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Microbial reduction of Cr(VI) in the presence of chromate conversion coating constituents

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ABSTRACT

The reduction of Cr(VI) by the metal-reducing bacterium *Shewanella oneidensis* MR-1 was evaluated, to determine the potential for exploiting Cr(VI) bioreduction as a means of treating chromate conversion coating (CCC) waste streams. Inclusion of Cr(VI) at concentrations ≥1 mM inhibited aerobic growth of *S. oneidensis*, but that organism was able to reduce Cr(VI) at a concentration of up to 1 mM under anaerobic, nongrowth conditions. *S. oneidensis* reduced Cr(VI) in the presence of common CCC constituents, with the exception of ferricyanide, when these CCC constituents were included at concentrations typical of CCC waste streams. Ferricyanide inhibited neither aerobic growth nor metabolism under aerobic, nitrate- or iron-reducing conditions, suggesting that the ferricyanide-depended inhibition of Cr(VI) reduction is not due to broad metabolic inhibition, but is specific to Cr(VI) reduction. Results indicate that under some conditions, the activities of metal-reducing bacteria, such as *S. oneidensis*, could be exploited for the removal of Cr(VI) from CCC waste streams under appropriate conditions.

KEYWORDS

Biological reduction; chromate conversion coatings; chromium bioremediation; chromium reduction; wastewater treatment

Introduction

Chromate conversion coatings (CCCs) are extensively used as surface treatments on aluminum and magnesium alloys (Twite and Bierwagen 1998; Kendig and Buchheit 2003). CCCs are applied via spraying, brushing, electrostatic spraying, or dipping a component into a treatment bath (Breu, Guggenbichler, and Wollmann 2008). Chromate is responsible for corrosion protection, producing a mixed trivalent/hexavalent chromium oxo-hydroxide on metal surfaces that inhibits the oxygen reduction reaction and increases the pitting potential of the alloy (Zhao, Frankel, and McCreery 1998; Frankel 2001). Other CCC constituents include fluoride and ferricyanide, which serve to destabilize Al(III) oxides and facilitate the reaction between chromate and bare aluminum (Frankel 2001; Zhao et al. 2001).

Given the health and environmental hazards associated with Cr(VI), that element must be safely removed from waste streams that result from CCC application activities (Costa and Klein 2006; Wise, Peyton, and Petersen 2002; Sellamuthu et al. 2011). Removal of Cr from waste streams is generally accomplished by reduction of relatively soluble Cr(VI) to Cr(III) via chemical additions, followed by filtration of sparingly soluble Cr(III) phases using a physical or electrical membrane (Beleza, Boaventura, and Almeida 2001; Roundhill and Koch 2002; McGuire et al. 2006); however, these approaches may be expensive, hazardous, and impractical (Barrera-Diaz, Lugo-Lugo, and Bilyeu 2012).

A variety of microorganisms may reduce relatively soluble and toxic Cr(VI) to relatively insoluble Cr(III) phases (Marsh, Leon, and McInerney 2000; Barrera-Diaz, Lugo-Lugo, and Bilyeu 2001; Daulton et al. 2007; Liu et al. 2011). Microbially mediated reductive immobilization of Cr may be used as an approach to in situ remediation of contaminated aquifers and sediments (e.g., Tokunaga et al. 2003; Kamaludeen et al. 2003). The microbial activities leading to the reductive

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removal of soluble Cr(VI) from solution as relatively insoluble Cr(III) phases may be similarly cost-effective for removal of Cr from CCC waste streams (Agrawal, Kumar, and Pandey 2006). However, these waste streams will likely contain higher concentrations of Cr (VI) than might be encountered in contaminated water bodies, as well as other CCC chemical constituents that may inhibit microbiological activities (Palmer and Wittbrodt 1991; Dakiky et al. 2002). As such, it is difficult to predict the applicability of microbial Cr(VI) reduction to the removal of that element from CCC waste streams. To date, work has concentrated on dilute chromate concentrations to simulate Cr(VI) levels in contaminated soils, wastewater streams, and aquifers rather than the more concentrated effluents that are generated during CCC applications. In this article, we evaluated the potential for the metal-reducing bacterium *Shewanella oneidensis* MR-1 to reduce Cr(VI) at concentrations relevant to CCC waste streams. Additionally, the potential inhibitory effects of other CCC constituents on Cr(VI) reduction by *S. oneidensis* were evaluated.

