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The role of iron in hexavalent chromium reduction by municipal landfill leachate

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ABSTRACT

The function of iron (ferric (Fe(III)) and ferrous (Fe(II))) in the hexavalent chromium (Cr(VI)) reduction mechanism by bacteria in municipal landfill leachate (MLL) was assessed. Evidence of an "electron shuttle" mechanism was observed, whereby the Cr(VI) was reduced to trivalent chromium (Cr(III)) by Fe(II) with the resulting Fe(III) bacterially re-reduced to Fe(II). Typically, investigations on this electron shuttle mechanism have been performed in an artificial medium. As MLL comprises an elaborate mixture of bacteria, humic materials and organic and inorganic species, additional complexities were evident within the cycle in this study. Bioavailability of the Fe(III) for bacterial reduction, availability of bacterially produced Fe(II) for chemical Cr(VI) reduction and hydrolysis of Fe(II) and Fe(III) become prevalent during each phase of the shuttle cycle when MLL is present. Each of these factors contributes to the overall rate of bacterial Cr(VI) reduction in this media. This work highlights the need to consider local environmental conditions when assessing the bacterial reduction of Cr(VI).

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1. Introduction

Chromium is a common pollutant in the environment resulting from widespread industrial use [1,2]. The toxicity of chromium depends on its oxidation state. Hexavalent chromium (Cr(VI)) species, such as CrO_4^{2-} and $Cr_2O_7^{2-}$, are toxic, mutagenic and carcinogenic [2,3], whereas trivalent chromium (Cr(III)) is essential for humans [3,4]. Cr(VI) has high redox potential, is soluble in water and can actively react with the immediate environment [2,5].

Cr(VI) can be reduced to Cr(III) by Fe(II) and sulfide via chemical reaction and the reduction rates vary with experimental parameters such as pH, temperature, dissolved oxygen and the ratios of Cr(VI) and Fe(II) or sulfide [6–8]. The chemical reduction of Cr(VI) by Fe(II) has been reported to be relatively fast, taking from tens of seconds to several hours to reach completion [6,7,9]. Sedlak and Chan [7] and Kim et al. [8] reported the kinetics of chemical reduction of Cr(VI) by Fe(II) and sulfide to be first-order with respect to Cr(VI) and Fe(II) or sulfide. Fe(II) and sulfide are common in reducing environments, with Fe(III)-reducing and sulfate-reducing bacteria being reported to reduce Fe(III) to Fe(II) and sulfate to sulfide [10–14], respectively. The iron-reducing bacterium, *Shewanella alga* strain BrY, is capable of converting Cr(VI) to Cr(III) through the microbial reduction of Fe(III) to Fe(II) [15,16]. Sulfide generated by sulfate-reducing bacteria is also capable of reducing Cr(VI) [17–19]. In these reactions, a bacterial process produces Fe(II) or sulfide species which in turn reduce Cr(VI) via an indirect chemical reaction [15,17,20].

Many bacteria are capable of reducing Cr(VI) to Cr(III) through direct microbial reaction, either enzymatically or non-enzymatically. *Escherichia coli* ATCC 33456 can reduce Cr(VI) via an enzymatic reaction on the cell surface [21,22]. Cytochrome c_3 has been identified as the enzyme responsible for Cr(VI) reduction by *Desulfovibrio vulgaris* [23]. A *Pseudomonad* (CRB5), isolated from a decommissioned wood preservation site, also demonstrated an ability to reduce Cr(VI) through an enzymatic reaction [24]. Some facultative anaerobic bacteria, such as *Pantoea agglomerans* SP1, can use Cr(VI) as an alternate electron acceptor for their anaerobic growth [25,26].

Despite the knowledge of Cr(VI) reduction by chemical and microbial processes, there remains limited understanding of Cr(VI) reduction by microbial communities in the natural environment. Landfill leachate generated from municipal landfills typically contains high concentrations of humic substances, large quantities of inorganic and organic pollutants, as well as a consortium of bacteria [27,28]. Previous works have shown municipal landfill leachates (MLL) can reduce Cr(VI), whereas non-putrescible landfill leachate

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can not [29,30]. The reduction of Cr(VI) by MLL was observed to be due to bacterial presence and was related to the concentrations of bacterial biomass, organic matter and initial Cr(VI) concentration [29]. This study continues the prior work, seeking to clarify the mechanism for Cr(VI) reduction in MLL through the role played by Fe(III)/Fe(II), as well as investigating the effects of sulfate on Cr(VI) reduction.

