale, long-term source of perchlorate contamination makes Henderson, Nevada one of the most highly contaminated sites in the U.S.

Biological reduction of perchlorate to chloride under anoxic conditions occurs readily and it has been demonstrated in laboratory and full-scale reactors (Hatzinger et al., 2000; Herman and Frankenberger, 1999; Kim and Logan, 2001; Liu and Batista, 2000; Logan, 2001). Remediation of perchlorate contaminated sites using biological reduction is a potential means of cleaning the sediments and water from the LVW area and Lake Mead. The persistence of high levels of perchlorate for many years suggests that biological removal may be limited by environmental factors. In this study, the limitations to perchlorate biodegradation in the contaminated area are evaluated. The investigation includes: (a) evaluation of the numbers, diversity, and identification of isolates using 16S rDNA sequencing of indigenous PRB present in native sediments and waters collected from the LVW and Lake Mead; (b) examination of electron donor limitations; and (c) investigation of the presence of competing electron acceptors (e.g., oxygen and nitrate).

MATERIALS AND METHODS

Sample Collection

Soil samples were collected along the LVW and water samples from Lake Mead (Figure 1). The wash is the main drainage channel for the Las Vegas Valley and it carries wastewater effluent, dry and wet weather runoff and the contaminated seepage from the Basic Management Industrial (BMI) complex, all of which enters into Lake Mead. Soil samples were collected from the banks along the LVW using a clean metal shovel that had been rinsed with sterile deionized water. Flame-sterilizing was contraindicated because of the potential fire hazards associated with surrounding dry brush and debris, therefore samples were collected after soil was turned over three times. A sterile wooden tongue depressor was used to collect soil samples that were placed in sterile Whirlpack bags. Water samples were collected from Lake Mead and the Las Vegas Bay using a Van Dorn sampler and transferred to sterile high density polyethylene containers. The water samples were collected at the epilimnion and hypolimnion regions of Lake Mead at several locations. Sediments and waters were transported back to the laboratory on ice in a cooler.

Media Composition

All media were prepared using ultra pure water (Technic Water Systems, Lab 5 Extended) and research-grade chemicals in the amounts indicated below. To prepare 1 liter of (20X) Phosphate-buffer: K_2HPO_4 * $3H_2O$ (2.03 g), NaH_2PO_4 (0.85 g) $NH_4H_2PO_4$ (0.50 g). To prepare a (100X) mineral salts solution: $MgSO_4$ * $7H_20$ (100 mg), EDTA (3 mg), $ZnSO_4$ * $7H_2O$ (2.0 mg), $CaCl_2$ * $2H_2O$ (1.0 mg), $FeSO_4$ * $7H_2O$ (4.0 mg),