Materials and methods

Growth and incubations

Shewanella oneidensis MR-1 was routinely cultivated on solid or liquid tryptic soy media (TSA and TSB, respectively) consisting of tryptic soy powder (20 g/L) and Bacto agarose (15 g/L) for TSA. Cr(VI) toxicity and reduction experiments were conducted in a minimal medium described by Myers and Nealson (1990) that included 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; 50 mM), sodium lactate (15 mM), $(NH_4)_2SO_4$ (9 mM), K_2HPO_4 (5.7 mM), KH_2PO_4 (3.3 mM), $NaHCO_3$ (2 mM), $MgSO_4$ (1.0 mM), $CaCl_2$ (0.5 mM), yeast extract (0.05 g/L), casamino acids (0.1 g/L), L-arginine HCl (20 mg/L), L-glutamate (20 mg/L), vitamins, and trace metals (Tanner 2007). The pH of the medium was adjusted to 7.4 with HCl. When appropriate, the O_2 was removed from minimal medium by purging with O_2-free N_2, and the medium dispensed into serum bottles that contained a headspace of N_2 and were sealed with rubber stoppers.

For experiments to assess the influence of Cr(VI) on growth of *S. oneidensis*, a 10% inoculum of TSB-grown cells was added to minimal medium that contained $K_2Cr_2O_7$ at concentrations of 0, 0.01, 0.1, 1.0, and 10 mM Cr(VI). Cell density was determined by measuring absorbances at 600 nm using a spectrophotometer. For experiments to determine (i) the influence of oxygen on Cr(VI) bioreduction, (ii) the maximum Cr(VI) concentration that could be bioreduced, and (iii) the influence of other CCC constituents on Cr(VI) bioreduction, *S. oneidensis* was grown to late log phase in TSB medium. Cells were harvested by centrifugation and resuspended in sterile medium. These steps were repeated twice more before resuspension and addition of the cells to minimal medium to achieve a density (A_{600}) of 0.7. For experiments to determine the impact of oxygen on Cr(VI) reduction, cell suspensions were incubated in oxic or anoxic minimal medium described above that was amended with 0.5 mM $K_2Cr_2O_7$ to achieve a Cr(VI) concentration of 1 mM. For experiments to determine the maximum concentration of Cr(VI) that could be reduced by *S. oneidensis*, cell suspensions were incubated in anoxic minimal medium that was amended with $K_2Cr_2O_7$ to achieve Cr(VI) concentrations of 0.1, 1.0, and 10.0 mM Cr(VI), respectively. All incubations were conducted in triplicate.

CCC components were included in minimal medium based on the composition of Alodine 1200S. Commercial CCC baths typically contain chromate (45–90 mM), potassium fluoroborate (KBF_4; 12–36 mM), potassium ferricyanide ($K_3[Fe(CN)_6]$; 4.6–13.7 mM), sodium fluoride (NaF; 17.9–35.7 mM), and potassium zirconium fluoride (K_2ZrF_6; 2.6–5.29 mM) (Breu, Guggenbichler, and Wollmann 2008). When CCCs are applied, the excess coating is subsequently rinsed with water. This results in CCC waste streams containing 0.4–10 mM chromate, 0.1–3.6 mM KBF_4, 0.4–1.4 mM $K_3[Fe(CN)_6]$, 0.2–3.6 NaF, and 0.02–0.5 mM K_2ZrF_6 (Dakiky et al. 2002). In this work, CCC components were provided to minimal medium at concentrations of 0.2 mM KBF_4, 0.5 mM $K_3[Fe(CN)_6]$, 0.2 mM NaF, and/or 0.05 mM K_2ZrF_6.

Analytical approaches

Subsamples were removed from incubations, and cell densities were determined by measuring A_{600} of cultures using a Helios Zeta ultraviolet-visible (UV-Vis) spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Before measurement of dissolved Cr(VI), Fe(II), nitrate, and lactate concentrations, cells and other solids were removed from suspensions by

centrifugation. Dissolved Cr(VI) was quantified spectrophotometrically as described by Sandell (1959) or Xia et al. (2000). The method described by Xia et al. (2000) was used in cases where ferricyanide was included in media, since Fe(III)-containing compounds at concentrations exceeding 18 μM may interfere with Cr(VI) quantification using the method described by Sandell (1959). Dissolved Fe(II) was determined by colorimetrically ferrozine assay (Stookey 1970). Lactate was quantified by high-performance liquid chromatography (HPLC), using and a Shimadzu LC-10A HPLC system (Shimadzu Scientific Instruments, Columbia, MD) equipped with an Aminex HPX-87H column (300 mm × 7.8 mm; Bio-Rad Laboratories, Hercules, CA) with UV (254 nm) detection (SPD-10A), with a mobile phase of 0.008 N H_2SO_4 at a flow rate of 0.6 ml/min. Nitrate concentrations were determined using Dionex ion chromatography system 1100 with an AS14 column and conductivity detection (Thermo Scientific, Memphis, TN).