2. Materials and methods

2.1. Landfill leachate

The MLL used in this study was taken from a municipal landfill in Australia with its characteristics provided in Table 1. It possessed a pH of 7.7 with a pH buffer capacity of 6.6 mmol H^+/pH unit, a redox potential of −310 mV and contained high total organic and inorganic carbon levels as well as a range of inorganic pollutants. This included a 6.5 mg L^{-1} iron content. It contained a high concentration of bacterial biomass. An optical microscopy examination showed the bacteria in the MLL were rod shaped (length <10 μm). The dominant bacterial species was identified as *Bacillus megaterium* by API 50 CH fermentation assays (BioMérieus) [31].

2.2. Cr(VI), Fe(III), Fe(II), sulfate solutions and bacterial growth medium

A 1000 mg L^{-1} stock Cr(VI) solution (pH 4.2) was prepared by dissolving $K_2Cr_2O_7$ in deionised water. Stock solutions (5000 mg L^{-1}) of Fe(III) (pH 1.7), Fe(II) (pH 4.3) and sulfate (pH 5.9) were prepared by dissolving $FeCl_3{\cdot}6H_2O$, $FeCl_2$ and Na_2SO_4, respectively, in deionised water. A chemically defined growth medium was prepared by dissolving 5.0 g glucose, 1.0 g $(NH_4)H_2PO_4$, 5.0 g NaCl, 0.2 g $MgSO_4$ and 0.2 g K_2HPO_4 in 1 L of deionised water [32]. The pH of the medium was adjusted to the same pH (7.7) as the MLL using NaOH and sterilised by autoclaving at 121 °C for 15 min. This medium contained essential nutrients for bacterial growth but no iron.

Table 1
Municipal landfill leachate characteristics[a]

pH	7.7
Calcium	140
Chromium	<0.1
Iron (total)	6.5
Iron(II)	5.2
Potassium	630
Sodium	2300
Chloride	2100
Sulfate	2.1
Ammonia-N	570
Nitrate	0.038
Nitrite	0.002
Total Kjeldahl nitrogen	830
Total phosphorus	6.1
Total organic carbon (TOC)	1600
Total carbon	4600
Total inorganic carbon (TIC)	3000
BOD_5	2600
COD	4900
Conductivity (ms cm^{-1})	16
Redox potential (mV)	−310
pH buffer capacity (mmol H^+/pH unit)	6.6
Heterotrophic count (CFU mL^{-1}, 21 °C/3 days)	110000

[a] Leachate collected on one occasion. All values are in mg L^{-1} except where indicated.

2.3. Leachate preparation

Landfill leachate was initially filtered through Whatman 541 filter paper (pore size 20–25 μm), which allowed the bacteria to pass while removing larger particulate matter. The filtered MLL was treated with Chelex-100 (100–200 mesh, sodium form) using a solid/liquid ratio of 4 g/100 mL. This treatment reduced the background concentrations of Fe(II) and total iron to 2.2 and 2.6 mg L^{-1}, respectively. A growth curve of the Chelex treated bacteria was determined by spiking 10% treated MLL in a Lurua–Bertani medium and compared to that of untreated bacteria. The similar growth curves (not shown) indicated the growth activity of the bacteria was not affect by the Chelex treatment. The sterilised leachate was obtained by autoclaving the sample and had the same TOC level as non-sterilised MLL. Sterilisation of the Chelex-100 treated MLL oxidised the residual Fe(II) to Fe(III) and was assumed to have no effect on organic matter in MLL and its ability to form metal complexes. The prepared leachates were stored at 4 °C and warmed to room temperature (22 °C) for 24 h prior to use.

