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Oxidation state of chromium associated with cell surfaces of *Shewanella oneidensis* during chromate reduction

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Abstract

Employing electron energy loss spectroscopy (EELS) and X-ray photoelectron spectroscopy (XPS), we demonstrate that in both aerobic and anaerobic culture *Shewanella oneidensis* cells are capable of chromate reduction. No Cr(VI) or Cr(V) species were identified at the cell surfaces in Cr $2p_{3/2}$ core photoelectron spectra. More chromium was associated with cell surfaces recovered from anaerobic medium than aerobic. Multiplet-splitting models derived for Cr(III) and Cr(IV) were employed to determine contributions from each ion to Cr $2p_{3/2}$ photopeaks collected from the various cell treatments. Whilst in all cases Cr(III) was the major ion associated with cell surfaces, a significant contribution was identified due to Cr(IV) in anaerobically grown cells. The Cr(IV) contribution was far less when cells were grown aerobically. Moreover, when anaerobically grown cells were exposed to oxygen very little re-oxidation of Cr-precipitates occurred, the precipitates were again identified as a mixture of Cr(III) and Cr(IV). A positive relationship was observed between amounts of chromium and phosphorous associated with cell surfaces resulting from the various treatments, suggesting the precipitates included Cr(III)-phosphate. The fact that Cr(IV) remained associated with precipitates following re-oxidation suggests that under anaerobic conditions the intermediate ion is afforded sufficient stability to be incorporated within the precipitate matrix and thus conferred a degree of protection from oxidation.
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1. Introduction

Anthropogenic input of chromium to the environment results from a number of industrial and related processes [1]. Since high valence state chromium species (i.e. Cr(VI) and Cr(V)) are both mobile and toxic they present a significant cause for concern. Chromate is a known carcinogen [2], however, the pentavalent ion although unstable, is thought to mediate DNA cleavage [3] and therefore represents a potential and pernicious problem.

Immobilization of oxidized Cr species can be achieved by reduction to the less soluble, and thus less mobile, stable trivalent form. This is accomplished by various mechanisms, loosely divided into abiogenic and biogenic processes. Mineral surfaces

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including goethite (α-FeOOH) [4], amorphous FeS [5] and ferrous-containing phyllosilicates [6,7] have been identified as sites of abiotic reduction of chromate and as sites for precipitate nucleation [8]. The common reductive agent in these processes is the ferrous ion [9,10] and this may also be generated by Fe(III)-reducing organisms in anaerobic sediments. However, direct bacterial reduction of chromate, both aerobic and anaerobic is also well documented in a wide range of bacteria [11–13].

Environmental chromium chemistry is usually considered in terms of the stable end-members; Cr(VI) reduction to Cr(III) and vice versa. This however is an over simplification of a complex chemistry which can include the intermediate but less stable Cr(V) and Cr(IV) ions. Both intermediates have been identified during Cr(III) oxidation at birnessite ($MnO_{1.75}(OH)_{0.25}$) surfaces [14] and the pentavalent ion has been identified during direct reduction of chromate ions by the aerobe *Pseudomonas ambigua* strain G-1 [15] and anaerobic Cr(VI)-reduction by the facultative anaerobe *Shewanella oneidensis* strain MR-1 [16]. The identification of intermediate chromium phases both in abiotic oxidation and biotic reduction mechanisms suggests both processes proceed via successive, one-electron steps. Here, we present electron energy loss and X-ray photoelectron spectroscopic evidence for the presence of both Cr(III) and Cr(IV) phases associated with surfaces of *S. oneidensis* strain DSP10 in the presence of chromate under both aerobic and anaerobic conditions. Furthermore, the reduction products appear resistant to re-oxidation once formed.

2. Experimental

2.1. Shewanella culture conditions

The facultative anaerobe *S. oneidensis* strain DSP10 was used for all experiments described here. The organism was originally isolated from Lake Oneida, NY and has previously been classified as *Alteromonas putrefaciens* and *S. putrefaciens*. When growing anaerobically, *Shewanella* spp. are able to utilize a wide range of inorganic and organic molecules as terminal electron acceptors including iron and manganese oxides, and fumarate and oxidized sulfur species [17].

In all cases, DSP10 cells were grown at 30 °C in a defined growth medium [18] containing; 9 mM $(NH_4)_2SO_4$, 5.7 mM $K_2HPO_4{\cdot}3H_2O$, 3.3 mM KH_2PO_4, 2 mM $NaHCO_3$, 1 mM $MgSO_4{\cdot}7H_2O$, 500 μM $CaCl_2{\cdot}2H_2O$, 70 μM $Na_2EDTA{\cdot}2H_2O$, 60 μM H_3BO_3, 10 μM NaCl, 6 μM $FeSO_4{\cdot}7H_2O$, 5 μM $Ni(NH_4)(SO_4)_2{\cdot}6H_2O$, 4 μM $Na_2MoO_4{\cdot}2H_2O$, 1.5 μM Na_2SeO_4, 1.3 μM $MnSO_4{\cdot}H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$ and 0.2 μM $CuSO_4{\cdot}5H_2O$. The amino acids *L*-arginine, *L*-serine and *L*-glutamic acid were added at a concentration of 0.02 mg ml^{-1}, and 32 mM sodium lactate was added as a carbon source and electron donor. Electron acceptors were either 32 mM fumarate or 100 μM CrO_4^{2-} (as $K_2Cr_2O_4$). Medium pH was adjusted to pH 7 with 10 N KOH.

Four treatments were studied in an attempt to understand Cr(VI) reduction under both aerobic and anaerobic conditions and determine the stability of the reduced forms to re-oxidation. In each case, cells were grown in M1 medium with addition of the appropriate electron acceptor for 30 days before being harvested by centrifugation. Cells were grown anaerobically in M1 medium containing either fumarate (no chromate) or potassium chromate. Additionally, cells were grown aerobically in M1 medium containing Cr(VI). Finally, a batch of anaerobically grown cells and associated reduction products were washed and prepared aerobically.