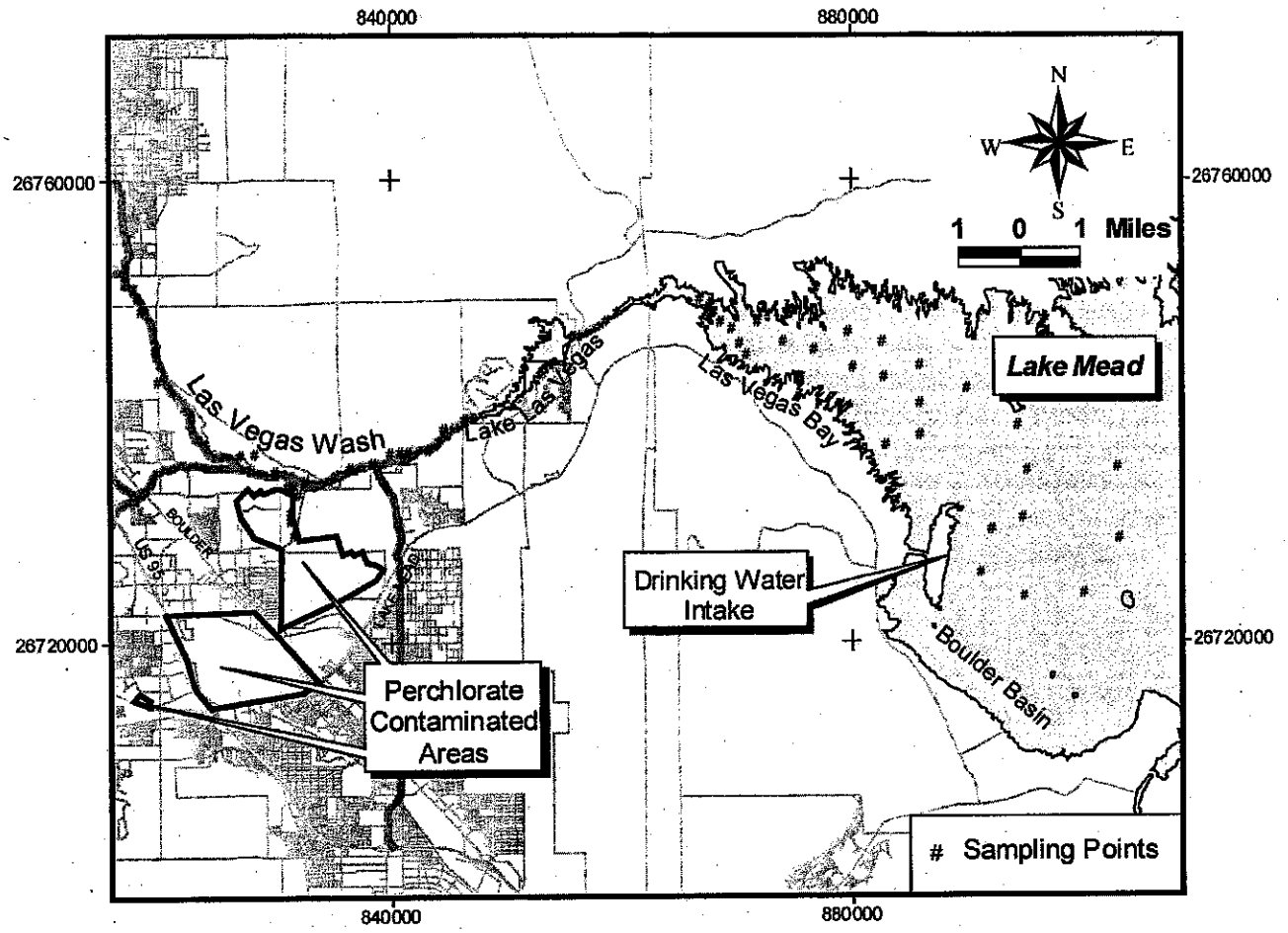

FIGURE 1 Map of Las Vegas Valley Wash and Lake Mead sampling sites.

$Na_2MoO_4 * 2H_2O$ (0.4 mg), $CuSO_4 * 5H_2O$ (0.2 mg), $CoCl_2 * 6H_2O$ (0.4 mg), $MnCl_2 * 4H_2O$ (1.0 mg), $NiCl_2 * 6H_2O$ (0.1 mg), $NaSeO_3$ (0.1 mg), H_3BO_3 (0.6 mg). To prepare a $NaClO_4$ stock solution (1 liter): (12.32 g) to produce a final concentration of 10,000 mg/L. To prepare a $NaCH_3COOH$ stock solution (1 liter): $NaCH_3COOH * 3H_2O$ (23.05 g) to produce a final concentration of 10,000 mg/L Perchlorate-Acetate (PA) medium contained each of the following stock solutions: 50 ml of phosphate-buffer, 10 ml of mineral salts, 10 ml of sodium perchlorate and 50 ml of sodium acetate. Growth medium was solidified with 15 g/L of agar.

Enumeration of Perchlorate-Reducing Bacteria

Most Probable Number (MPN) for Soil

To facilitate removal of bacteria from sediment particles, sediment (10 g) was placed into 90 ml of 0.1% sodium pyrophosphate (Sigma) and incubated on a rotary shaker for 1 hour at 25°C. After incubation, the sediment slurry was used for MPN analysis. A 5-tube MPN was prepared for each dilution in the series. Dilutions were prepared from each sediment sample to achieve 10^{-1}–10^{-4}. Sediment MPN tubes were incubated for 6 weeks in the anaerobic chamber at 25°C. In order to accurately determine whether PRB were present in the samples the initial and final concentration of perchlorate was measured using ion chromatography (Dionex DX-120) with an AS-11 column and 49 mM NaOH as the eluent. An MPN tube was scored as positive if the initial concentration of perchlorate was reduced by one-half.

Most Probable Numbers (MPN) for Lake Mead Water

Water samples were prepared, incubated and analyzed as for soil described above. However, the

addition of sodium pyrophosphate was not necessary for water samples.

Bacterial Isolation and Characterization

Enrichment cultures consisted of soil or water inoculum transferred to sterile 180 ml serum bottles that contained mineral salts medium with acetate and perchlorate (PA) or from isolated colonies on plate count enrichments. After inoculation, the serum bottle was filled to the neck, fitted with a butyl rubber stopper and crimped with an aluminum seal. Samples from enrichments were subcultured onto solid PA medium. All enrichment culture and isolation procedures were conducted in an anaerobic chamber (Coy Laboratories, type A) at 25°C. Three to four successive transfers were made to fresh solid PA to achieve purity. Purity was confirmed by microscopic examination of wet mount preparations and observations of colony characteristics.