2.4. Spiking MLL with Fe(III), Fe(II) or sulfate

To investigate the role of iron in Cr(VI) reduction, a series of MLL (75 mL) was spiked with 5000 mg L^{-1} Fe(III) (0–1.0 mL), followed by 1000 mg L^{-1} Cr(VI) (5 mL) and then diluted to 100 mL with deionised water. This gave samples containing 75% (v/v) MLL, 50 mg L^{-1} Cr(VI) and 0–50 mg L^{-1} Fe(III) (excluding background iron, the same hereinafter). The sample containing MLL (75%, v/v, the same hereinafter), 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III) was prepared in duplicate. The same procedure was used to prepare samples containing (i) MLL, 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II); (ii) MLL and 50 mg L^{-1} Cr(VI); (iii) MLL and 50 mg L^{-1} Fe(III); (iv) sterilised MLL, 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III); (v) sterilised MLL, 10 mg L^{-1} Cr(VI) and (vi) 50 mg L^{-1} Fe(II) and sterilised MLL and 50 mg L^{-1} Fe(III). Limiting the spiking concentrations of Fe(III) and Fe(II) to 50 mg L^{-1} gives a maximum pH decrease of 0.1 units due to the buffer capacity of the MLL.

The procedure for spiking MLL with sulfate was similar to the procedure for Fe(III), whereby samples comprising MLL and 50 mg L^{-1} Cr(VI) were spiked with various concentrations of sulfate (0–200 mg L^{-1}, excluding background sulfate). A control sample containing sterilised MLL, 50 mg L^{-1} Cr(VI) and 100 mg L^{-1} sulfate was also prepared.

2.5. Cr(VI) and Fe(III) reduction and bacterial growth

To overcome analytical problems deriving from the naturally dark colour of the leachate, a chemically defined growth medium was used to investigate the relationships between the chemical reduction of Cr(VI), the microbial reduction of Fe(III) and the bacterial growth. These involved preparing samples comprising Fe(III) (10–50 mg L^{-1}), 10% (v/v) MLL and 80% (v/v) growth medium. The sample containing 50 mg L^{-1} Fe(III), 10% (v/v) MLL and 80% (v/v) growth medium and another sample containing 10 mg L^{-1} Cr(VI), 50 mg L^{-1} Fe(III), 10% (v/v) MLL and 80% (v/v) growth medium were prepared in duplicate. Control samples containing Cr(VI), Fe(II) and sterilised MLL in the growth medium were also prepared.

2.6. Analytical methods

Aliquots (2.0 mL) of the respective sample were retained in the dark in airtight tubes without headspace and at room temperature (22 °C) with continuous shaking (250 rpm). At a selected time interval, a sample was analysed for Cr(VI) concentration. Depending on

the investigated parameter, Fe(II) and/or sulfate content were also determined.

Cr(VI) and Fe(II) concentrations were ascertained by the diphenylcarbazide and the phenanthroline methods [33,34], respectively, using UV–vis Spectrophotometry (CARY 400, Varian, Australia). Bacterial growth was monitored using optical density (OD) readings, measured at 600 nm by spectrophotometry. Sulfate was determined by ion chromatography (DX600, DIONEX, USA) [35].

3. Results and discussion

3.1. Effect of iron on Cr(VI) reduction

The influence of Fe(II) and Fe(III) loadings in MLL on Cr(VI) reduction is provided in Fig. 1. Profile 1(a) shows no Cr(VI) reduction is evident under sterilised conditions and in the presence of Fe(III). This is to be expected as both Cr(VI) and Fe(III) are in their highest oxidation states. Alternately, when in the presence of Fe(II) in sterilised leachate (Profile 1(f)), Cr(VI) is rapidly reduced according to Eq. (1).

$$Cr(VI) + 3Fe(II) \rightarrow Cr(III) + 3Fe(III) \quad (1)$$

Although both free and hydroxylated forms of Fe(II) can reduce Cr(VI), $Fe(OH)_2$ is reported to be more reactive [7]. At MLL pH (7.7), the concentration of free Fe(II) ion is low, with Fe(II) predominantly present as insoluble $Fe(OH)_2$ ($K_{sp} = 1.6 \times 10^{-14}$) [36]. A fraction of Fe(II) may also exist as Fe(II)–organic complexes due to the high organic matter content of MLL. In Profile 1(f) Fe(II) is in excess and complete Cr(VI) reduction is achieved within 90 min.