2.2. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was used to establish the microstructure of and spatial relationship between the bacteria and chromate reduction products and was chosen for its high resolution capabilities. Harvested cells were fixed in 1 ml, 5% aqueous solution of glutaraldehyde (Grade I, Sigma) overnight at room temperature. Fixed cells were washed three times in distilled water, and treated with 2% osmium tetroxide (aqueous solution) for 1 h. After washing three times in distilled water, the pellets were processed through a gradual ethanol dehydration sequence (in 35, 50, 75, and 95% ethanol in deionized water), 3–10 min washes in 100% ethanol, and 3–10 min washes in propylene oxide. The specimen was embedded in Spurr low viscosity epoxy resin. Increasing concentrations of Spurr resin in propylene oxide were infiltrated into the pellets at mixtures of 1:1, 2:1,

3:1 and 100% resin for 1 h each. Cell pellets were infiltrated a second time with fresh Spurr resin for 1 h and cured at 70 °C for 2 days. Specimens of 80 nm thickness were sectioned from the embedded blocks using a Leica UltraCut UCT microtome and mounted on amorphous carbon coated Cu TEM grids. A JEOL JEM-3010 transmission electron microscope operating at 300 keV with a LaB_6 filament was used in this study. This instrument is capable of electron energy loss spectroscopy (EELS). Experimental conditions for EELS analysis can be found in [13].

2.3. X-ray photoelectron spectroscopy (XPS)

XPS was employed to determine the valence state of Cr associated with DSP10 cell surfaces. When necessary, cell preparatory procedures were carried out under an oxygen-free (95% N_2, 5% H_2) atmosphere to ensure chemical integrity, otherwise no other precautions were taken. Prior to mounting for XPS, cells were harvested from the growth medium (20 ml) by centrifugation and washed once in chilled distilled, deionised water (resitivity >18 Ω m) [19] previously filtered (0.2 μm) and purged with O_2-free N_2 (if required). The cells were harvested a second time by centrifugation and resuspended in 20 μl H_2O, placed on a Si wafer and allowed to dry under a stream of N_2 over silica desiccant for 7 h. Once dry, anaerobic samples were transported to the spectrometer in a portable gas tight chamber, aerobic samples under ambient atmosphere. Cr(III) reference compounds were also mounted under an anaerobic environment. Two compounds were employed, Cr(III) acetate ($(Cr_3(OH)_2(OOCCH_3)_7$, Strem Chemicals Inc., Newburyport, MA) and Cr(III) phosphate ($CrPO_4{\cdot}4H_2O$, Alfa Aesar, Ward Hill, MA).

Spectra were collected on a Perkin-Elmer Physical Electronics Division Model 5600 ci spectrometer(Perkin-Elmer, Eden Prairie, MN). The spectrometer was calibrated employing the Au $4f_{7/2}$, Cu $2p_{3/2}$ and Ag $3d_{5/2}$ photopeaks with binding energies of 83.99, 932.66 and 368.27 eV, respectively. A consistent 400 μm spot size was analyzed on all surfaces using a monochromatized Al Kα ($h\nu = 1486.6$ eV) X-ray source at 300 W and a pass energy of 46.95 eV for survey scans, and 11.75 eV for high-resolution scans. The system was operated at a base pressure of 10^{-8} to 10^{-9} torr. A consistent emission angle was used throughout ($2\theta = 45°$). Sample charging was compensated for by employing a 5 eV electron flood gun and referencing the C–$H_{aliphatic}$ C 1s binding energy to 284.8 eV [19,20]. Cr valence state was determined employing multiplet-splitting models derived for the $2p$ photopeaks of the Cr(IV) and Cr(III) ions [21,22] affording more detailed valence state analysis than EELS spectra can currently provide. The applicability of these models to resolving contributions from various Cr ions has been demonstrated [14,23]. Peaks were fit using a 70:30 Gaussian:Lorenztian peak shape and a consistent full width at half maximum (1.4 eV). The Cr(IV) ion is represented by four multiplet peaks, the Cr(III) ion by three. All peak shifts and relative intensities are as described in [14]. By indexing the binding energy shift and intensity of each multiplet peak to the principal peak for the respective ion the fitting procedure is simplified, only those parameters pertaining to the principal peaks need be adjusted iteratively. All peak fitting was preceded by baseline subtraction employing the Shirley algorithm [24]. Since there is evidence that Cr species undergo photoreduction during X-ray exposure [25], spectral collection times were therefore standardized for all samples at 30 min.

3. Results and discussion

3.1. XPS of surfaces of fumarate grown cells

Low resolution XPS spectra of *S. oneidensis* strain DSP10 cells grown anaerobically with fumarate as electron acceptor indicate that other than C, N and O, no significant contributions were present from other elements associated with cell surfaces (Fig. 1a). High resolution spectra collected from the Cr $2p_{3/2}$ core region indicate that indeed there is no Cr associated with the cell surfaces (Fig. 1b).The C 1s core region (Fig. 1c) indicates that contributions are evident from a number of moieties as judged by the width of the photopeak.

Contributions from C–H, C–N/C–O, and C=O groups commonly associated with bacterial surfaces [19] are present. High resolution spectra collected from the P 2p region (Fig. 1d) also indicate that phosphate groups were present at the cell surface [26] despite low resolution spectra indicating otherwise.