Electron microscopy

Samples were prepared for electron microscopy by fixation in a sodium cacodylate–buffered (1% vol) solution of glutaraldehyde (2% vol), followed by fixation in a cacodylate-buffered solution of osmium tetroxide (2% vol). Following fixation, samples intended for examination by scanning electron microscopy (SEM) were rinsed with water, and dehydrated in an acetone series, followed by critical point drying (Quorum Technologies, model K850; Lewes, United Kingdom). Dried samples were mounted and coated with a gold-palladium mix with a Polaron E5000 sputter coater (Quorum Technologies; Lewes, United Kingdom). Samples intended for examination by transmission electron microscopy (TEM) were suspended in molten 2% agarose solution, which was then allowed to solidify. The solidified samples were sectioned, immersed in water, and dehydrated in an acetone dehydration series. Samples were infiltrated with plastic resin in a solution of 90% acetone–10% Embed-812 resin (Electron Microscopy Science, Hatfield, PA) for 14 h, followed by immersion of samples in 100% resin. Samples were transferred to molds, covered in 100% resin, and placed in a 60°C oven for 48 h. After removal from the oven, samples were cooled, sectioned using a Reichert OMU-3 ultramicrotome with a diamond knife (Reichert Microscope Services, Depew, NY), and mounted on 300 mesh copper grids. Sections were stained with 1% methanolic uranyl acetate for 8 min and lead citrate for 15 minutes (Reynolds 1963). Selected samples were not stained with uranyl or lead so that cell-associated Cr(III) could be visualized.

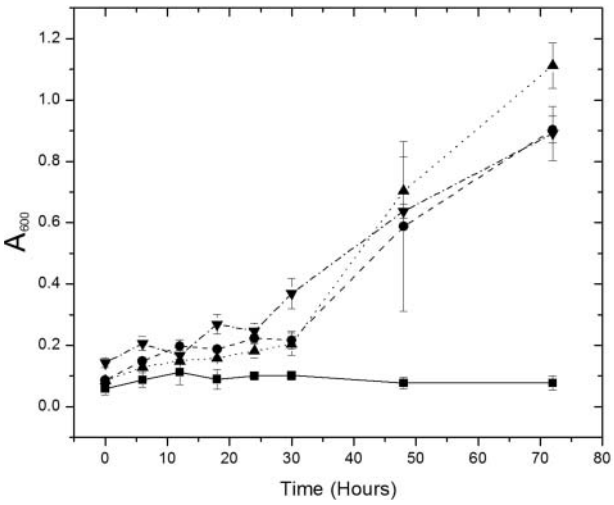

Figure 1. Optical density of *S. oneidensis* MR-1 in aerobic medium (▲) without Cr(VI) or amended with (▼) 0.01 mM, (●) 0.1 mM, or (■) 1 mM Cr(VI). Data points are averages of OD of triplicate cultures, and error bars represent one standard deviation.

SEM was carried out at the Environmental Scanning Electron Microscopy Laboratory in the Department of Geosciences, The University of Akron, using an FEI Quanta 200 (FEI Company, Hillsboro, OR) environmental scanning electron microscope with an energy-dispersive x-ray spectroscopy (EDS) attachment (EDAX QUANTA-200/400; EDAX, Mahwah, NJ). TEM was performed at The Ohio State University Ohio Agricultural Research Development Center Molecular and Cellular Imaging Center (OARDC MCIC) in Wooster, Ohio, using a model H 7500 transmission electron microscope by Hitachi (Hitachi High-Technologies, Hitachinaka, Japan).

Results and discussion

Cr(VI) reduction by S. oneidensis *MR-1*

To determine growth-limiting concentrations of Cr(VI) for *S. oneidensis*, growth was evaluated in minimal medium amended with Cr(VI) at concentrations ranging from 0.01 to 1 mM. Aerobic growth in media amended with 0.01 and 0.1 mM Cr(VI) was similar to that observed in Cr(VI)-free medium, but no aerobic growth was observed in medium

amended with 1 mM Cr(VI) (Figure 1). No growth could be observed in medium in which Cr(VI) served as the sole potential terminal electron acceptor (not shown).