Primary enrichments for PRB were incubated anaerobically in mineral salts medium, modified from Van Ginkel (1995) containing acetate and perchlorate, at a concentration of 500 mg/L and 100 mg/L, respectively. All strains grew anaerobically at 25°C in a mineral salts medium, with acetate as the sole electron donor and sodium perchlorate as the sole electron acceptor. After two weeks of incubation at 25°C, samples were transferred to solid basal medium. Subsequent subcultures were transferred at least three times until colony characteristics remained consistent. Cells grown anaerobically on perchlorate-acetate agar at 25°C developed colonies within two weeks. Characterization of each isolate was based on microscopy, morphology, motility, size and colony characteristics.

16S Ribosomal RNA Sequencing

DNA from each isolate was extracted using heat treatment. Cell suspension, in 1.5 ml PA broth, was transferred to a sterile, Eppendorf tube. The cell suspension was heated to 95°C for 5 min, rapidly cooled and then stored at −20°C until further analysis. Samples were sent to the Nevada Genomics Center in Reno, Nevada for sequence analysis. Sequencing and fragment analyses were performed on an Applied Biosystems (ABI) Prism 3730 DNA Analyzer.

Carbon Utilization

Carbon utilization by pure isolates was completed using a gram-negative BIOLOG™ assay. BIOLOG™ plates were prepared using cells suspended in perchlorate broth (100 mg/L). Cell suspension (100 μl) was transferred to 94 wells and 100 μl sterile medium was transferred to one well as a control. Wells were covered with sterile mineral oil to confer anaerobiosis (BioMerieux Vitek, Inc.) The BIOLOG™ plates were incubated at 25°C for two weeks.

RESULTS

Perchlorate-Reducing Bacteria Enumeration and Characterization

MPN results include data for both the Lake Mead waters and the LVW sediments, and are shown in Tables 1 and 2, respectively. The MPN index for Lake Mead water samples ranged from less than 20 to 230 PRB/100 ml. For a creek water in Pennsylvania, Wu et al. (2001) reported 1–17 PRB/100ml of sample. However, counts ranging from 10^6–10^8/100 ml have been reported in the literature for swine waste and raw wastewater (Wu et al., 2001; Coates et al., 1999a). The MPN index for PRB in the LVW sediment samples ranged from less than 20 to 10^5 PRB/g soil, on dry weight basis. Previous studies revealed MPN counts of 10^3 PRB/g from pristine soil and petroleum-contaminated soil samples (Coates et al., 1999a). The PRB numbers found for LVW sediments are, in some locations, up to two orders of magnitude greater than previously reported PRB for sediments and soils. In other locations, the numbers are comparable. In Lake Mead, PRB numbers are much smaller than those determined in the LVW. An exception was found in the Las Vegas Bay, which is the confluence of the LVW and Lake Mead, where PRB counts as high as 2.3×10^2/100 ml of water were found. Unexpectedly, the lowest PRB numbers were found within the contaminated site that contains the highest perchlorate concentrations. Recent investigations have revealed that the low microbial counts in the contaminated site may be due to the presence of high salinity (Batista et al., 2005). The highest PRB numbers were found along the Wash before and after the contaminated site where perchlorate concentrations are low.

Characterization of each isolate was based on microscopy, morphology, motility, size and colony

TABLE 1 Most Probable Number of Perchlorate Reducing Bacteria (PRB), Perchlorate and Dissolved Oxygen (DO) Concentrations in Lake Mead Water Samples

Sample ID	MPN[1] Index/100 ml	[a]ClO_4^- µg/L	DO mg/L	Sample ID	MPN[1] Index/100 ml	[a]ClO_4^- µg/L	DO[4] mg/L
WLVB1[2]	80	Bay 140–160	8.0	WLVBE5[2]	80	Bay 140–160	8.5
WLVB2[2]	110		7.42	WLVB6 [2]	80		8.0
WLVB3[b][2]	13	Bay 70–80	7.6	WLVB7[2]	2.3×10^2		0.31
WLVB4[2]	80		ND[3]	WLVB8 [2]	20		0.91
				WLVB9 [2]	80		8.05
WLM1	<20	Lake Mead 0–13	8.1	WLM17	<20	Lake Mead 0–13	4.52
WLM2	40		8.12	WLM18	<20		6.95
WLME3-*E*	20		12.0	WLM19	<20		5.98
WLM4	20		6.7	WLME20-*E*	<20		6.93
WLM5	20		9.65				
WLM6	20		7.09	WLM21	<20		6.17
WLM7	<20		5.7	WLM22	<20		6.47
WLM8	<20		8.35	WLME23-*E*	<20		7.11
WLM9	<20		8.20				
WLME10-*E*	40		862	WLM23	<20		6.11
WLM11	<20		6.95	WLM24	<20		7.94
WLM12	<20		7.0	WLM25	<20		6.24
WLM13	<20		7.6	WLM26	40		7.16
WLM14	<20		7.8	WLME27-*E*	40		8.36
WLM15	<20		7.8				
WLME16	<20		8.5				