Profile 1(b) indicates that under non-sterilised conditions Cr(VI) is reduced at a comparatively higher rate over the first day than over subsequent days. At the end of the first day the Cr(VI) concentration has decreased to 37 mg L^{-1} whereby over the following 10 days the reduction rate slows to a constant value of 2.3 ± 0.4 mg L^{-1} day^{-1}. Decreasing Cr(VI) concentration in the non-sterilised MLL compared with the sterilised leachate indicates the bacteria are necessary for promoting Cr(VI) reduction [29].

The addition of Fe(III) to the non-sterilised leachate may have promoted a slight increase in the rate of Cr(VI) reduction over the first day, decreasing the Cr(VI) concentration to 33 mg L^{-1} for 10 mg L^{-1} Fe(III) and 31 ± 2 mg L^{-1} for 50 mg L^{-1} Fe(III). Beyond the first day, the rates slow to 2.5 ± 0.2 and 2.3 ± 0.2 mg L^{-1} day^{-1}, respectively, which are similar to the non-spiked system.

Although the apparent increase in initial Cr(VI) reduction with increasing Fe(III) loading agrees with the findings by Xu et al. [37], who reported the addition of Fe(III) (5–30 mg L^{-1}) significantly enhanced Cr(VI) reduction by *Cellulomonas flavigena*, the similar reduction rate for each profile after day 1 suggests the differences are not significant in our system. The findings illustrate increasing Fe(III) concentration in the MLL has little effect on the rate of Cr(VI) reduction, particularly after day 1. This implies the Fe(III) concentration is not governing the rate of Cr(VI) reduction in this system. This is further discussed in Section 3.3.

The addition of 50 mg L^{-1} Fe(II) to the non-sterilised leachate (Profile 1(e)) also invokes an initial decrease in Cr(VI) concentration (to 25 mg L^{-1}) over the first day whereby the rate again slows to a value similar to the non-spiked and Fe(III)-spiked systems. The initial larger decrease in Cr(VI) concentration compared with the non-spiked and Fe(III)-spiked systems may be ascribed to direct chemical reduction of the Cr(VI) by the Fe(II). According to Eq. (1), 50 mg L^{-1} Fe(II) is capable of reducing 16.7 mg L^{-1} Cr(VI). The difference between the non-spiked/Fe(III)-spiked leachates and the Fe(II)-spiked leachate is 9 ± 3 mg L^{-1} indicating a portion of the Fe(II) chemically reduces the Cr(VI). Beyond the initial chemical reduction by Fe(II) it appears the Cr(VI) reduction rate is controlled by the same factors present in the Fe(III) systems.

3.2. Fe(III) reduction by MLL

Variations in Fe(II) concentration with time during Cr(VI) reduction in sterilised and non-sterilised MLL are provided in Fig. 2. On adding 50 mg L^{-1} Fe(III) to non-sterilised MLL the Fe(II) concentration increases over 2 days to approximately 40 mg L^{-1} whereby it remains constant. The final Fe(II) concentration (80% of initial Fe(III) concentration) suggests the Fe(III) is not completely reduced by this system. The reasons are further discussed in Section 3.3. Profile 2(b) shows when Fe(III) is added to sterilised MLL there is no Fe(II) produced. Comparing Profile 2(b) with Profile 2(a) it is apparent bacteria are required for Fe(III) reduction.

In the presence of 50 mg L^{-1} Cr(VI) (Profile 2(c)) 2 mg L^{-1} Fe(II) is detected, suggesting the background Fe is entirely present as Fe(II) in which induces the Cr(VI) reduction observed in Profile 1(b). On adding 5 mg L^{-1} Fe(III) to the Cr(VI)-containing MLL (Profile 2(d)),

