J Ind Microbiol Biotechnol (2008) 35:1571–1579
DOI 10.1007/s10295-008-0399-5

ORIGINAL PAPER

Bioremoval of hexavalent chromium from water by a salt tolerant bacterium, *Exiguobacterium* sp. GS1

Benedict C. Okeke

Received: 6 November 2007 / Accepted: 7 July 2008 / Published online: 29 July 2008

Abstract Pollution of terrestrial surfaces and aquatic systems by hexavalent chromium, Cr(VI), is a worldwide public health problem. A chromium resistant bacterial isolate identified as *Exiguobacterium* sp. GS1 by 16S rRNA gene sequencing displayed high rate of removal of Cr(VI) from water. *Exiguobacterium* sp. GS1 is 99% identical to *Exiguobacterium acetylicum*. The isolate significantly removed Cr(VI) at both high and low concentrations (1–200 μg mL^{-1}) within 12 h. The Michaelis–Menten K_m and V_{max} for Cr(VI) bioremoval were calculated to be 141.92 μg mL^{-1} and 13.22 μg mL^{-1} h^{-1}, respectively. Growth of *Exiguobacterium* sp. GS1 was indifferent at 1–75 μg mL^{-1} Cr(VI) in 12 h. At initial concentration of 8,000 μg L^{-1}, *Exiguobacterium* sp. GS1 displayed rapid bioremoval of Cr(VI) with over 50% bioremoval in 3 h and 91% bioremoval in 8 h. Kinetic analysis of Cr(VI) bioremoval rate revealed zero-order in 8 h. *Exiguobacterium* sp. GS1 grew and significantly reduced Cr(VI) in cultures containing 1–9% salt indicating high salt tolerance. Similarly the isolate substantially reduced Cr(VI) over a wide range of temperature (18–45 °C) and initial pH (6.0–9.0). The T_{opt} and initial pH_{opt} were 35–40 °C and 7–8, respectively. *Exiguobacterium* sp. GS1 displayed a great potential for bioremediation of Cr(VI) in diverse complex environments.

Keywords Hexavalent chromium · Water and soil pollution · Bioremediation · *Exiguobacterium* sp. salt tolerance

B. C. Okeke (✉)
Department of Biology, Auburn University Montgomery,
P.O. Box 244023, Montgomery, AL 36124, USA
e-mail: bokeke@aum.edu

Introduction

Hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)) are the most prevalent species of chromium in the natural environment [11]. Cr(III) is relatively insoluble in water and exhibits little or no toxicity. In mammals Cr(III) promotes effective glucose, protein, and lipid metabolism [1]. The hexavalent form (Cr(VI)) is, however, highly soluble and mobile in water and displays toxic, mutagenic, and carcinogenic effects to living systems, including microorganisms, at low concentrations [23]. Cr(VI) is an irritant at relatively high concentrations [11]. It has also been linked to morphological changes and growth reduction in plants [21]. Major sources of Cr(VI) pollution include effluents from leather tanning, chromium electroplating, wood preservation, alloy preparation and nuclear wastes due to its use as a corrosion inhibitor in nuclear power plants [40]. Although some living organisms require Cr as an essential element, its toxic, mutagenic, and carcinogenic nature render it hazardous [10, 20]. Health problems associated with Cr pollution of terrestrial surfaces and aquatic systems is of increasing world-wide concern. Discharge of Cr(VI) into surface waters is regulated by both the European Union and US EPA to below 50 μg L^{-1} [16]. The United States Environmental Protection Authority (US EPA) set the maximum contaminant level (MCL) for total chromium including Cr(VI) and Cr(III) at 100 μg L^{-1} of water [11].

Widespread anthropogenic contamination of water and soils by hexavalent chromium Cr(VI) has spurred the development of physicochemical and bioremediation treatment technologies for Cr(VI) removal or detoxification. Physicochemical treatment technologies include ion exchange adsorption, electrodialyses, precipitation, and chemical reduction [6, 23]. The drawback of these conventional methods include high energy expenditure in the

process, use of expensive and toxic chemical reductants [22] as well as inefficient removal of low concentrations of Cr(VI) in wastewater [24, 39]. Biological methods (bioremediation) for Cr(VI) removal involve the use of microorganisms (microbial bioremediation) and plants (phytoremediation). Bioremediation is a more attractive option in that the technology is relatively cheap and environmentally compatible [37]. Mechanisms of Cr bioremediation are by microbial metabolism [7, 8] including dissimilatory removal and detoxification as well as biosorption [20]. Indirect reaction with metabolites such as H_2S is a possible mechanism [27, 29]. Microbially mediated bioremoval of chromium from high valence to low valence states have been reported in some microorganisms including *Arthrobacter crystallopoietes* [7], *Bacillus* spp. [5, 9], *Providencia* sp. [40], *Enterobacter* spp. [12], *Streptomyces* spp. [15], *Micrococcus roseus* and *Escherichia coli* [19], *D. desulfuricans and D. vulgaris* [32], *Shewanella alga* [18] and other bacterial isolates [4, 6, 10, 30, 31]. Biomass related removal of Cr(VI) has also been reported in *Chlamydomonas* [1] and in fungi [3]. However, there is paucity of information on microorganisms that can remove Cr from more diverse environments especially in saline aquatic systems and in both high and low temperature conditions encountered in natural environments. In this study six environmental isolates of Cr(VI) resistant bacteria were examined for rapid removal of Cr(VI) in aqueous media. One isolate identified by 16S ribosomal RNA gene sequencing as *Exiguobacterium* species GS1 with high capacity for Cr(VI) bioremoval was further characterized.