[a]Historical perchlorate levels in the Las Vegas Bay and other portions of Lake Mead has been measured on 578 frozen water samples and the concentration range given above has been determined (Boralessa, 2001).

[1]MPN = Most Probable Number for perchlorate reducing bacteria; [2]Las Vegas Bay; [3]not determined; [4]DO = dissolved oxygen; "*E*": epilimnion.

TABLE 2 Most Probable Number of Perchlorate Reducing Bacteria (PRB), Perchlorate, Total Organic Carbon (TOC), and Nitrate in Sediment Samples of the Contaminated Site and Along the Las Vegas Wash

Sample site identification	Soil sample ID	Perchlorate (μg/g of soil)	TOC (μg/g of soil)	NO_3^- (μg/g of soil)	MPN/g sediment
SUS[(1)]	S1	1.27	136.3	80.9	1.6×10^5
SUS[(1)]	S2	0	85.82	22.16	$\geq 1.6 \times 10^5$
SUS[(1)]	S3	0	84.55	16.26	$\geq 1.6 \times 10^5$
SUS[(1)]	S4	0	79.09	3.27	$\geq 1.6 \times 10^5$
SUS[(1)]	S5	0	71.76	24.47	6.7×10^3
SCS[(2)]	S1	6.44	87.69	12.79	<20
SCS[(2)]	S2	405.9	85.44	6.69	<20
SCS[(2)]	S3	138.5	71.28	0	<20
SCS[(2)]	S4	707.2	85.3	0	40
SDSA[(3)]	S1	0	241.5	6.07	1.3×10^3
SDSA[(3)]	S2	0	196.9	0	1.6×10^5
SDSA[(3)]	S3	0	261.4	0	1.1×10^5
SDSA[(3)]	S4	0	147.2	0	3.5×10^4
SDSA[(3)]	S5	0	190.6	0	3.0×10^3
SDSB[(4)]	S1	0	169.4	0	3.3×10^3
SDSB[(4)]	S2	0	166.9	0	2.4×10^4
SDSB[(4)]	S3	0	163.2	0	$\geq 1.6 \times 10^4$
SDSB[(4)]	S4	24.4	217.7	0	3.4×10^2
ST[(5)]	S1	0	91.15	0	4.2×10^4
ST[(5)]	S2	0	109.4	0	5.5×10^4

[(1)]Soil upstream; [(2)]soil contaminated site; [3]soil downstream; [4]soil downstream; [5]soil tributary.

characteristics. The sizes of isolated colonies from Lake Mead waters and LVW sediments, on perchlorate-acetate agar, ranged from pinpoint to large. Most of the colonies were white, shiny, translucent and entire. Noticeable exceptions included tan or yellow colored colonies which were characteristic of isolates W4413C or S51013A from Lake Mead water (W) or Las Vegas Wash sediments (S), respectively. Isolate purity was verified using phase-contrast microscopy (Nikon OPTIPHOT). All strains were motile; however, strains W3413A and W4716A exhibited hypermotility. Each of the isolates was rod-shaped and S51013A grew in chains. Cell size ranged from 1.0 μm × 3.0 μm to 1.5 μm × 7.0 μm. All cells were gram negative and rod shaped. All isolates were catalase and oxidase positive with the exception of W3330A, which was oxidase negative. The results of this study demonstrate that most PRB were characteristically similar (e.g., gram negative, rod-shaped, catalase positive). However, there were exceptions found with cell size, motility and oxidase. The PRB inhabit both LVW sediments and Lake Mead waters. Previously, Zhang et al. (2001) demonstrated that bacteria capable of perchlorate reduction can also be isolated from LVW waters. Together, these two investigations provide additional support that PRB are more commonly characteristically similar, ubiquitous, and present in varying numbers in sediments and waters near Las Vegas.