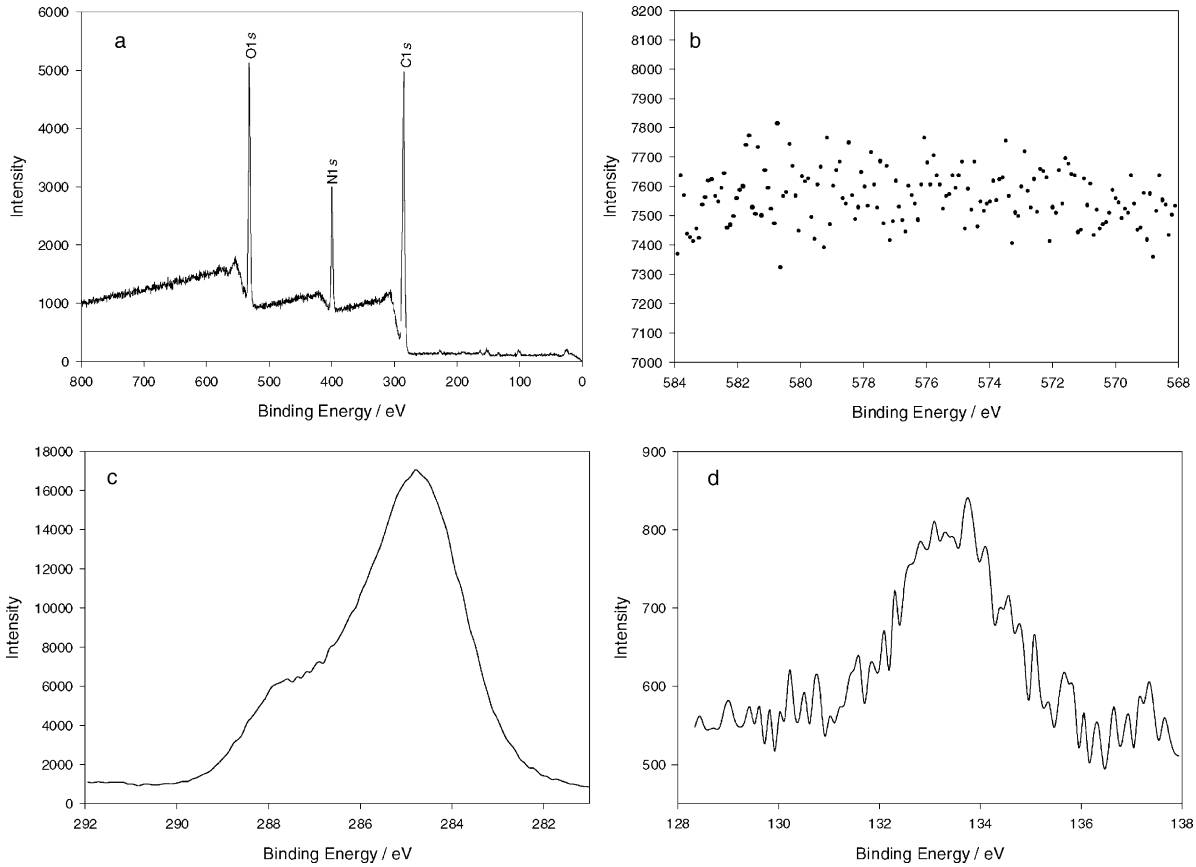

Fig. 1. X-ray photoelectron spectra collected from *S. oneidensis* cells grown anaerobically with fumarate as terminal electron acceptor: (a) low resolution spectrum suggests that the only significant photopeaks are due to O, C and N associated with the cell surface; (b) high resolution spectrum of the Cr $2p_{3/2}$ core region indicates that no chromium is associated with cell surfaces, (c) C 1s core region demonstrates a broad peak envelope indicative of various C-moities from C–H aliphatic at 284.8 eV to C=O at 287.8 eV, (d) P 2p core region indicates the presence of phosphate groups at the cell surface. All spectra were referenced to a C 1s C–H aliphatic photopeak at 284.8 eV.

At least a portion of this is likely to be derived from the growth medium.

3.2. Cell surface microstructure of chromium associated cells

Cross sectional images of *S. oneidensis* cells recovered from anaerobic cultures containing chromate as the electron acceptor by TEM (Fig. 2a) indicated cells heavily encrusted with electron-dense precipitates. No evidence of intracellular precipitates is found, suggesting restriction to the bacterial outer surface. Precipitates ranged in size from approximately 10 to 200 nm. Selected area diffraction indicated the precipitates were predominantly amorphous, perhaps due to a high degree of hydration however, precipitates appeared stable under the electron beam. Microanalysis of isolated precipitates proximal to cell walls demonstrate they are chromium-rich based on the presence of strong Cr–L_3 ($2p_{3/2}$) and Cr–L_2 ($2p_{1/2}$) peaks in EELS spectra (Fig. 2b). The measured chemical shift, i.e. mean position of the Cr–L_3 peaks at 576.0 ± 0.4 eV, is consistent with Cr(III) [13]. The EELS edge onset, the sudden rise in intensity preceding each of the $L_{2,3}$ peaks, represents the ionization threshold which approximately corresponds to the inner-shell binding energy measured by XPS.