Materials and methods

Chemicals

Potassium dichromate (99%) was purchased from Acros Organics (Fair Lawn, NJ, USA), sym-diphenylcarbazide (97%) was procured from Pfaltz and Bauer, Inc (Waterbury, CT, USA), oligonucleotides were synthesized by Sigma-Genosys (The Woodlands, TX, USA), ethanol (95%), sodium chloride (100%), sodium hydroxide (98.4%) were purchased from Fisher Scientific (Rochester, NY, USA) and hydrochloric acid (36.5%) was from EM Science (Gibbstown, NJ, USA).

Screening of Cr(VI) resistant microorganisms for Cr(VI) removal

Six chromium resistant bacterial isolates were selected from enrichment cultures of Cr(VI) resistant bacteria [38]. Tryptic soy broth (TSB) was contaminated with 500 μg mL^{-1} Cr(VI) from $K_2Cr_2O_7$ stock (35.35 mg/mL; 1 mL = 12.5 mg Cr). The Cr(VI) stock was prepared in distilled water and filter sterilized (0.22 μm) before predetermined aliquots were added to cultures and controls to the desired concentration of Cr(VI). The inoculum was prepared by inoculating TSB containing 0.5 μg mL^{-1} Cr(VI) in Fisher brand 16 mm × 125 mm borosilicate glass culture tubes with three loops of each of the six bacteria from 24 h old colonies grown on typtic soy agar (TSA). Inocula were incubated at 30 °C for 12 h. Inocula cells were recovered by centrifugation and resuspended in 5 mL TSB. The initial OD_{600} was determined (0.98, 0.48, 0.50, 0.94, 0.47, and 0.61 for GS1, GS2, GS3, PB1, PB2 and PB3, respectively). Seven milliliters of TSB in Fisher brand 16 × 125 mm borosilicate glass culture tubes equipped with polypropylene caps, were contaminated with Cr(VI) at 200 μg mL^{-1}. The inoculum suspension were normalized to an OD_{600} of approximately 0.5 with sterile TSB and 200 μl was used to inoculate the Cr(VI) contaminated medium. Cultures were incubated aerobically in an orbital incubator (Lab-Line instruments, Melrose Park, IL.) at 30 °C and 120 rpm for 6 h.

Cr(VI) analyses and biomass determination

Hexavalent chromium was determined by the diphenylcarbazide method [2] using UV-9100 spectrophotometer (Bestech, Irvine, CA) at λ_{540}. A Cr(VI) standard curve was prepared in the range 25–800 μg mL^{-1}. To achieve concentrations within the linear range of the standard, dilutions of culture supernatants were subjected to Cr(VI) analysis and remaining Cr(VI) concentrations were calculated from the Cr(VI) standard curve. Biomass was determined by measuring absorbance at λ_{600} against sterile TSB blank without Cr(VI).

Molecular characterization of isolate GS1

DNA extraction

Isolate GS1 was identified by 16S ribosomal RNA gene sequencing as follows. The isolate was cultivated by streaking on tryptic soy agar with incubation at 30 °C for 24 h for evaluation of culture purity. Discrete bacterial colonies were then suspended in nuclease-free water (Promega, Madison, WI). Cells were recovered by centrifugation. DNA was extracted from the cells using Promega wizard genomic DNA purification kit (Promega, Madison, WI) with slight modification. Briefly, cells were re-suspended in 600 μl of nucleic acid lysis solution, incubated at 80 °C for 5 min and allowed to cool to room temperature. RNase solution (3 μl) was added and incubated at 37 °C for 20 min. Protein precipitation solution (200 μl) was added and the tubes incubated on ice for 5 min. Following centrifugation, the supernatant was transferred to a tube and ice cold 95% ethanol

was added. The precipitate was recovered by centrifugation. The pellet was washed with 70% ethanol at ambient temperature and resuspended in rehydration solution.

Polymerase chain reaction amplification (PCR) of 16S rRNA gene and DNA sequencing

Bacterial universal primers corresponding to *E. coli* positions 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) were used for PCR amplification of the 16S ribosomal DNA [25]. The PCR reaction mixture consisted of 25 µl of PCR master mix (Promega, Madison, WI), genomic DNA template (2 µl), primer 27F (5 µl = 25 pmol), primer 1492r (5 µl = 25 pmol) and made up to 50 µl final volume with nuclease-free water. The 16S rRNA gene was amplified using a 35-cycle PCR (initial denaturation, 95 °C for 5 min; subsequent denaturation, 95 °C for 0.5 min; annealing temperature, 50 °C for 1 min; extension temperature, 72 °C for 1 min and final extension, 72 °C for 5 min). The PCR amplification products were analyzed by electrophoresis on a 1% agarose gel. Millipore Montage PCR filter units (Millipore, Billerica, MA) were used to remove primers, salts, and unincorporated dNTPs according to the manufacturer's instructions except that an additional 400 µl of sterile nuclease free water was added to wash off residual PCR ingredients. DNA cycle sequencing was performed using BigDye terminator kit (Applied Biosystems, Foster City, CA) with sequencing primers 1492r and 519r (5′-GWATTACCGCGGCKGCTG-3′) in independent reactions (UCR Genomics Institute, Riverside, CA).

DNA sequence similarity and phylogenetic analysis

Genbank BLAST (N) was used for homology searches. Evolutionary position amongst related organisms was analyzed by *MyRDP* Release 9.50 [34]. The ribosomal RNA gene sequence was submitted to Genbank under accession number EF 608145.

Morphology and oxygen requirement

Cells were cultivated on tryptic soy agar overnight (18 h) and then microscopically examined (1,000×) after Gram staining. To evaluate oxygen requirement for growth, cells were streaked on tryptic soy agar and incubated under strict anaerobic condition at 30 °C for 3 days using a BBL GasPak anaerobic system (Becton-Dickinson, Cockeysville, MD).