A phylogenetic analysis was conducted using partial 16S rRNA sequencing of approximately 600 base pairs. The phylogenetic trees constructed for Lake Mead water and LVW isolates can be found in Figures 2 and 3, respectively. 16S rRNA sequencing of certain perchlorate-reducing strains revealed that

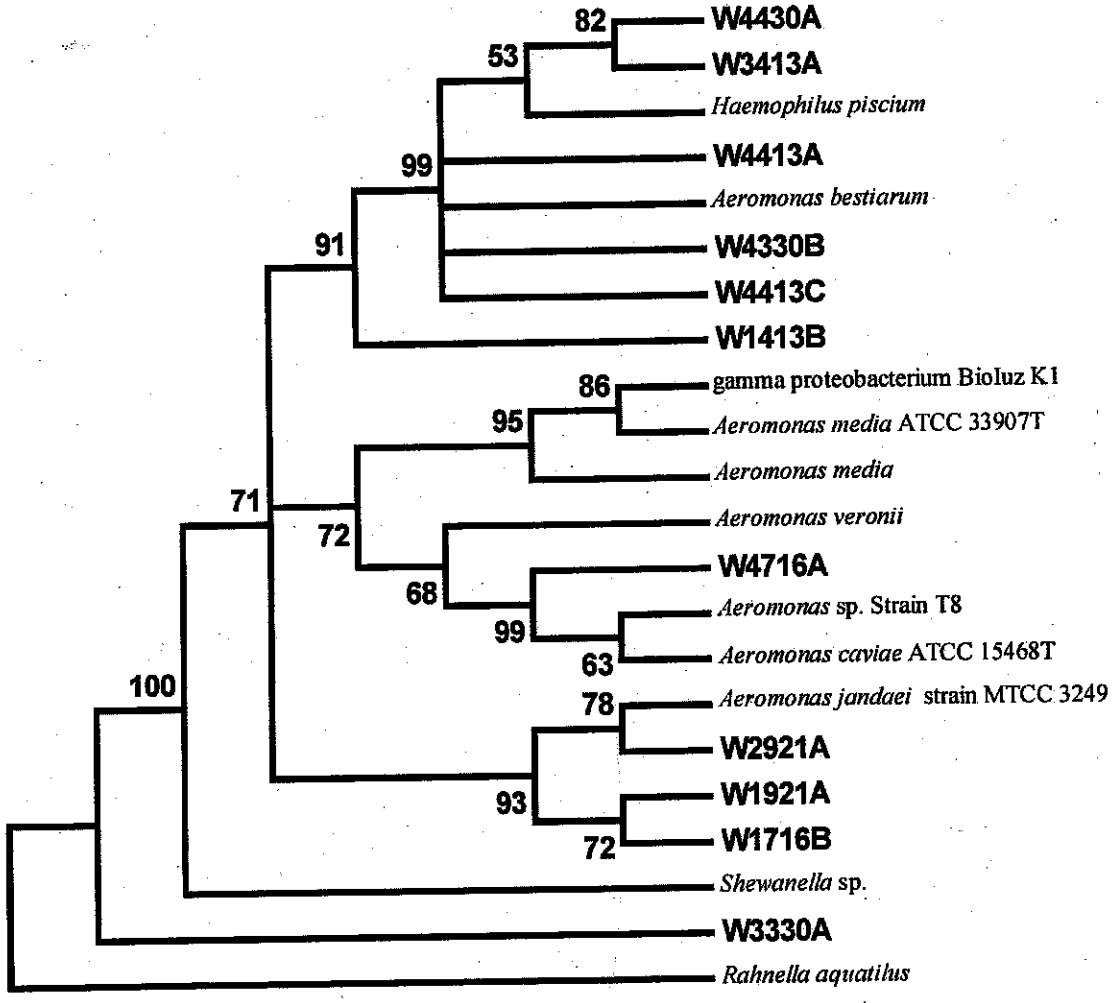

FIGURE 2 Phylogenetic tree (based on neighbor-joining method analysis of 16S rDNA Sequences) of perchlorate-reducing bacteria isolated from Lake Mead Waters and related species.