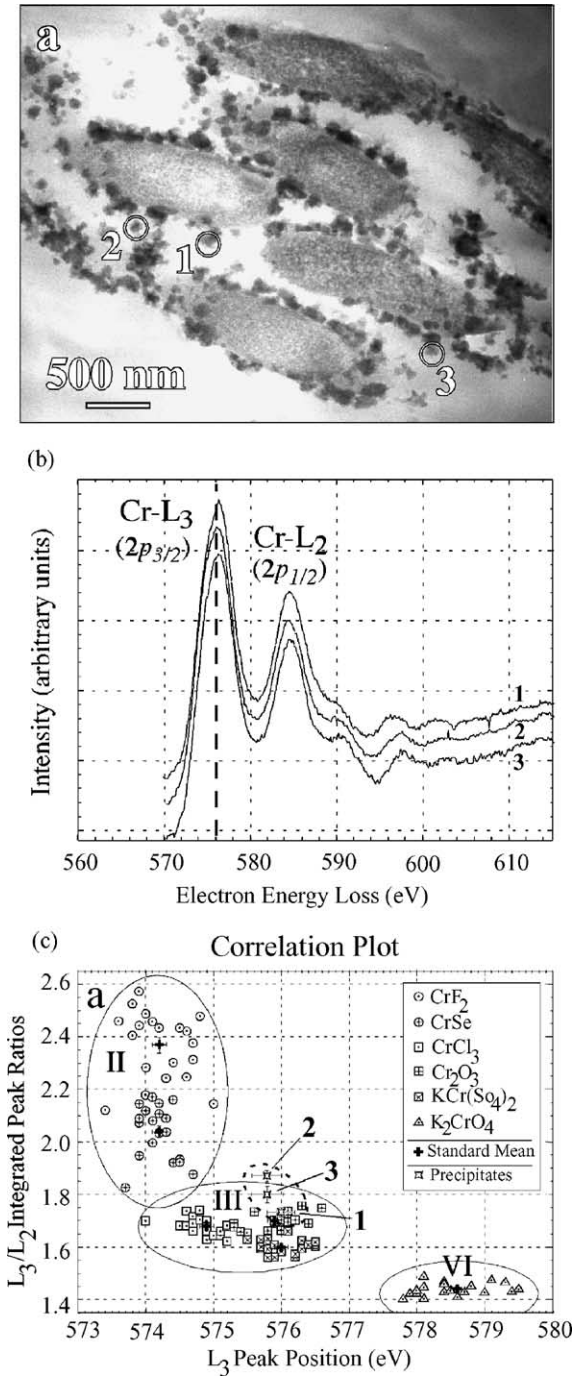

Fig. 2. *S. oneidensis* strain DSP10 cells imaged by TEM in approximately 80 nm thick, thin-section; (a) cell is encrusted with an electron-dense particulate material, (b) replicate EELS spectra from regions 1 and 2 in (a) indicate the presence Cr in trivalent form. Circles in (a) indicate the electron probe diameter.

3.3. Chromium phases associated with Shewanella cells

Low resolution XPS spectra of cells grown anaerobically with chromate as the sole terminal electron acceptor identified a significant contribution due to Cr at the surfaces of the cells (Fig. 3a). Other elements present in the growth medium were also identified as Ca, K, P and Mg in contrast to the spectra collected from fumarate grown cells (Section 3.1). Lack of significant contributions above 578.5 eV to the high resolution Cr $2p_{3/2}$ spectrum (Fig. 3b) suggests a generally reduced sample with a lack of Cr(V) and Cr(VI) ions [25,27,28]. The broad peak envelope was therefore modeled employing multiplet-splitting patterns derived for the Cr(IV) and Cr(III) ions. The best fit to the experimental spectrum was provided by a Cr(IV) contribution at 576.2 eV, consistent with CrO_2 [29], and a contribution due to Cr(III) at 577.1 eV consistent with CrOOH [25,29]. The Cr(III) contribution represented 56.1 at.% and the Cr(IV) contribution 43.9 at.% of the fitted region (but not the total peak area). However, as demonstrated by the residuals illustrated in Fig. 3b, there was a significant lack of fit of the model to the experimental peak envelope, especially in the low binding energy region (ca. 575 eV). Such a lack of fit could result from a contribution due to a Cr compound other than an oxide or hydroxide which is not accounted for in the fit.

Besides carbon, which as acidic moieties of bacterial exopolymers may complex metal species (for example, [30,31]), another potential complexant present at the cell surface is phosphorous (Fig. 3a). Two model compounds were thus studied in an attempt to demonstrate the possibility that a Cr-species other than oxy(hydroxide) was present at the surface of the cells. The high resolution C 1s spectrum collected from the cell surface is shown in Fig. 3c together with the same region collected form Cr(III)-acetate. A contribution at elevated binding energy (287–290 eV) is evident in the spectrum collected from the cell surface consistent with that of Cr(III)-acetate and of ester and carboxyl groups. With regard to phosphorous, the P 2p region collected from DSP10 cells is shown in Fig. 3d together with the same region collected from Cr(III)-phosphate. The peak positions are in good agreement. Although not unequivocal evidence, spectra collected from the two reference