Effect of chromium concentration

Effect of chromium concentration was determined using the enrichment medium contaminated with 1, 25, 50, 75, 100, 125, 150, 175, and 200 µg mL^{-1} of Cr(VI) using aliquots of filter-sterilized (0.22 µm) potassium dichromate stock solution. The sterile Cr(VI) media were inoculated with 100 µl aliquots of *Exiguobacterium* sp GS1 (OD_{600} = 0.91) and incubated aerobically at 30 °C for 12 h with orbital shaking (120 rpm). Inoculum preparation, biomass determination and chromium analysis were as described under screening of Cr(VI) resistant microorganisms.

Time course of hexavalent chromium bioremoval

Sterile enrichment medium was aseptically contaminated with Cr(VI) to a final concentration of 8,000 µg L^{-1} using the stock chromium solution. The Cr(VI) contaminated sterile medium was then inoculated with 500 µL of *Exiguobacterium* sp. cell suspension (OD_{600} = 0.94) and incubated aerobically with orbital shaking (120 rpm) to keep cells suspended. Cultures were terminated at different time intervals (t_0, t_1 t_2, t_3, t_4, t_5, t_6, t_7, t_8, and t_{12}; subscripts are hours). Remaining Cr(VI), biomass and final pH were determined. The relationship between optical density (OD) readings and cell dry weight was estimated by measuring OD_{600} of dilutions of cultures and drying at 60 °C for 24 h.

Determination of kinetic parameters

Data obtained from the effect of substrate concentration on Cr(VI) bioremoval was used to calculate the half saturation constant (K_m) and the maximum velocity (V_{max}) using hyperbola regression equation, $y = ax/(b + x)$, equivalent of Michaelis–Menten equation, $V = V_{max}[S]/(K_m + [S])$ [33]. SigmaPlot 10 software (Systat, San Jose, CA) was employed. Time course data of Cr(VI) bioremoval fitted a zero-order model and the kinetic constant (k) was calculated from linear regression curve.

Evaluation of Exiguobacterium sp heat killed biomass for Cr(VI) removal

Cr(VI) bioremoval culture medium was inoculated with an overnight culture of *Exiguobacterium* sp GS1 as described under time course experiment. Cultures were incubated with orbital shaking at 30 °C for 8 h and sterilized at 121 °C for 20 min. Medium pH was then aseptically adjusted to 7.3 using predetermined amounts of 1 M NaOH. The media containing dead biomass and controls (without biomass) were spiked with Cr(VI) to a final concentration of 8,000 µg L^{-1}, vortex mixed to fully resuspend dead cells and incubated as described under time course experiment.

Effect of culture parameters

The effects of temperature, pH, and salinity on bacterial growth and Cr(VI) bioremoval by *Exiguobacterium* sp. were examined using the enrichment medium artificially contaminated with 40 μg mL^{-1} Cr(VI) using aliquots of filter-sterilized (0.22 μm) potassium dichromate stock solution. For evaluation of the influence of temperature, cultures were incubated at 18–45 °C. In the experiment in which the effects of pH were examined, sterilized enrichment medium was adjusted to pH 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0 by addition of pre-determined amounts of filter-sterilized (0.22 μm) 1 M NaOH or 1 M HCl and incubated at 30 °C. The effect of salt concentration was examined using enrichment medium made with varying concentrations of NaCl (1, 3, 5, 7, and 9%). Preparation of inoculum, media inoculation, and biomass determination were as described under screening of Cr(VI) resistant microorganisms for Cr(VI) removal except that the initial OD of the inoculum suspension was 0.91 and 100 μL of inoculum was used. Cultures were incubated aerobically with orbital shaking (120 rpm) at 30 °C for 12 h. Cr(VI) was determined by the diphenylcarbazide method.

Statistical validation of treatment effects

The means and standard deviations ($N - 1$) of independent replicate treatments were calculated. Significance of factor effect ($P < 0.05$) and non-significance ($P > 0.05$) were assessed as necessary by ManWhitney non-parametric test using InStat 3 statistical software (GraphPad, San Diego, CA, USA).

Results

Cr(VI) removal from water by isolates

Table 1 presents an evaluation of Cr(VI) removal from water by six Cr(VI) resistant bacteria. Cr(VI) removal was highest in cultures of isolate GS1 (57.06%). Growth of most of the isolates was significantly inhibited by 200 μg mL^{-1} Cr(VI). Isolate GS1 was the most resistant to Cr(VI).

Identity of isolate GS1

Chromium resistant isolate GS1 is a Gram positive rod with rounded ends. Colonies are yellow to orange on TSA plates. GS1 grows both aerobically and anaerobically. Growth under aerobic condition is more rapid than under strict anerobic condition. Approximately 1.45 kb of the 16S rDNA of isolate GS1 was sequenced from a band amplified by polymerase chain reaction (Fig. 1). Blast analysis revealed significant similarity to *Exiguobacterium* spp., 99% similarity to *E. acetylicum* and *E. antarcticum*, and 98% similarity to *E. oxidotolerans* and *E. aestuari*. Isolate GS1 was therefore identified as *Exiguobacterium* sp. GS1. Figure 2 presents a phylogenetic tree showing the evolutionary position of *Exiguobacterium* sp. GS1 amongst related organisms based on 16S rRNA gene sequence.

Effect of Cr(VI) concentration on GS1

Concentrations of Cr(VI) in the medium in the range 1–75 μg mL^{-1} generally showed no substantial inhibition of growth. Significant growth decreases of 11.26, 14.94, 17.47, 19.31, and 30.34% were, however, respectively, observed at 100, 125, 150, 175, and 200 μg mL^{-1}. Isolate GS1 significantly removed Cr(VI) at both high and low concentrations (1–200 μg mL^{-1}) in 12 h. In cultures spiked with 1 μg mL^{-1} no Cr(VI) was detected in 12 h. The K_m and V_{max} for Cr(VI) bioremoval were calculated to be 141.92 and 13.22 μg mL^{-1} h^{-1}, respectively, by non-linear regression analysis (Fig. 3).