The poor growth observed under anaerobic conditions may be attributable to Cr(VI) toxicity (Viamajala, Peyton, and Petersen 2003; Parker, Borer, and Bernier-Latmani 2011). Anaerobic Cr(VI)-dependent growth was not detected; this may have been attributable to insufficient electron acceptor (1 mM) to support growth. However, there is no previous evidence that *Shewanella* spp. may exploit Cr(VI) respiration for growth, and several studies suggest that *Shewanella* spp. are incapable of Cr(VI) respiration-dependent growth (Middleton et al. 2003; Bencheikh-Latmani et al. 2007), despite similarities in transcription profiles of cells exposed to Cr(VI) and U(VI), which does support cell growth (Bencheikh-Latmani et al. 2005).

Since *S. oneidensis* did not grow in solutions with Cr(VI) concentrations likely in CCC waste streams, the ability of MR-1 to reduce Cr(VI) under conditions not linked with growth was tested. To make this evaluation, incubations containing a high density of aerobically grown cells in minimal medium under oxic and anoxic conditions were monitored. Cr(VI) was reduced under anaerobic conditions only, suggesting that oxygen inhibited Cr(VI) reduction (Figure 2A). The similarly high standard reversible potential of the CrO_4^{2-}-Cr(VI)/Cr_2O_3-Cr(III) redox couple (1.36 V vs. standard hydrogen electrode [SHE]) to the O_2-O (0)/H_2O-O(−II) redox couple (1.27 V vs. SHE) indicates that competitive inhibition of Cr(VI) reduction by O_2 is an unlikely explanation for the lack of Cr(VI) reduction by *S. oneidensis* under aerobic condition (Figure 2B). Several organisms reduce Cr(VI) under aerobic conditions (Shen and Wang 1994; McLean, Beveridge, and Phipp 2000; Nepple, Kessi, and Bachofen 2000; Shakoori, Makhdoom, and Haq 2000). However, even when organisms are capable of Cr(VI) reduction under aerobic conditions, reduction rates are generally greater under anaerobic conditions (McLean, Beveridge, and Phipp 2000; Nepple, Kessi, and Bachofen 2000; Shakoori, Makhdoom, and Haq 2000). For example, Middleton et al. (2003) showed that *S. oneidensis* could reduce approximately 100 μM Cr(VI) under aerobic conditions, perhaps as a mechanism of detoxification. Results presented here are in agreement with previous studies that indicate that *S. oneidensis* and other organisms cannot readily reduce Cr(VI) in the presence of dissolved oxygen (Chen and Hao 1996; Viamajala et al. 2002; Zhang et al. 2014). In the current work, *S. oneidensis* was capable of reducing Cr(VI) at concentrations $\leq$1 mM under anaerobic conditions, the largest value reported for Cr(VI) reduction by *S. oneidensis*. However, compared with other Cr(VI)-reducing organisms, including *Enterobacter cloacae, Bacillus* spp., or *Pseudomonas aeriguinosa* (Komori et al. 1990; Ezaka and Anyanwu 2011; Ganguli and Trapathi 2002), no Cr(VI) reduction was observed in incubations that contained 10 mM Cr(VI) (Figure 2B).

Examination of cells recovered from anaerobic incubations containing 1 mM Cr(VI) by SEM and EDS indicated that electron-dense deposits on cells were rich in Cr (SI C and D) compared with normal cells (SI A and B). Characterization of these precipitates by x-ray diffraction revealed that they were amorphous Cr phases (not shown), likely a hydrated chromium hydroxide, as has been reported by Daulton et al. (2002). Most of the Cr was

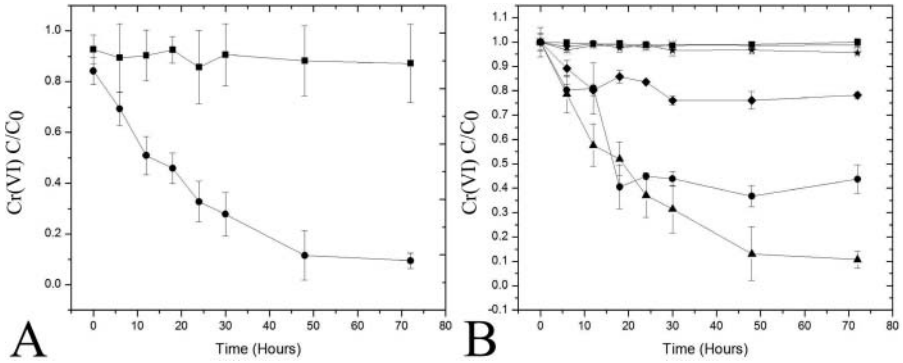

Figure 2. Cr(VI) concentrations (A) in (■) aerobic and (●) anaerobic media inoculated with *S. oneidensis* MR-1 and (B) in anaerobic incubations with (●) 0.2 mM, (▲) 1 mM, and (■) 10 mM Cr(VI). Cr(VI) concentrations in uninoculated media containing 0.2 mM, 1 mM, and 10 mM Cr(VI) are represented by ♦, ★,∗ and UÚ respectively.