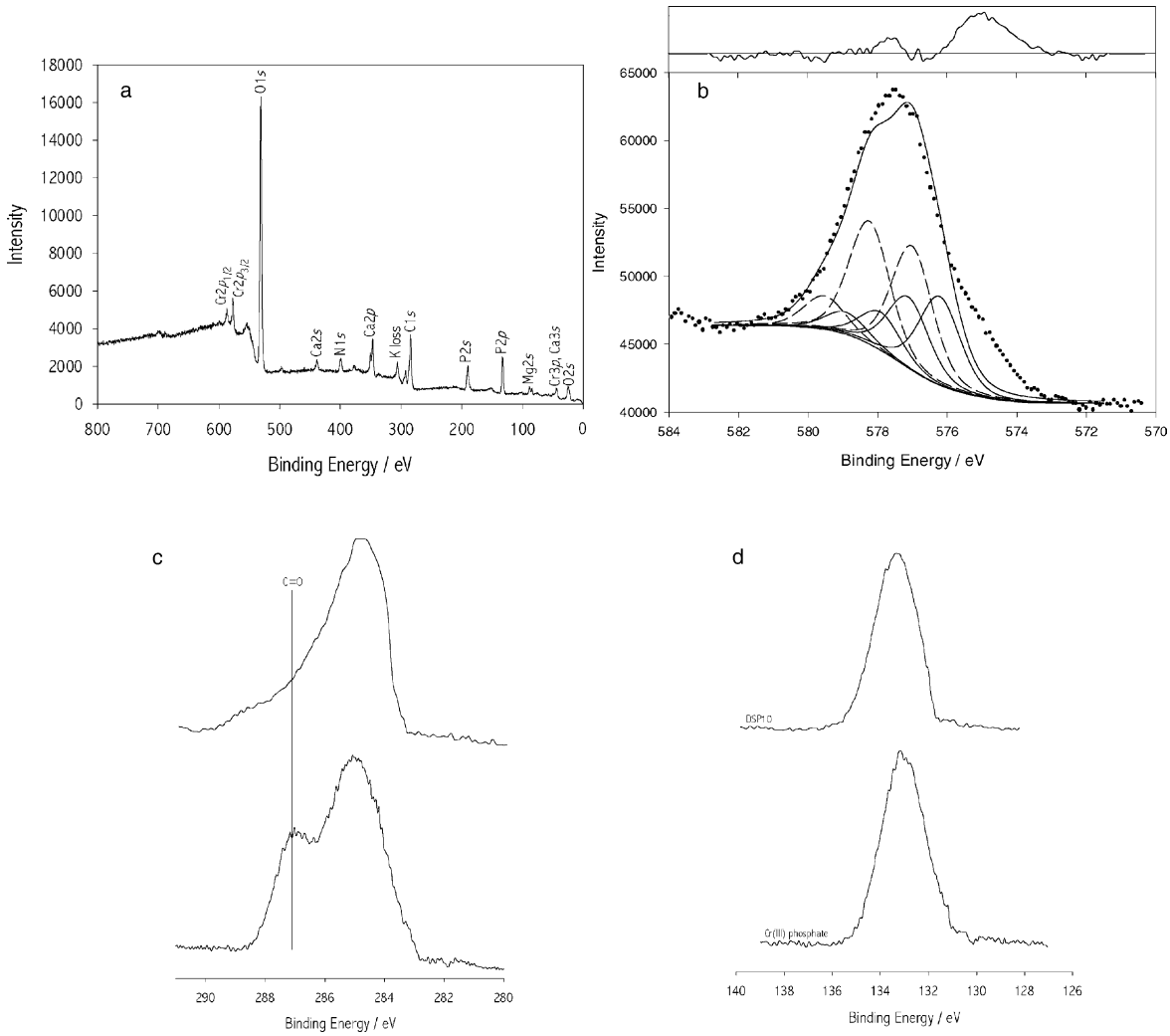

Fig. 3. X-ray photoelectron spectra collected from *S. oneidensis* cells grown anaerobically with chromate as terminal electron acceptor; (a) low resolution spectrum indicates contributions from a number of growth medium components including Cr and P, (b) the high resolution Cr $2p_{3/2}$ spectrum indicates a lack of peaks above 578.5 eV suggestive of a generally reduced sample, the peak envelope is therefore modeled employing multiplet-splitting models derived for the Cr(III) (solid fitted curves) and Cr(IV) (dashed fitted curves) ions. The original data is represented by the data points whilst the model is represented by the solid curve, the residuals resulting from the fitted model are shown above the main figure, contributions due to Cr(III) at 577.1 eV representing 56.1 at.% and Cr(IV) at 576.2 eV representing 43.9 at.% proved the best fit to the peak envelope. All fitted curves had a full width at half maximum of 1.4 eV. See text for a discussion of the fitted models and the lack of model agreement with the data, (c) C 1s core region collected from DSP10 cells together with that collected from Cr(III)-acetate, (d) P 2p core region collected from DSP10 cells and that collected from Cr(III)-phosphate. All spectra were referenced to a C 1s C–H aliphatic photopeak at 284.8 eV.

compounds suggests that Cr(III) may be present at the cell surface either in an organic complex or as a phosphate. Fig. 4 details the Cr $2p_{3/2}$ core region collected on the two compounds. Fitting of the multiplet splitting models is problematic since unlike Cr_2O_3, where Cr(III) can be considered to be largely ionicly bonded within an octahedral field and therefore modeled as the free ion, in the two reference compounds the line shape may be altered due to axial field distortion [23]. Without fitting a model to

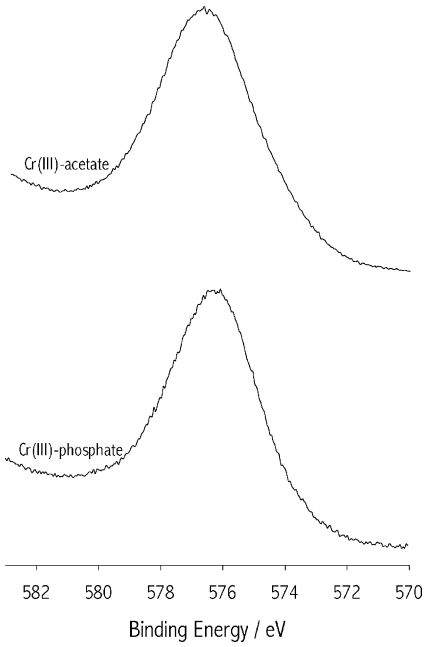

Fig. 4. High resolution Cr $2p_{3/2}$ spectra collected from Cr(III)-acetate (top) and Cr(III)-phosphate (bottom). Both spectra indicate a contribution at 575 eV which could account for the lack of fit of the multiplet-splitting models to the spectrum collected from chromate exposed cells. All spectra were referenced to a C 1s C–H aliphatic photopeak at 284.8 eV.

represent the multiplet splitting in the two compounds, however, contributions at reduced binding energy (ca. 575 eV) are evident in both spectra. Thus, the possibility exists that Cr may be present at the cell surface in the form of an organic complex or Cr(III)-phosphate phase, or both.