Dynamics of Cr(VI) bioremoval in water

The dynamics of Cr(VI) removal from water by *Exiguobacterium* GS1 is presented in Fig. 4a. Sterile control media contaminated with Cr(VI) remained relatively stable during the 8 h incubation. Cr(VI) contaminated media inoculated with *Exiguobacterium* sp. GS1 displayed rapid bioremoval of Cr(VI) with over 50% bioremoval in 3 h and 91% bioremoval was recorded in 8 h. Depletion of Cr(VI) from an initial concentration of 8,000 μg L^{-1} to below accurate detection by the analytical method employed (<25 μg L^{-1})

Table 1 Bioremoval of hexavalent chromium from water by Cr(VI) resistant isolates. Initial chromium concentration was 200 μg mL^{-1}. Cultures were incubated for 6 h at 30 °C

Isolate	Source	Biomass (λ_{600})	% Growth inhibition	Remaining Cr(VI) (μg mL^{-1})	% Cr(VI) removal
GS1	Grass land soil	0.887 ± 0.02	15.52	85.89 ± 7.96	57.06
GS2	Grass land soil	0.267 ± 0.05	40.66	153.47 ± 9.95	23.26
GS3	Grass land soil	0.187 ± 0.06	52.05	155.58 ± 16.92	22.21
PB1	Plant bed soil	0.177 ± 0.04	59.12	214.02 ± 13.93	0
PB2	Plant bed soil	0.573 ± 0.03	43.26	93.63 ± 12.94	53.18
PB3	Plant bed soil	0.236 ± 0.03	45.50	203.46 ± 8.96	0

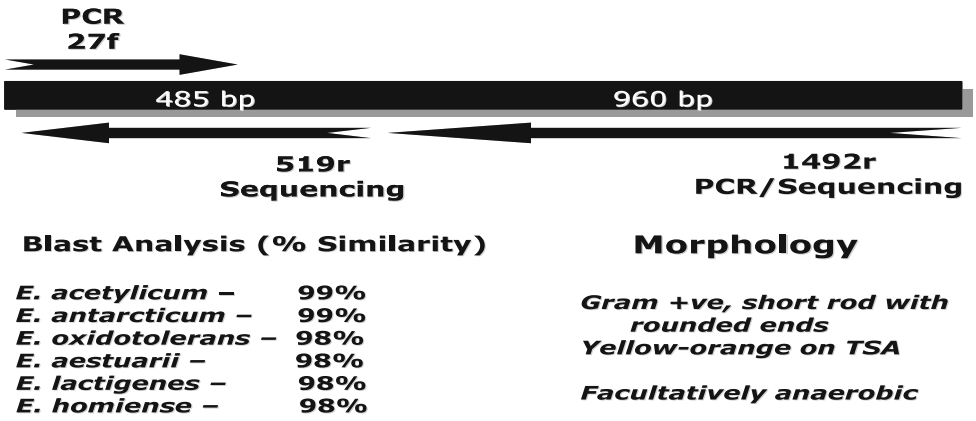

Fig. 1 Schematic representation of the identification of *Exiguobacterium* sp. GS1 by PCR amplification of 16S rRNA gene. Morphological characteristics and oxygen requirements are given

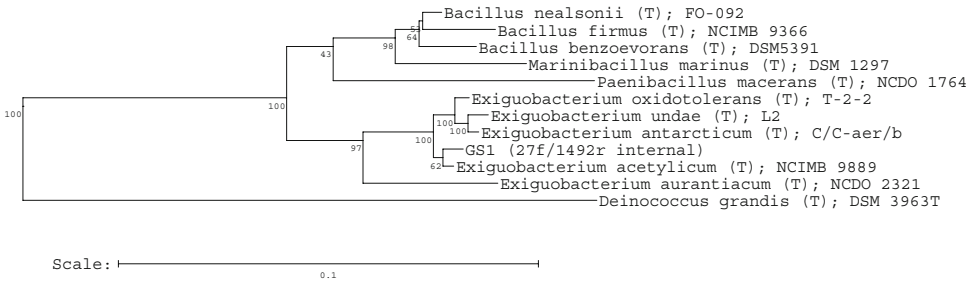

Fig. 2 Evolutionary position of *Exiguobacterium* sp. GS1 amongst related organisms based on 16S rRNA gene sequence (1,445 nucleotides). The scale represents the evolutionary distance value. Number at each node is the bootstrap out of 100 analyses

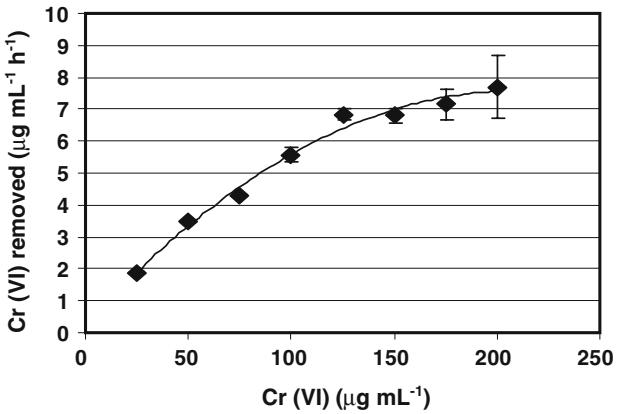

Fig. 3 Effect of Cr(VI) concentration on rate of Cr(VI) bioremoval by *Exiguobacterium* GS1. Data was subjected to hyperbola non-linear regression analysis to calculate K_m and V_{max}

was observed after 12 h. Biomass measurement revealed a typical bacterial growth pattern. Bacterial biomass development increased rapidly and logarithmic growth was maintained for 4 h (Fig. 4a). Thereafter bacterial biomass increased slightly maintaining a pseudo stationary phase for the rest of the incubation period. Kinetic analysis of Cr(VI) bioremoval from water at 8,000 μg L^{-1} revealed a zero-order model in 8 h (Fig. 4b) with k and r^2 values of 0.71 μg mL^{-1} h^{-1} and 0.99, respectively. The relationship between culture biomass OD_{600} readings and dry weight can be predicted from the regression curve ($y = 4.33x + 23.73$; where y and x are dry weight and biomass readings, respectively).