deposited on cell membranes (SI E-H), suggesting that this may be the location of Cr(VI) reduction, consistent with results reported previously (Myers et al. 2000; Daulton et al. 2002, 2007). However, Middleton et al. (2003) observed both intracellular and extracellular Cr deposits upon reduction by *S. oneidensis*. The observation here of electron-dense precipitates along the exterior of cells are similar to Cr(III) precipitates identified by Daulton et al. (2002, 2007). These findings could represent a mechanism of inhibition, whereby cells are encased in biogenic precipitates.

Whereas *S. oneidensis* cells grown in the absence of Cr(VI) exhibited typical rod-shape morphology, cells recovered from incubations that contained 1 mM Cr(VI) were filamentous (SI C). This observation may provide some insight into the mechanisms of growth inhibition by Cr(VI). Whereas Cr(VI) may inhibit enzymatic activity and induce cell death (e.g., incubations that contained 10 mM Cr(VI)), exposure of cells to Cr(III) (whether biogenic or exogenous) may result in disruption of cell division, resulting in the filamentous cells (Middleton et al. 2003; Bencheikh-Latmani et al. 2005; Zhang et al. 2014) observed in the current work (SI C). However, *S. oneidensis* appears to retain enzymatic activity (including Cr (VI)-reducing activity) upon exposure to Cr(III) (Parker, Borer, and Bernier-Latmani 2011). It is unclear whether *S. oneidensis* can anaerobically respire Cr(VI) (Myers et al. 2000). The unusual cell morphologies observed by SEM indicate that retention of enzymatic activity in the presence of 1 mM Cr(VI) (Figure 2) facilitated an increase in cell volume that would be associated with growth, but production of Cr(III) inhibited cell division, thus stunting appreciable reproduction.

In the current study, it is illustrated that although Cr(VI) concentrations $\geq$1 mM inhibit aerobic growth of *S. oneidensis* MR-1, organisms may remain active and metabolize Cr(VI) at concentrations of up to 1 mM under anoxic conditions. However, Cr(VI) concentration of 10 mM renders *S. oneidensis* MR-1 inactive with respect to Cr(VI) reduction. The finding that *S. oneidensis* remains enzymatically active, and anaerobically reduces Cr(VI) at 1 mM, represents the highest Cr(VI) concentration for which that organism has been observed to remain active (previous work used concentrations in the range 100–200 μM; Middleton et al. 2003; Viamajala, Peyton, and Petersen 2003; Bencheikh-Latmani et al. 2005, 2007; Parker, Borer, and Bernier-Latmani 2011). The finding that *S. oneidensis* MR-1 is capable of Cr(VI) reduction at 1 mM indicates that this organism may be exploited for reductively precipitating Cr from CCC waste streams (Dakiky et al. 2002). As such, Cr(VI) reduction by *S. oneidensis* simulating the chemistry of a commercial CCC waste stream was evaluated.

Biological Cr(VI) reduction in CCC waste streams

After establishing the maximum concentrations at which *S. oneidensis* could reduce Cr(VI), the ability of *S. oneidensis* to reduce Cr(VI) when exposed to solutions containing other CCC components was investigated. Incubations were conducted in minimal medium amended with CCC additives KBF_4, $K_3[Fe(CN)_6]$, NaF, and K_2ZrF_6 individually and in combination, at concentrations reflecting a dilute CCC waste stream (Breu, Guggenbichler, and Wollmann 2008). *S. oneidensis* reduced Cr(VI) in the presence of KBF_4, NaF, and K_2ZrF_6 at rates and to extents similar to those observed in CCC constituent–free medium (Figure 3). In contrast, Cr(VI) reduction was inhibited in the presence of $K_3[Fe(CN)_6]$, whether individually or included with the other CCC constituents (Figure 3). An explanation for the limited Cr(VI) reduction in the presence of ferricyanide could be that ferricyanide-Fe(III) may be reduced to Fe(II) by *S. oneidensis* to the exclusion of Cr(VI) reduction (Liu et al. 2011). However, the standard reversible potential of the ferricyanide-Fe(III)/ferrocyanide-Fe(II) couple is 0.35 V vs. SHE, compared with the reduction potential of CrO_4^{2-}-Cr(VI)/Cr_2O_3-Cr(III) couple, which is 1.36 V vs. SHE (values calculated from Dean [1998]); thus, Cr(VI) reduction would be a more thermodynamically favorable reductive process. Additionally, no evidence of the insoluble ferrocyanide phase, "Prussian Blue," was observed, indicating that ferricyanide-Fe(III) was not reduced. As such, it is unlikely that inhibition of Cr(VI) reduction by ferricyanide was caused by preferential reduction of ferricyanide-Fe(III). Ferricyanide is generally considered nontoxic. For example, growth of *Escherichia coli* was only inhibited at ferricyanide concentrations above 25 mM (Liu et al. 2009a, 2009b), and ferricyanide may be used as an