The asymmetric peak shape evident in the Cr $2p_{3/2}$ region collected from the cells surface (Fig. 3b) may arise due to differential sample charging. We made every effort to ensure that this did not occur; a flood gun was employed to compensate for the monochromatic source, and the sample was shielded. However, since the other core regions do not share the asymmetry we conclude that it is inherent to the Cr core-region spectrum, indeed the shape is consistently observed in different batches of cells grown anaerobically in the presence of chromate. Asymmetry of Cr 2p photopeaks has been reported for various CrP phases (i.e. $Cr_{93}P_7$–$Cr_{72}P_{28}$) [32] however, such structures are inconsistent with P 2p spectra collected from cells which suggest the presence of PO_4^{2-} ions (Fig. 3d) [26].

Surfaces of cells grown aerobically in the presence of chromate ions were relatively less rich in Cr (relative to the C 1s photopeak) than surfaces of anaerobically incubated cells (Fig. 5a, cf. Fig. 3a). It is also the case that less of other medium components were associated with the cell surface, of particular note phosphorous. Despite being grown in an aerobic medium, the Cr associated with the cell surface was in a reduced form. As before the Cr $2p_{3/2}$ photopeak was best described by a combination of Cr(III) and Cr(IV) multiplet splitting models, the Cr(IV) contribution at 575.1 eV representing 23.7 at.% and the Cr(III) at 577.1 eV representing 76.3 at.% of the total peak area. The fitted model was a reasonable fit to the photopeak envelope and there was no indication of a lack of fit at low binding energy as observed with the anaerobically grown cells. However, the binding energy of the putative Cr(IV) contribution is significantly (0.8 eV) lower than previously reported values [14], we therefore remain cautious regarding its assignment, however no Cr(III) binding energy has been reported below 576.3 eV [14] and Cr(IV) remains the best assignment for this fitted contribution.

3.4. Reoxidation of Cr-phases associated with cell surfaces

Possible re-oxidation of cell associated precipitates during washing was following by preparation under aerobic conditions. XPS revealed a sample similar to cells grown and prepared under anaerobic conditions. Relatively high levels of Cr were associated with cell surfaces (Fig. 6a). High resolution Cr $2p_{3/2}$ spectra indicated once more a lack of oxidized species, the photopeak was adequately fit using the Cr(III) at 577.3 eV (59.4 at.%) and Cr(IV) at 575.6 eV (40.6 at.%) models. There was therefore a Cr(IV) contribution of similar quantity as that derived for the Cr associated with anaerobically grown cells. The increased contribution from Cr was accompanied by an increased contribution due to phosphorous and the P 2p photopeak again suggested the presence of PO_4^{2-} groups (Fig. 6d).

3.5. Influence of growth conditions upon reduced phase chemistry

In all instances where chromate was added to the growth medium, no Cr(VI) or Cr(V) phases were

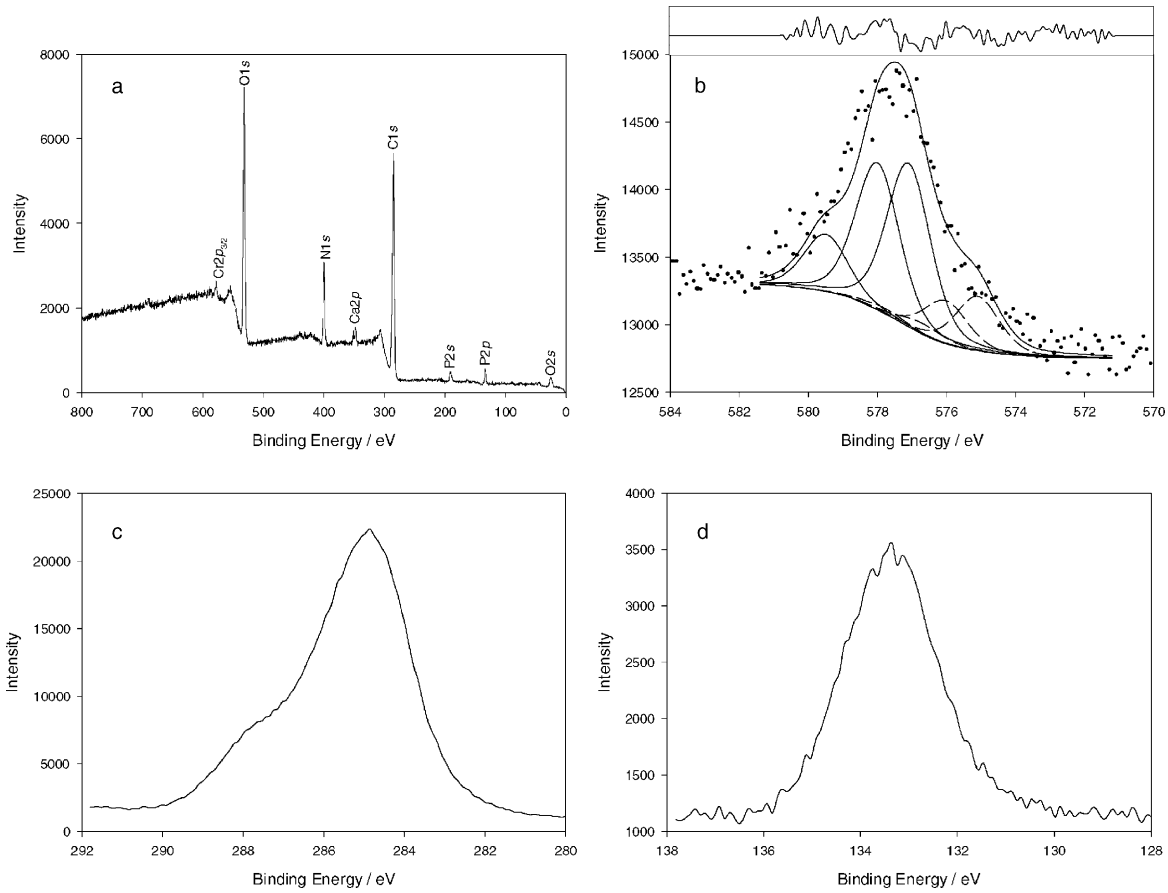

Fig. 5. X-ray photoelectron spectra collected from *S. oneidensis* cells grown aerobically in the presence of with chromate; (a) low resolution spectrum indicates contributions from Cr and P, (b) high resolution Cr $2p_{3/2}$ spectrum is again modeled employing Cr(III) and Cr(IV) models, contributions are as described for Fig. 2, (c) C 1s core region spectrum again exhibits a contribution between 287 and 288 eV consistent with C=O groups of Cr(III)-acetate, and (d) phosphate groups are again evident from the P 2p core region. All spectra were referenced to a C 1s C–H aliphatic photopeak at 284.8 eV.