Effect of heat killed biomass on Cr(VI) bioremoval

During the 8 h incubation of *Exiguobacterium* dead biomass in Cr(VI) medium, Cr(VI) concentration remained relatively stable. Abiotic loss of Cr(VI) was approximately 6.6% after 8 h. Biomass levels similarly remained stable. Initial biomass (OD_{600}) reading was 1.18 and 1.16 was recorded after 8 h incubation at 30 °C, indicating no cell growth.

Effect of temperature and media pH

Cr(VI) bioremoval and growth profiles at different temperatures are illustrated in Fig. 5a and b, respectively. *Exiguobacterium* sp. GS1 substantially reduced Cr(VI) over a wide range of incubation temperature (18–45 °C). Bioremoval of Cr(VI) was highest at 35–40 °C and slightly decreased at 45 °C. Bacterial biomass development was maximal at 30–35 °C and significantly decreased at 45 °C. Cr(VI) bioremoval by *Exiguobacterium* at different initial pH levels is presented in Table 2. Cr(VI) removal was similar from pH 6 to 8 and only declined significantly at pH 9. Growth of GS1 apparently increased with increasing initial culture pH (Fig. 6). However, initial culture pH generally decreased during the 12 h incubation.

Effect of salt concentration

The influence of salinity on Cr(VI) bioremoval and bacterial biomass development are illustrated in Fig. 7a and b, respectively. *Exiguobacterium* sp. GS1 significantly

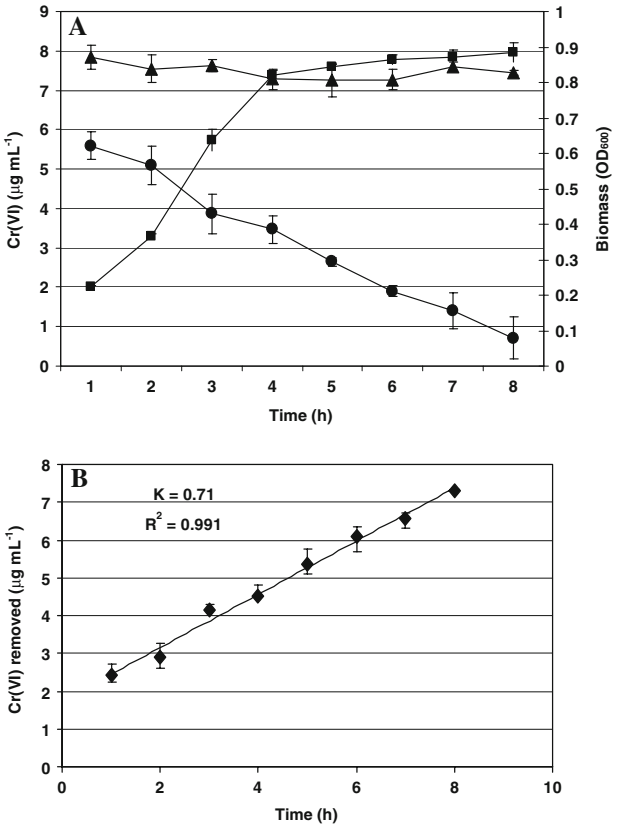

Fig. 4 **a** The dynamics of Cr(VI) bioremoval in water over 8 h incubation at 30 °C. Remaining Cr(VI) in culture (*filled circle*), remaining Cr(VI) in control (uninoculated) (*filled triangle*) and biomass (*filled square*). Initial chromium concentration was 8 μg mL^{-1}. **b** Kinetic analysis of the rate of Cr(VI) removal. $K_{0\text{-order}}$ = d*y*/d*x* which was calculated from the regression curve $y = 0.705x + 1.7417$

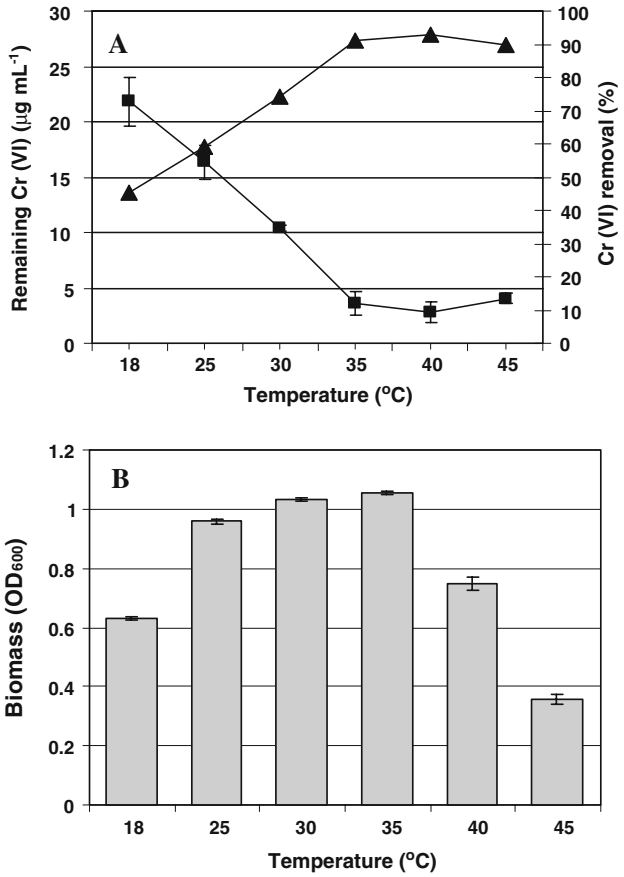

Fig. 5 Effect of temperature on Cr(VI) bioremoval (**a**), with growth (**b**) of *Exiguobacterium* sp. GS1. Remaining Cr(VI) (*filled square*) and % removal (*filled triangle*). Initial chromium concentration was 40 μg mL^{-1}

removed Cr(VI) in cultures containing 1–9% salt (NaCl) indicating salt tolerance. Generally Cr(VI) bioremoval and growth were inversely related to the concentration of sodium chloride. Approximately 76.5 and 63.1% removal were, respectively, observed at NaCl concentrations of 5 and 9% in cultures of *Exiguobacterium* sp. GS1 incubated for 12 h. Substantial biomass development was noted at all concentrations of NaCl. Optical density readings (OD_{600}), however, declined to 0.284 at 9% NaCl.