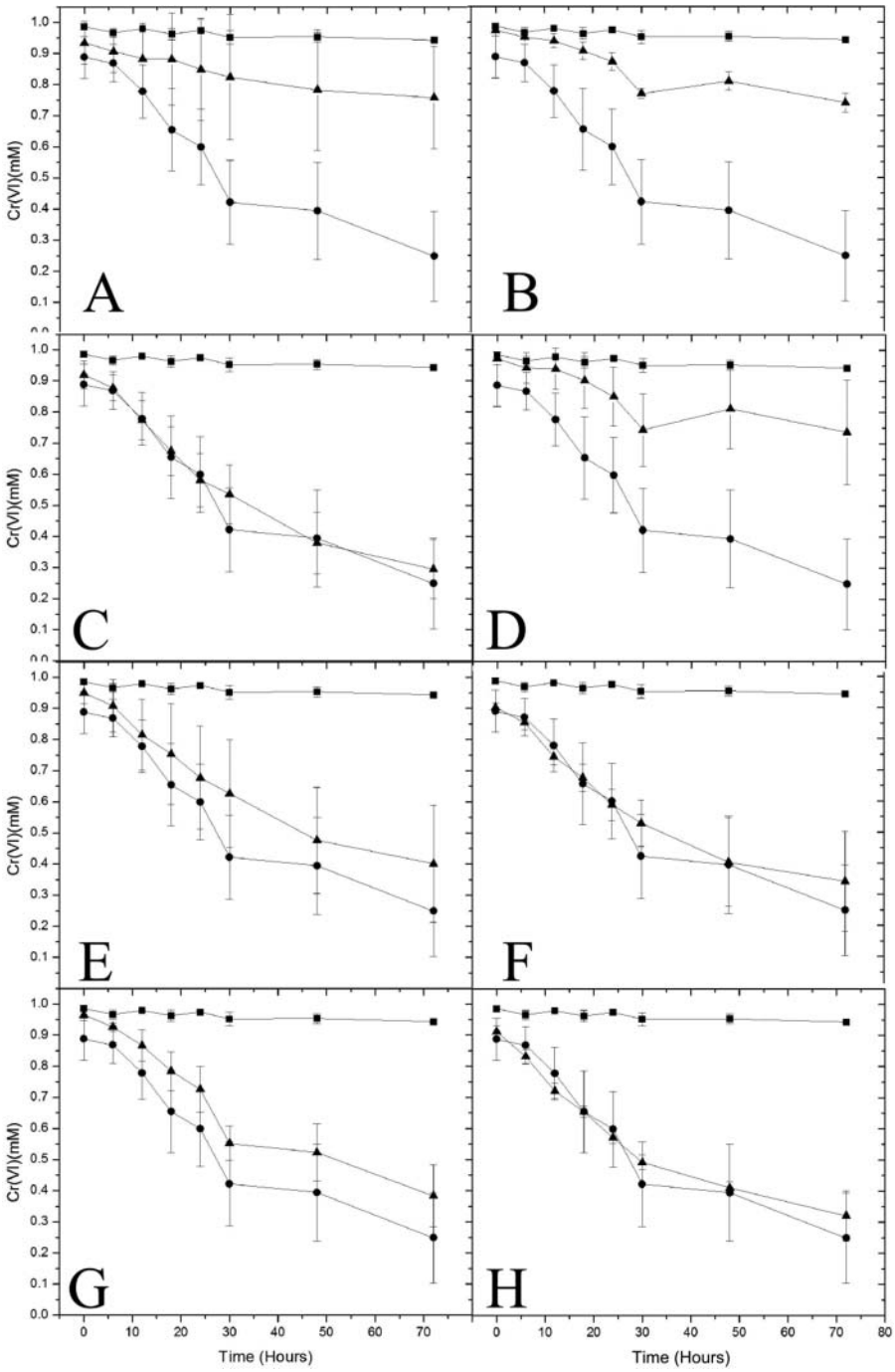

Figure 3. Cr(VI) concentrations in anaerobic incubations of *S. oneidensis* MR-1 with CCC components, including KBF4, NaF, K3[Fe(CN)6], K2ZrF6 (all CCC components). A) K3[Fe(CN)6]; (B) KBF4, K2ZrF6; (C) NaF, K3[Fe(CN)6]; (D) KBF4; (E) NaF, KBF4, K2ZrF6 (F) KBF4; (G) or K2ZrF6 (G). Cr(VI) concentrations in uninoculated medium are depicted by ■, while incubations containing *S. oneidensis* in CCC component-amended and -free media are depicted by ♦ and ▲, respectively.

electron shuttle in microbial fuel cells (MFCs) (Bullen et al. 2006).

To evaluate the potential toxicity of ferricyanide to *S. oneidensis*, the growth of that organism was monitored in minimal medium amended with 0.5, 5, 25, and 50 mM $K_3[Fe(CN)_6]$. *S. oneidensis* grew in medium containing ferricyanide concentrations as high as 50 mM (Figure 4), indicating that ferricyanide does not globally inhibit metabolism by *S. oneidensis*, even at concentrations that far exceed those expected in CCC waste streams. To further evaluate the influence of ferricyanide on the metabolism of *S. oneidensis* MR-1 under conditions not linked with growth, a high density of aerobically grown cells were incubated in