identified by XPS of cell surfaces. Even under aerobic conditions cells appear able to directly reduce chromate however, the amount of Cr-species associated with the cells was considerably reduced compared to anaerobic reduction. Concomitant with this reduction of associated Cr-phases there was also a reduction in P-species. This circumstantial evidence suggests that at least a portion of the reduced Cr may be present as a P-containing compound, P 2p photopeaks suggest the presence of phosphate groups, and are consistent with spectra collected from $CrPO_4{\cdot}4H_2O$. We have modeled the Cr $2p_{3/2}$ photopeak envelope using multiplet-splitting models derived for Cr(III) (i.e. CrOOH and Cr_2O_3) and Cr(IV) (i.e. CrO_2). The implied association of $CrPO_4$ with cell surfaces could explain the lack of fit of the applied multiplet-splitting models to the photopeak envelope collected from anaerobically grown cells since the phosphate is unlikely to conform to models of the free ions. Phosphate compounds have previously been identified in association with *Shewanella*, specifically vivianite ($Fe_3(PO_4)_2{\cdot}8H_2O$) [33]. Notwithstanding this lack of model agreement, it is interesting to note that under aerobic conditions there is a reduced contribution from Cr(IV). Stable forms of chromium are the hexa-and trivalent ions, intermediate phases are less stable [1]. It would appear

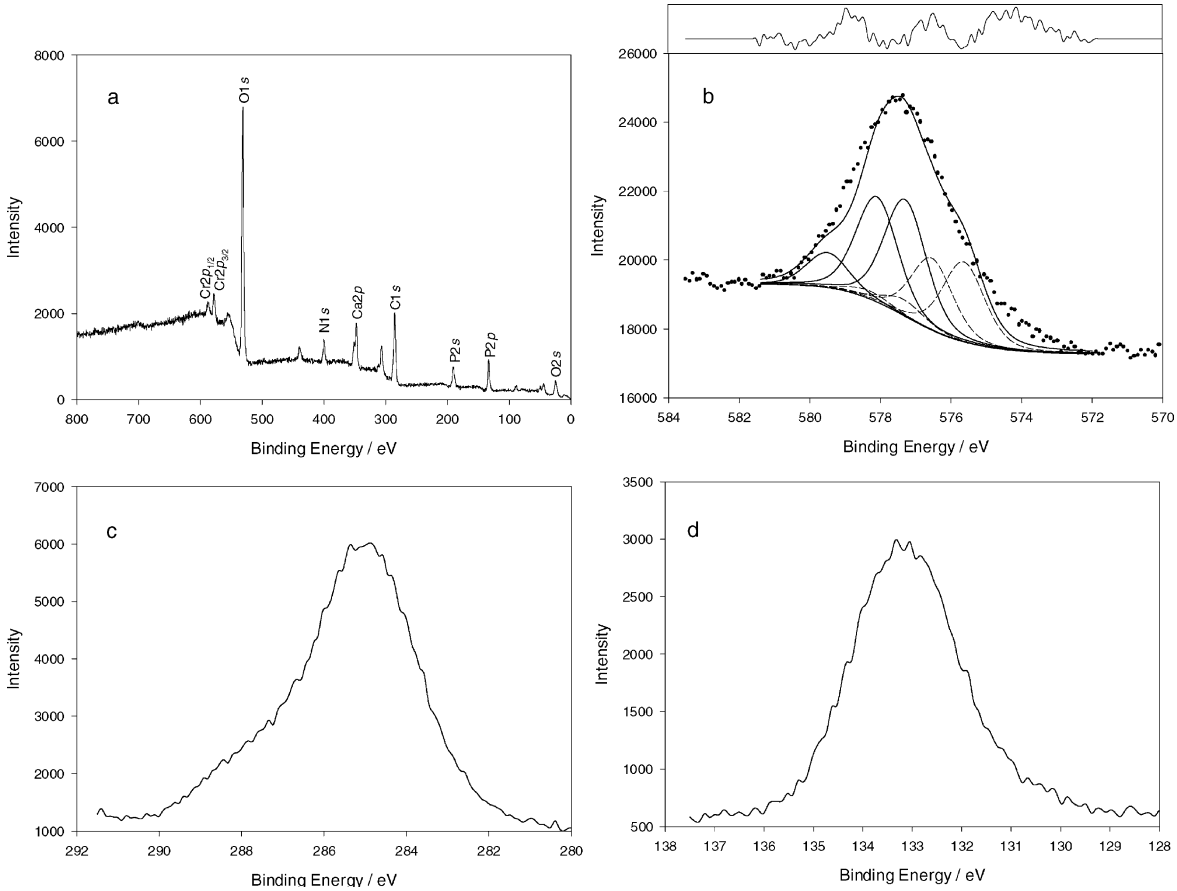

Fig. 6. X-ray photoelectron spectra collected from *S. oneidensis* cells grown anaerobically with chromate as electron acceptor exposed to oxygen during washing and preparation; (a) low resolution spectrum again indicates the presence of significant contributions due to Cr and P, (b) high resolution Cr $2p_{3/2}$ spectrum indicates a lack of oxidized species and is consistent with the spectrum collected from anaerobic cells (Fig. 2), C 1s (c) and P 2p (d) high resolution spectra are consistent with those collected from anaerobic cells (Fig. 2). All spectra were referenced to a C 1s C–H aliphatic photopeak at 284.8 eV.