Table 2 Effect of pH on Cr(VI) bioremoval by *Exiguobacterium* sp. GS1. Initial chromium concentration was 40 μg mL^{-1}

	Remaining Cr(VI) (μg mL^{-1})	Δ Cr(VI) (μg mL^{-1})	Cr(VI) removal (%)	Final pH
6.0	8.82 ± 1.18	31.18	77.95	5.85 ± 0.07
6.5	8.51 ± 1.44	31.49	78.73	5.74 ± 0.12
7.0	8.49 ± 0.08	31.51	78.78	5.76 ± 0.03
7.5	7.85 ± 1.38	32.15	80.38	5.80 ± 0.01
8.0	8.36 ± 0.21	31.63	79.09	5.91 ± 0.02
9.0	12.99 ± 1.24	27.01	67.53	6.45 ± 0.01

Discussion

Bacterial removal of hexavalent chromium is an attractive bioremediation strategy for Cr(VI). In this study, *Exiguobacterium* sp. GS1 displayed rapid bioremoval of hexavalent chromium from water. *Exiguobacterium* species are cryotolerant bacteria [17, 41–43] frequently isolated from ancient permafrost sediments and more recently from diverse environments [43] but have not been reported to reduce hexavalent chromium.

Growth of *Exigubacterium* sp. GS1 was indifferent to 1–75 μg mL^{-1} Cr(VI) and only 25% decrease in biomass concentration was recorded at 200 μg mL^{-1} in 12 h. *Streptomyces griseus* removed hexavalent chromium but growth was significantly inhibited above 25 μg mL^{-1} [26]. Losi et al. [29] reported Cr(VI) concentration of 12 μg mL^{-1} to be inhibitory to soil bacteria in liquid cultures. Chromium toxicity to microorganisms has been attributed to alteration

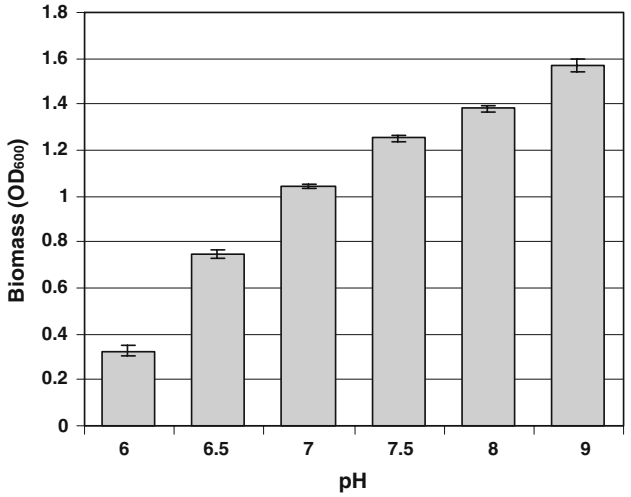

Fig. 6 Biomass development during Cr(VI) bioremoval in cultures of *Exiguobacterium* sp. GS1 at different initial pH

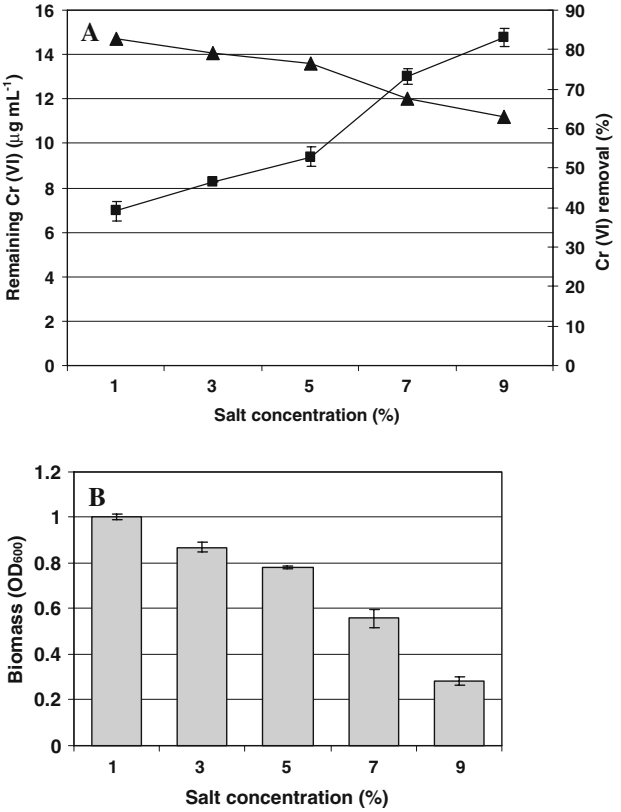

Fig. 7 Effect of salt concentration on Cr(VI) bioremoval (**a**), and growth (**b**). Remaining Cr(VI) (*filled square*) and % removal (*filled triangle*). Initial chromium concentration was 40 µg mL^{-1}

of nucleic acid structure as well as physiological and metabolic processes [30].