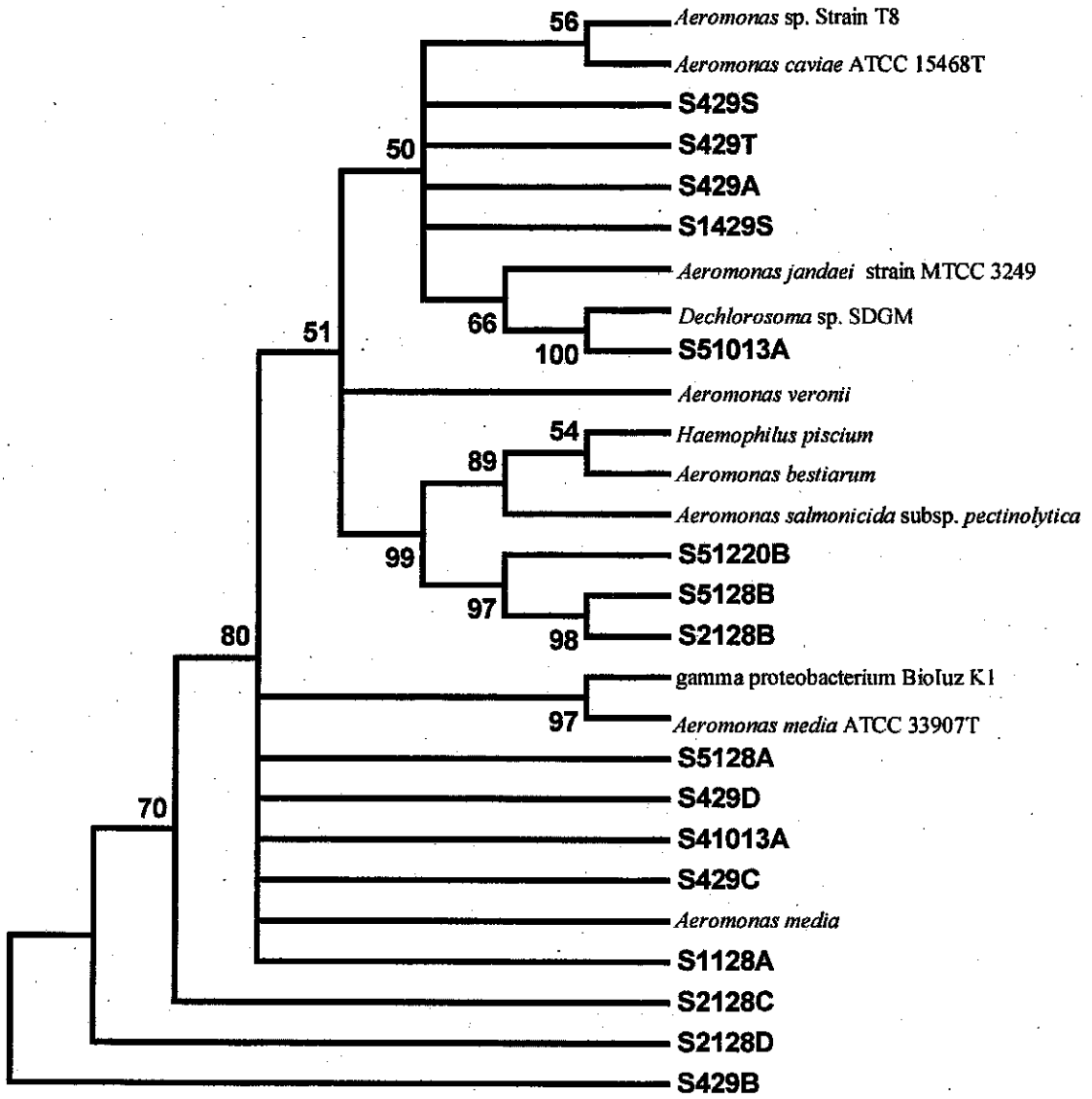

FIGURE 3 **Phylogenetic tree (based on neighbor-joining method analysis of 16S rDNA sequences) of perchlorate-reducing bacteria isolated from Las Vegas Wash sediments and related species.**

several of the isolates were closely related to genera that have not previously been shown to be capable of perchlorate-reduction. Novel genera include, *Aeromonas, Shewanella* and *Rahnella.* Many of the isolates were not closely related to any previously described bacteria but were closely related to members of the *Proteobacteria.* Several perchlorate-reducing isolates have been previously reported to represent new genera in the class *Proteobacteria* (Coates et al., 1999a). One isolate was closely related to the genus *Dechlorosoma,* which was the same genus isolated by Coates et al. (1999a). Two genera, *Dechloromonas* and *Azospira,* are believed to be the principal PRB in the environment (Coates and Achenbach, 2004). Conversely, in the LVW and Lake Mead the dominant PRB were *Aeromonas* sp.