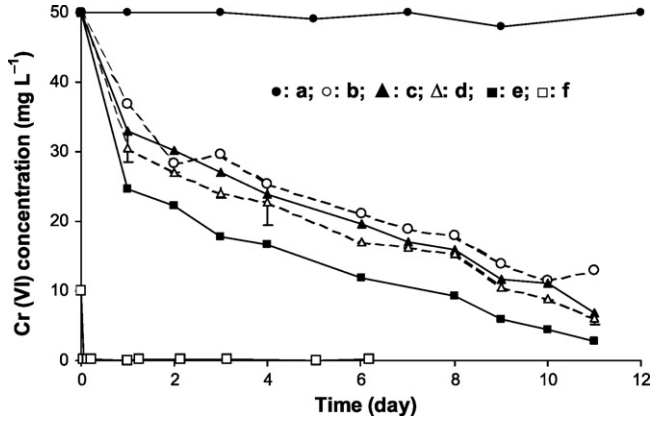

Fig. 1. Variation in Cr(VI) concentration with respect to time in: (a) sterilised MLL containing 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III); (b) MLL containing 50 mg L^{-1} Cr(VI); (c) MLL containing 50 mg L^{-1} Cr(VI) and 10 mg L^{-1} Fe(III); (d) MLL containing 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III); (e) MLL containing 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II); (f) sterilised MLL containing 10 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II). MLL concentration: 75% (v/v). Results in Profile (d) are means and S.D.'s from duplicates.

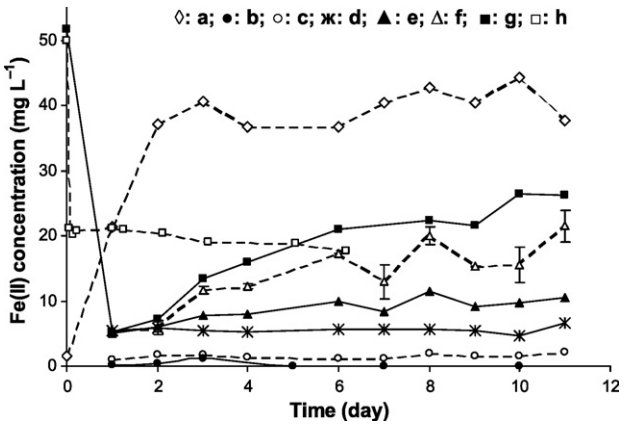

Fig. 2. Variation in Fe(II) concentration with respect to time in: (a) MLL containing 50 mg L^{-1} Fe(III); (b) sterilised MLL containing 50 mg L^{-1} Fe(III); (c) MLL containing 50 mg L^{-1} Cr(VI); (d) MLL containing 50 mg L^{-1} Cr(VI) and 5 mg L^{-1} Fe(III); (e) MLL containing 50 mg L^{-1} Cr(VI) and 10 mg L^{-1} Fe(III); (f) MLL containing 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III); (g) MLL containing 50 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II); (h) sterilised MLL containing 10 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II). MLL concentration: 75% (v/v). Results in Profile (f) are means and S.D.'s from duplicates.

by day 1 the Fe(II) concentration has increased to approximately 5 mg L^{-1}. Apart from the background Fe contribution, 3 mg L^{-1} (60%) spiked Fe(III) is reduced. Adding 10 mg L^{-1} Fe(III) (Profile 2(e)) sees an increase in the Fe(II) concentration to approximately 8 mg L^{-1} by day 4 while adding 50 mg L^{-1} Fe(III) (Profile 2(f)) induces an increase in Fe(II) concentration to around 20 mg L^{-1} by day 11 whereby the concentration has not yet stabilised. Compared with the 80% Fe(III) reduction after 2 days seen in Profile 2(a), it is apparent the percentages and rates of Fe(III) reduction are lower in the presence of Cr(VI). This suggests a portion of the formed Fe(II) has been consumed by Cr(VI) (Eq. (1)) or Cr(VI) is toxic to the bacteria and decreases its ability to reduce Fe(III).

Adding 10 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II) to sterilised leachate (Profile 2(h)) results in a decrease in the Fe(II) concentration by 30 mg L^{-1} in agreement with the stoichiometry of Eq. (1). Under non-sterile conditions and in the presence of 50 mg L^{-1} Cr(VI) (Profile 2(g)) the Fe(II) concentration decreases to 5 mg L^{-1} on day 1 whereby it begins to increase in a similar manner to the Fe(III)-spiked systems. The initial decrease in Fe(II) can be explained by the chemical reaction with Cr(VI), which is also evident in Profile 1(e) (Fig. 1), with the resulting Fe(III) undergoing reduction in a manner similar to the Fe(III)-spiked solutions.