Kinetic analysis of the effects of chromium concentration revealed a K_m and V_{max} of 141.92 and 13.32 µg mL^{-1} h^{-1}, respectively. In a study [35] of bacterial removal of hexavalent Cr in water, higher K_m values of 13–1,730 mM were observed. Camargo et al. [6] reported on five Cr(VI) reducing bacterial isolates which displayed K_m values of 0.271 mg L^{-1}(2.61 µM) to 1.51 mg L^{-1} (14.50 µM) and a V_{max} of 88.4 µg L^{-1} h^{-1} (14.17 nmol min^{-1}) to 489 µg L^{-1} h^{-1} (78.36 nmol min^{-1}). The velocity of Cr(VI) bioremoval from water by *Exiguobacterium* sp. GS1 is superior to that of a potent Cr(VI) remover, *Bacillus* sp. ES29, which displayed a V_{max} of 0.489 µg L^{-1} h^{-1} [6].

The dynamics of Cr(VI) bioremoval from water by GS1 revealed rapid depletion of Cr(VI) with slight lag period of biomass development and Cr(VI) bioremoval. Cheung and Gu [10] attributed the lag period to acclimatization to Cr(VI). The negligible amount of Cr(VI) depletion observed in the non-inoculated control or with heat killed cells of GS1 indicated no substantial abiotic destruction of chromate and further suggests biological removal. The kinetic model that fit GS1 data, the abiotic studies, effect of salt, temperature and pH profiles, and effect of Cr(VI) concentration are all indications that the major mechanism of Cr(VI) removal is attributable to microbial metabolism.

Cr(VI) removal over a wide range of culture conditions was observed with GS1. As far as could be established there is no report on bacterial removal of Cr(VI) at low temperature. Other studies reported drastic reduction of Cr(VI) removal at high temperature [6, 26]. *Acidiphilium cryptum* strain JF-5 [14], only removed Cr(VI) from pH 1.7–4.7 and optimally at pH 3.2. Hexavalent chromium is a common contaminant of waste water [10] which contains high solute. Ion exchange treatment processes for removal hexavalent chromium [6, 23] and other contaminants [13] also produce wastes that are high in salt [13, 36]. Such high solute wastes suppress bacterial growth in treatments to remove contaminants [28]. Very interestingly GS1 removed Cr(VI) in high salt media suggesting it could be useful for Cr(VI) bioremediation in saline environments. At high salt concentration, acidic pH and high temperature, biomass readings were low although substantial Cr(VI) removal was observed. Such culture conditions cause cell lysis which can lower optical density. Moreover, lysis of cells release enzymes [8] that remove Cr(VI).

In conclusion, *Exiguobacterium* sp. GS1 can be considered a halotolerant, thermotolerant, psychroactive, and alkalitolerant facultatively anaerobic bacterium. *Exiguobacterium* sp. GS1 displayed rapid bioremoval of Cr(VI) from water under diverse environmental conditions. The Cr(VI) bioremoval properties of this organism make it a unique potential organism for bioremediation of environmental pollutants in diverse complex environments. Further studies will focus on the metabolic factors that catalyze Cr(VI) bioremoval in *Exiguobacterium* sp. GS1.

Acknowledgments Thanks to Shakena Crenshaw, Jeffery Laymon, Charles Oji, Mark Losi and Pete Hall. This study was in part supported from Auburn University Montgomery faculty grant-in-aid, the School of Sciences start-up fund and a grant from Ecomat Inc CA.