Ninety four different carbon sources were tested using perchlorate as the electron acceptor and determined that the Las Vegas Wash/Lake Mead water isolates demonstrated a broad ability to use various carbon sources. All PRB isolated in this study were capable of utilizing the following electron donors: acetate, fumarate, pyruvate, L-glutamate, L-malic acid, L–alanyl–L–glutamine, β–methyl–D–glucoside, and the combination of L–valine + L–aspartate. All of the isolates were able to utilize glycerol as an electron donor with the exception of strain S51013A. These results differ from those found by Coates et al. (1999a), because

none of the perchlorate-reducing isolates tested in their study was able to utilize glycerol in the presence of chlorate. A previous study also demonstrated that the perchlorate-reducing isolates shared the ability to utilize acetate, fumarate, malate and pyruvate when chlorate was the electron acceptor (Coates et al., 1999a). Moreover, Bruce et al. (1999) also demonstrated that perchlorate-reducing strain, CKB, was able to utilize acetate, fumarate and malate as electron donors in the presence of chlorate. However, none of the isolates in this study were able to utilize L-methionine or itaconic acid when tested on a broad range or carbon sources. Percent carbon utilization revealed findings that all isolates were able to utilize a large percent of the 94 electron donors in the BIOLOG^{TM} microplate; 37 to 54% or 28 to 78% for soil and water isolates, respectively.

Electron Donor and Acceptor Limitations

Perchlorate and dissolved oxygen (DO) values for each water sample are recorded in Table 1. Perchlorate, total organic carbon (TOC), and nitrate (NO_3^-) values for each soil sample are recorded in Table 2. Along the LVW, sediment samples were grouped by site: upstream (SUS), within contaminated area (SCS), downstream of the contaminated area (SDSA and SDSB), and tributary confluence with contaminated area (ST). Notice that PRB numbers within Lake Mead furthest from the Wash discharge are very low (Table 1). In general, Lake Mead is highly oxygenated and perchlorate reduction is unlikely because oxygen is preferred to perchlorate as an electron acceptor. The highest PRB counts in Lake Mead were found in the Las Vegas Bay. This is expected because the Las Vegas Bay water consists of treated wastewater effluent and untreated urban runoff that are rich in nutrients. In this area, some of the lowest oxygen levels were also found (Table 1). Nitrate levels along the Las Vegas Wash were highest upstream from the contaminated and varied from 3.27–80.9 μg/g, within the range found in agricultural sediments ((Laverman et al., 2002). Nitrate concentrations within and downstream of the contaminated site were low. This is due to denitrification that occurs in the sediments along the Wash as nitrate-containing Las Vegas Wash water is transported to Lake Mead.

Extensive investigation of historic perchlorate concentrations in the Las Vegas Bay has been performed (Boralessa, 2001) based on 900 frozen water samples taken in Lake Mead from 1990 to 2000. As expected, in Lake Mead, the highest perchlorate concentrations are found within the Las Vegas Bay, the discharge point of the LVW. Perchlorate concentrations are higher in the hypolimnion than in the epilimnion layers owing to the higher total dissolved solids (TDS) concentration of the Wash water compared to Lake Mead's (Holdren et al., 1998), which causes the perchlorate plume of the Wash to sink. In the inner Las Vegas Bay, extending about 3 km from the discharge of the LVW, perchlorate concentrations varied from 70–80 μg/L and from 140–160 μg/L in the epilimnion and hypolimnion, respectively. About 5 km from the discharge point, perchlorate concentrations dropped to 40 μg/L and 95 μg/L in the epilimnion and hypolimnion, respectively. At Hoover Dam, 22 km from the discharge point, perchlorate concentrations are below 12 μg/L. No perchlorate is found in several points of the Lake due to dilution or lack of hydraulic connection to the LVW. The persistence of perchlorate in Lake Mead and the Colorado River may be explained by the competition of oxygen with perchlorate as an electron acceptor and by the low dissolved organic carbon (DOC). The DOC levels in Lake Mead average 4.56 mg/L (Wei, 2004).

TOC values for the sediments along the Wash were very low and varied from 70–250 μg/g of soil (0.007–0.025%) primarily due to the lack of vegetation in the arid sediments of the LVW area. Unlike in arid environments, TOC of forested and agricultural areas can vary from 0.05% to 10% (Laverman et al., 2002). In summary, along the Wash, perchlorate is limited by electron acceptor and the presence of nitrate. Within the contaminated site, despite the high concentrations of perchlorate in the sediments, the number of PRB found is very low. This low concentration of PRB within the contaminated site has been associated with the high salinity (i.e conductivity > 300 mS/m) of the sediments in the area (Batista et al., 2005).

DISCUSSION

In this study, the limitations to natural perchlorate biodegradation in sediments along the Las Vegas Wash and Lake Mead waters in Henderson, Nevada were investigated. This site is critical to perchlorate cleanup efforts in the United States because it is the source of perchlorate to the Colorado River, the source of water for 30 million people in Nevada, Arizona, and California. For natural bioremediation to occur at this location using perchlorate as an electron acceptor, the

necessary components are the presence of PRB an electron donor and anaerobic conditions.