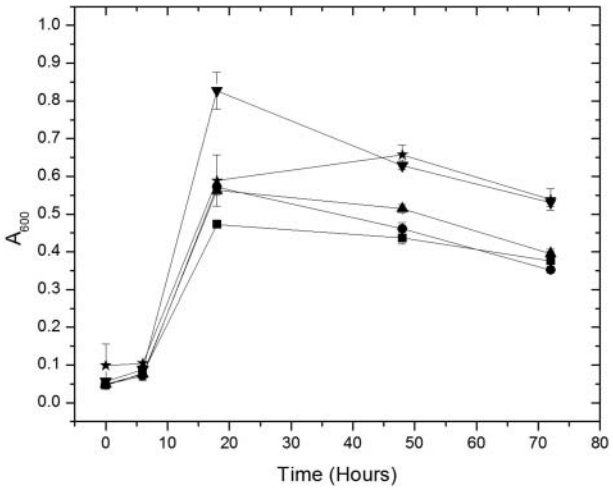

Figure 4. Optical density of *S. oneidensis* MR-1 in aerobic medium with 0 mM (■), 0.5 mM (●), 5 mM (▲), 25 mM (▼), or 50 mM (▼) ferricyanide.

minimal medium with or without potassium ferricyanide, and with O_2, nitrate, or Fe(III) (hydr)oxide as sole terminal electron acceptors. Under such conditions, the addition of potassium ferricyanide had no effect on lactate consumption under any terminal electron accepting conditions (Figure 5A–C) and did not influence nitrate reduction (Figure 5D) or iron reduction (Figure 5E) by *S. oneidensis* (Figure 5). Since ferricyanide inhibited Cr(VI) reduction, but not aerobic, nitrate- or Fe(III)-reducing metabolism by *S. oneidensis*, it is concluded that ferricyanide specifically disrupted Cr metabolism. These results indicate that the inhibition of Cr(VI) reduction by ferricyanide was not attributable to broad deactivation of cellular metabolism by ferricyanide, but rather, was specific to Cr(VI) reduction.