References

1. Arıca MY, Bayramoglu G (2005) Cr(VI) biosorption from aqueous solutions using free and immobilized biomass of *Lentinus sajor-caju*: preparation and kinetic characterization. Physicochem Eng A253:203–211
2. Bartlett RJ, James BR (1996) Chromium. In: Spark DL (ed) Methods of soil analysis Part 3, SSSA Book Series 5, SSSA, Madison, pp 683–701
3. Bayramoglu G, Celik G, Yalcin E, Yilmaz M, Arica MY (2005) Modification of surface properties of *Lentinus sajor-caju* mycelia by physical and chemical methods: evaluation of their Cr^{6+} removal efficiencies from aqueous medium. J Hazard Mater B119:219–229
4. Camargo FAO, Okeke BC, Bento FM, Frankenberger WT (2005) Diversity of chromium-resistant bacteria isolated from soils contaminated with dichromate. Appl Soil Ecol 29:193–202
5. Camargo FAO, Okeke BC, Bento FM, Frankenberger WT (2004) Hexavalent chromium reduction by immobilized cells and cell-free extract of *Bacillus* sp. ES 29. Bioremediation J 8:23–30
6. Camargo FA, Bento FM, Okeke BC, Frankenberger WT (2003) Chromate reduction by chromium-resistant bacteria isolated from soils contaminated with dichromate. J Environ Qual 32:1228–1233
7. Camargo FA, Bento FM, Okeke BC, Frankenberger WT (2004) Hexavalent chromium reduction by an actinomycete, *Arthrobacter crystallopoietes* ES 32. Biol Trace Elem Res 97:183–194
8. Camargo FA, Okeke BC, Bento FM, Frankenberger WT (2003) In vitro reduction of hexavalent chromium by a cell-free extract of *Bacillus* sp. ES 29 stimulated by Cu^{2+}. Appl Microbiol Biotechnol 62:569–573
9. Campos J, Martinez-Pacheco M, Cervantes C (1995) Hexavalent-chromium reduction by a chromate-resistant *Bacillus* sp. strain. Antonie Leeuwenhoek 68:203–208
10. Cheung KH, Gu JD (2003) Reduction of chromate (CrO_4^{2-}) by an enrichment consortium and an isolate of marine sulfate-reducing bacteria. Chemosphere 52:1523–1529
11. Chung J, Nerenberg R, Rittmann BE (2006) Bio-reduction of soluble chromate using a hydrogen-based membrane biofilm reactor. Wat Res 40:1634–1642
12. Clark DP (1994) Chromate reductase activity of *Enterobacter aerogenes* is induced by nitrite. FEMS Microbiol Lett 122:233–238
13. Clifford D, Liu X (1993) Ion exchange for nitrate removal. Amer Water Works Assoc J 85:135–143
14. Cummings DE, Fendorf S, Singh N, Sani RK, Peyton BM, Magnuson TS (2007) Reduction of Cr(VI) under acidic conditions by the facultative Fe(lll)-reducing bacterium *Acidiphilium cryptum*. Environ Sci Technol 41:146–152
15. Das S, Chandra AL (1990) Chromate reduction in *Streptomyces*. Experientia 46:731–733
16. Dermou E, Velissariou A, Xenos D, Vayenas DV (2005) Biological chromium(VI) reduction using a trickling filter. J Hazard Mater 126:1–3
17. Fruhling A, Schumann P, Hippe H, Straubler B, Stackebrandt E (2002) *Exiguobacterium undae* sp. nov. and *Exiguobacterium antarcticum* sp. nov. Int J Syst Evol Microbiol 52:1171–1176
18. Guha H, Jayachandran K, Maurrasse F (2001) Kinetics of chromium (VI) reduction by a type strain *Shewanella alga* under different growth conditions. Environ Pollut 115:209–218
19. Gvozdyak PI, Mogilevich NF, Ryl'skii AF, Grishchenko NI (1987) Reduction of hexavalent chromium by collection of strains of bacteria. Microbiologia 55:770–773
20. Iyer A, Mody K, Jha B (2004) Accumulation of hexavalent chromium by an exopolysaccharide producing marine Enterobacter cloaceae. Mar Pollut Bull 49:974–977
21. James BR, Bartlett RJ (1984) Plant–soil interactions of chromium. J Environ Qual 13:67–70
22. Komori KA, Rivas A, Toda K, Ohtake H (1990) A method for removal of toxic chromium using dialysis-sac cultures of a chlomate-reducing strain of *Enterobacter cloacae*. Appl Microbiol Biotechnol 33:117–119
23. Krishna KR, Philip L (2005) Bioremediation of Cr(VI) in contaminated soils. J Hazard Mater B121:109–117
24. Kurniawan TA, Chan GYS, Lo W-H, Babel S (2006) Physicochemical treatment techniques for wastewater laden with heavy metals. Chem Eng J 118:83–98
25. Lane D (1991) 16S/23S sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. John Wiley and Sons, New York, NY
26. Laxman RS, More S (2002) Reduction of hexavalent chromium by *Streptomyces griseus*. Miner Eng 15:831–837
27. Lee DC, Park CJ, Yang JE, Jeong YH, Rhee HI (2000) Screening of hexavalent chromium biosorbent from marine algae. Appl Microbiol Biotechnol 54:445–448
28. Logan BE, Wu J, Unz RF (2001) Biological perchlorate reduction in high-salinity solutions. Wat Res 35:3034–3038
29. Losi ME, Amrhein C, Frankenberger WT (1994) Environmental biochemistry of chromium. Rev Environ Contam Toxicol 36:91–121
30. Losi ME, Amrhein C, Frankenberger WT (1994) Bioremediation of chromate-contaminated groundwater by reduction and precipitation in surface soils. J Environ Qual 23:1141–1150
31. Losi ME, Frankenberger WT (1994) Chromium-resistant microorganisms isolated from evaporation ponds of a metal processing plant. Water Air Soil Pollut 74:405–413
32. Lovely DR, Phillips EJP (1994) Reduction of chromate by *Desulfovibrio vulgaris* and its c3 cytochrome. Appl Environ Microbiol 60:726–728
33. Mathews CK, Van Holde KE, Ahern KG (1999) Biochemistry, 3rd edn. Benjamin-Cummings Pub Co, San Francisco CA
34. M*yRDP* Release 9.50 (http://rdp.cme.msu.edu/misc/rel9info.jsp)
35. Oh YS, Choi SC (1997) Reduction of hexavalent chromium by *Pseudomonas aeruginosa* HP014. J Microbiol (Microbiol Soc Korea) 35:25–29
36. Okeke BC, Giblin T, Frankenberger WT (2002) Reduction of perchlorate and nitrate by salt tolerant bacteria. Environ Pollut 118:357–363
37. Okeke BC, Frankenberger WT (2003) Biodegradation of methyl tertiary butyl ether (MTBE) by a bacterial enrichment consortia and its monoculture isolates. Microbiol Res 158:99–106
38. Okeke BC, Laymon J, Crenshaw S, Oji C (2008) Environmental and kinetic parameters for Cr(VI) bioreduction by a bacterial monoculture purified from Cr(VI) resistant consortium. Biol Trace Elem Res 123:229–241
39. Park D, Yun Y-S, Ahn CK, Park JM (2007) Kinetics of the reduction of hexavalent chromium with the brown seaweed *Ecklonia* biomass. Chemosphere 66:939–946
40. Thacker U, Parikh R, Shouche Y, Madamwar D (2006) Hexavalent chromium reduction by *Providencia* sp. Proc Biochem 41:1332–1337
41. Vishnivetskaya T, Kathariou S, McGrath J, Gilichinsky D, Tiedje JM (2000) Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. Extremophiles 4:165–173
42. Vishnivetskaya TA, Petrova MA, Urbance J, Ponder M, Moyer CL, Gilichinsky DA, Tiedje JM (2006) Bacterial community in

ancient Siberian permafrost as characterized by culture and culture-independent methods. Astrobiology 6:400–414
43. Vishnivetskaya TA, Siletzky R, Jefferies N, Tiedje JM, Kathariou S (2007) Effect of low temperature and culture media on the growth and freeze-thawing tolerance of *Exiguobacterium* strains. Cryobiology 54:234–240