therefore, that under aerobic conditions intermediate ions, if present, are quickly oxidized and only Cr(III)-phases accumulate to any significant level. Evidence for the existence of intermediate phases is plentiful both from abiotic [14,34,35] and biotic [15,16] redox reactions. Under anaerobic conditions where the intermediates are conferred a degree of stability due to reduced oxidation it would appear that the Cr(IV) may become incorporated into the precipitate structure since re-oxidation of phases formed under anaerobic conditions does not result in a significant reduction of Cr(IV) contribution to the modeled Cr $2p_{3/2}$ photopeak.

4. Conclusions

Under all conditions examined, *S. oneidensis* strain DSP10 cells were able to reduce chromate to nominally Cr(III). Significant contributions due to Cr(IV) were identified in precipitates formed in anaerobic media which were not observed associated with cells grown aerobically. Furthermore, more Cr was observed associated with anaerobically grown cells than aerobically grown. Association between the amounts of Cr and Phosphorous suggest that the cell-associated precipitates likely contain Cr(III)-phosphate.

Acknowledgements

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References

[1] J. Kotaś, Z. Stasicka, Env. Poll. 107 (2000) 263.
[2] M. Cieslak-Golonka, Polyhedron 15 (1995) 3667.
[3] R.P. Farrell, R.J. Judd, P.A. Lay, N.E. Dixon, R.S.U. Baker, Chem. Res. Toxicol. 2 (1989) 227.
[4] H. Abdel-Samad, P.R. Watson, Appl. Surf. Sci. 108 (1997) 371.
[5] R.R. Patterson, S. Fendorf, M. Fendorf, Environ. Sci. Technol. 31 (1997) 2039.
[6] E.S. Ilton, D.R. Veblen, Geochim. Cosmochim. Acta 58 (1994) 2777.
[7] M.F. Brigatta, G. Franchini, C. Lugli, L. Medici, L. Poppi, E. Turci, Appl. Geochem. 15 (2000) 1307.
[8] N.J. Anderson, B.A. Bolto, L. Pawlowski, Nucl. Chem. Waste Manage. 5 (1984) 125.
[9] L.E. Eary, D. Rai, Environ. Sci. Technol. 22 (1988) 972.
[10] S. Fendorf, G. Li, Environ. Sci. Technol. 30 (1996) 1614.
[11] Y.-T. Wang, H. Shen, J. Ind. Microbiol. 14 (1995) 159.
[12] L. Fude, B. Harris, M.M. Urrutia, T.J. Beveridge, Appl. Environ. Microbiol. 60 (1994) 1525.
[13] T.L. Daulton, B.J. Little, K. Lowe, J. Jones-Meehan, Microsc. Microanal. 7 (2001) 470.
[14] D. Banerjee, H.W. Nesbitt, Geochim. Cosmochim. Acta 63 (1999) 1671.
[15] T. Suzuki, N. Miyata, H. Horitsu, K. Kawai, K. Takamizawa, Y. Tai, M. Okazaki, J. Bacteriol. 174 (1992) 5340.
[16] C.R. Myers, B.P. Carstens, W.E. Antholine, J.M. Myers, J. Appl. Microbiol. 88 (2000) 98.
[17] M.D. Blakeney, T.J. DiChristina, Microbiol. Res. 155 (2000) 87.
[18] C.R. Myers, K.H. Nealson, Science 240 (1988) 1319.
[19] H.C. van der Mei, J. de Vries, H.J. Busscher, Surf. Sci. Reports 39 (2000) 1.
[20] P. Swift, Surf. Interf. Anal. 4 (1982) 47.
[21] R.P. Gupta, S.K. Sen, Phys. Rev. B 10 (1974) 71.
[22] R.P. Gupta, S.K. Sen, Phys. Rev. B 12 (1975) 15.
[23] A.R. Pratt, N.S. McIntyre, Surf. Interf. Anal. 24 (1996) 529.
[24] D.A. Shirley, Phys. Rev. B 5 (1972) 4709.
[25] G.P. Halada, C.R. Clayton, J. Electrochem. Soc. 138 (1991) 2921.
[26] R. Franke, T. Chassé, P. Streubel, A. Meisel, J. Electron Spectrosc. Related Phenom. 56 (1991) 381.
[27] G.C. Allen, M.T. Curtis, A.J. Hooper, P.M. Tucker, J. Chem. Soc., Dalton Trans. 16 (1973) 1675.
[28] H. Konno, H. Tachikawa, A. Furusaki, R. Furuichi, Anal. Sci. 8 (1992) 641.
[29] I. Ikemoto, K. Ishi, S. Kinoshita, H. Kuroda, M.A. Alario Franco, J.M. Thomas, J. Solid State Chem. 17 (1976) 425.
[30] I.B. Beech, V. Zinkevich, R. Tapper, R. Gubner, R.J. Avci, J. Microbiol. Methods 36 (1999) 3.
[31] L.-S. Johansson, T. Saastamoinen, Appl. Surf. Sci. 144/145 (1999) 244.
[32] T.P. Moffat, R.M. Latanision, R.R. Ruf, Electrochim. Acta 40 (1995) 1723.
[33] J.M. Zachara, J.K. Fredrickson, S.-M. Li, D.W. Kennedy, S.C. Smith, P.L. Gassman, Am. Miner. 83 (1998) 1426.
[34] J.H. Espensen, Acc. Chem. Res. 3 (1970) 347.
[35] J.Y.-P. Tong, E.L. King, J. Am. Chem. Soc. 82 (1960) 3805.