The findings that high numbers of PRB reside along the Las Vegas Wash suggests that bioaugmentation is not necessary. This is mainly because PRB were enumerated in various concentrations and were able to survive, grow and metabolize in this environment by utilizing an alternative electron acceptor for anaerobic respiration. This is supported by Wu et al. (2001), who suggested that intrinsic remediation of perchlorate is possible if there are naturally existing perchlorate-respiring bacteria that can compete with other soil microorganisms for organic matter. This idea is also supported by Baker and Herson (1994), who concluded that bioaugmentation of perchlorate contaminated sediments might not be necessary because the microbial community becomes enriched with bacteria capable of reducing this contaminant.

Our findings reveal that the Las Vegas Bay has the lowest (dissolved oxygen) DO levels throughout the samples collected at Lake Mead. Samples collected downstream from the Las Vegas Bay had increasingly higher DO levels and decreasing numbers of PRB. Environmental effects of oxygen exposure on perchlorate respiration have been investigated and early findings by Attaway and Smith (1993) reported that perchlorate-reduction was inhibited by the presence of oxygen. The adverse effects of oxygen on perchlorate-respiring cultures were further supported by Chaudhuri et al. (2002), revealing that PRB were unable to recover and conduct perchlorate-respiration after exposure to oxygen. In an anaerobic bioreactor, the presence of oxygen has been shown to interfere with perchlorate-reduction (Coates et al., 1999b; Song and Logan, 2004). However, environmentally, the presence of oxygen introduced into sediments or sediments during perchlorate respiration might not be a significant problem because it could be rapidly utilized by aerobic organisms in the community. This idea is supported by Rikken et al. (1996) who proposed that perchlorate-reducing microorganisms can utilize chloro-oxo acids and oxygen simultaneously. These findings suggest that high DO levels may be a limiting factor for remediation of Lake Mead waters.

The contaminated LVW sediments and Lake Mead are the two sites of concern. The concentration of perchlorate in the sediments vary widely with location, making it nearly impossible to remediate. Moreover, bioremediation of Lake Mead waters is unfeasible due to high DO levels and the large volume of water. At the discharge point of the LVW into the Bay, a significant amount of fine sediments has been deposited. In this area, an anoxic/anaerobic environment has been established. This confluence of the LVW with Lake Mead constitutes a potential site for perchlorate bioremediation. Nitrate levels in the LVW average 12 mg/L NO_3–N and in the Las Vegas Bay they vary from 0.5–0.9 mg/L (Piechota and Batista, 2003). The lower nitrate levels in the Bay are the result of both dilution and denitrification in the fine sediments. Oxygen levels are low in this area and TOC concentrations vary from 3.2 to 7.0 mg/L. For biological reduction of perchlorate to occur, the nitrate has to be removed first because it is a preferred electron acceptor. Sufficient electron donor has to be added to provide for both nitrate and perchlorate reduction. Considering an average perchlorate concentration in the Las Vegas Bay of 150 μg/L, an average nitrate–N level of 0.7 mg/L, and acetate as the electron donor, the stoichiometric amount of acetate required would be 9.6 mg acetate/L. This estimate considers molar stoichiometric ratios of 2:44:1 and 3.3:1 for acetate/perchlorate and acetate/nitrate, respectively (Rittmann and McCarty, 2001). The findings of this study suggest that low TOC levels found in the Las Vegas Bay are not sufficient to support both perchlorate and nitrate reduction. According to Tipton et al., (2003) when compared to natural sediments, biostimulation of carbon-limited sediments increased the rate of perchlorate reduction when acetate or glucose was added as a carbon source. Therefore, biological reduction of perchlorate in the Las Vegas Bay is limited by the lack of sufficient electron donor and the presence of nitrate and oxygen, both of which are preferably used to perchlorate. In the areas of the Las Vegas Bay where oxygen levels are low, such as within the fine sediments in the confluence of the LVW and Lake Mead, perchlorate reduction could be accomplished if an electron donor is provided that will sustain both nitrate and perchlorate reduction.

In summary, the findings reported in this study demonstrate that metabolically diverse PRB are present in the contaminated site in various concentrations. In addition, the results provide strong support that the persistence of perchlorate in the contaminated site and in the Colorado River is fostered by the presence of other preferred electron acceptors (i.e oxygen and nitrate) and the lack of sufficient electron donor to support the reduction of both perchlorate and nitrate. Most importantly, this study identifies the area occupied by fine

sediments in the Las Vegas Bay, the confluence of the Las Vegas Wash and Lake Mead, as a potential location where perchlorate biodegradation could take place if an electron donor were provided. Bioaugmentation in this location has the potential to halt the transport of perchlorate to Lake Mead and the Colorado River.

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