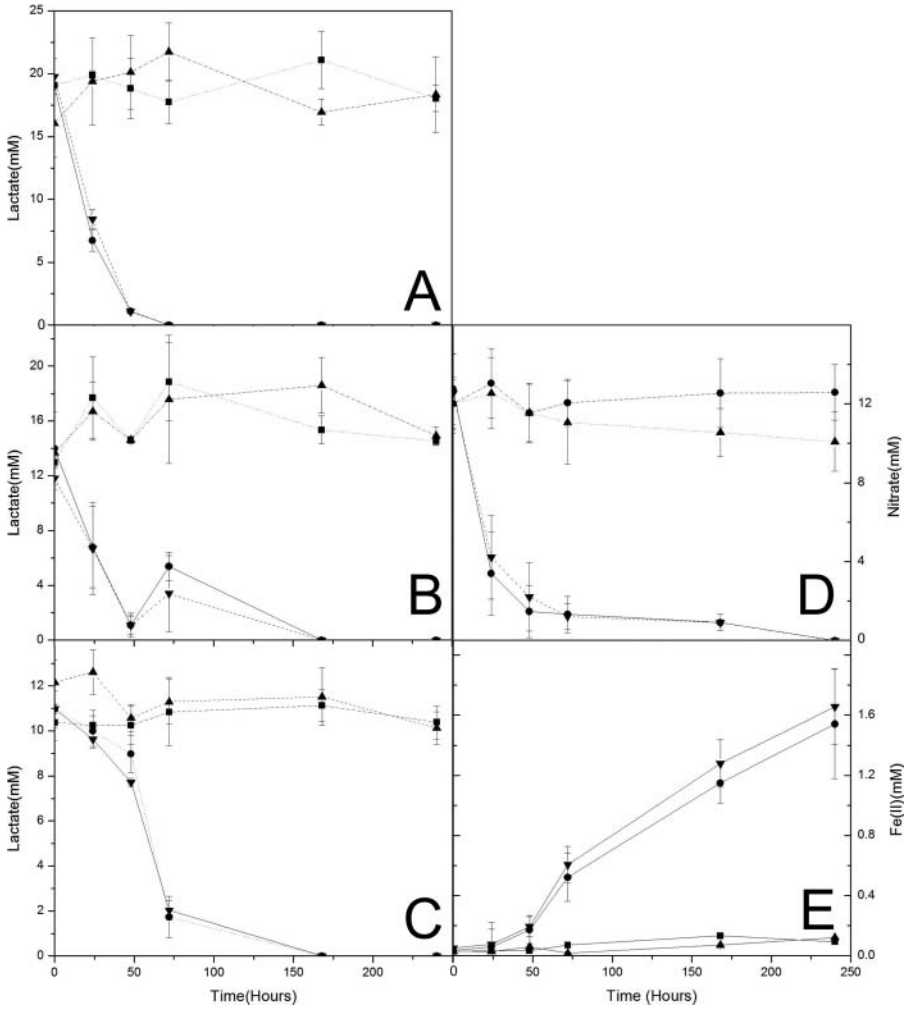

Figure 5. Effects of potassium ferricyanide on metabolism of Fe(III) (Top row); Cr(VI) (second row); $NO3^-$ reduction (third row); and aerobic respiration (bottom row). Each row consists of a terminal electron acceptor levels (except the bottom row) along with electron donor level (lactate). Each panel consists of the following set-up: ▼- electron acceptor, inoculated; ▲- electron acceptor, sterile; ●- electron acceptor with potassium ferricyanide, inoculated; ■- electron acceptor with potassium ferricyanide, sterile.

Implications for the biological treatment of CCC waste streams

The results presented herein indicate that the Cr(VI)-reducing activities of *S. oneidensis* MR-1 may be exploited to remove that element from CCC waste streams under appropriate conditions. However, results also indicate that it will likely be necessary to remove or dilute ferricyanide in the waste streams before reductively removing Cr(VI). Cr(VI) reduction by *S. oneidensis* in solutions with lower ferricyanide concentrations was observed in this research, indicating that this inhibition of Cr(VI) bioreduction may be dependent on ferricyanide concentration. As such, dilution of CCC waste streams may alleviate any inhibitory effects of ferricyanide on Cr(VI) reduction.

Conclusions

This work suggests that *Shewanella* spp. are capable of Cr(VI) reduction in growth medium with chromate and complex compounds associated with CCC waste streams. *S. oneidensis* reduced Cr(VI) at levels up to 1.0 mM in anaerobic conditions, supplemented with the electron donor lactate. Additionally, *S. oneidensis* anaerobically reduced Cr(VI) in presence of all CCC constituents except for potassium ferricyanide. Ferricyanide inhibited Cr(VI) reduction by *S. oneidensis* MR-1, but it was found to be nontoxic towards *S. oneidensis* up to concentrations as high as 50 mM, suggesting that the ferricyanide-depended inhibition of Cr(VI) reduction was not related to a broad metabolic inhibition. Ferricyanide in levels similar to a CCC waste stream did not disrupt the reduction of NO_3^-, O_2, and Fe^{3+} by *S. oneidensis* MR-1, indicating that ferricyanide specifically inhibits Cr(VI) reduction. These findings suggest biologically mediated reductive immobilization of Cr (VI) could be exploited commercially for remediation under appropriate conditions of CCC waste streams.

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