According to Fig. 1, the limited effect increasing Fe(III) concentration has on the Cr(VI) reduction rate indicates the Fe(III) loading is not rate limiting. Given the chemical reaction between Fe(II) and Cr(VI) is quick it is anticipated any Fe(II) produced by the bacteria should be rapidly consumed by the chemical reaction with Cr(VI), and resulting Fe(II) concentrations in the non-sterilised system should be low. Fig. 2 shows otherwise with the Fe(II) concentration increasing with the initial Fe(III) concentration and time. The increasing Fe(II) concentration with time suggests that, while Fe(II) is produced by the bacteria, it may not be readily available to chemically reduce Cr(VI). The Fe(II) can bond with functional groups, such as carboxyl groups (R–COO–) on bacterial cell walls [38] whereby its slow release governs the rate of Cr(VI) reduction.

3.3. Fe(III) and Cr(VI) reduction by growth medium

Fig. 3 illustrates the capacity of the bacteria for reducing Fe(III) in the absence of Cr(VI) in a chemically defined growth medium. Under sterilised conditions there is no growth in bacteria (Profile 3(d)) or change in Fe(II) concentration (Profile 3(h)). Under non-sterilised conditions and in the presence of 10 mg L^{-1} Fe(III), the bacteria undergo growth following a lag period of 1 day where no growth is observed (Profile 3(c)). Coinciding with the onset of bacterial growth is an increase in the Fe(II) concentration (Profile 3(g)). The Fe(II) concentration reaches 9 mg L^{-1} by day 2 where it stabilises at this value. Bacterial growth reaches a maximum by day 3 after which a slow decline in their numbers is observed. The bacterial growth curve agrees with the Monod kinetic model, developed by Liu et al. [38] for microbial reduction of Fe(III) to Fe(II) by *Shewanella putrefaciens* strain CN32. They found the Fe(II) concentration reached a maximum once bacterial growth ceased. The observed lag in the bacterial growth curve is typical [32] and represents the bacteria adjusting to the growth medium.

Increasing the Fe(III) loading does not significantly alter the bacterial growth profile as observed in Profiles 3(a and b) for 50 and 25 mg L^{-1} Fe(III), respectively. As the Fe(III) loading increases the amount of Fe(II) formed increases with the concentration stabilising at 19 mg L^{-1} after day 3 for 25 mg L^{-1} Fe(III) and at 30 mg L^{-1} after day 4 for 50 mg L^{-1} Fe(III). As was the case for the 75% (v/v) MLL system (Fig. 2) a discrepancy exists between the amount of Fe(III) added and the amount of Fe(II) formed.

At the medium and MLL, pH (7.7), Fe(III) exists predominantly as insoluble $Fe(OH)_3$ [39,40]. A fraction of Fe(III) may also exist as insoluble Fe(III) complexes due to the presence of organic matter [41]. The solubility of $Fe(OH)_3$ ($K_{sp} = 1.1 \times 10^{-36}$) [36] and activity of Fe(III) ion are very low [39] at pH 7.7. In this case, the bacterial Fe(III) reduction occurs on the particle surface of $Fe(OH)_3$ and Fe(III) complexes [14,42,43]. An increase in Fe(III) loading increases the particle size of Fe species, but may not provide a proportional increase in the total surface area. Furthermore, microbially generated Fe(II) species such as insoluble $Fe(OH)_2$ and/or products of the chemical reduction, such as $Cr(OH)_3$, may coat the particle surface of $Fe(OH)_3$ and Fe(III) complexes and consequently decrease Fe(III) availability for bacteria for continuous reduction. These explain why complete Fe(III) reduction is not achieved in Profiles 2(a) and 3(e–g). This is consistent with the rates of Cr(VI) reduction shown in Fig. 1, where they are quicker over day 1 than on subsequent days and the observation that Fe(III) loading does not govern Cr(VI) reduction rate. The similar Cr(VI) reduction rate (2.3–2.5 mg L^{-1} day^{-1}) after day 1 (Fig. 1) suggests the bioavailable Fe(III) in each system is similar and attains equilibrium regardless of the initial Fe(III) loading. The interaction between Fe(III) and the bacteria may be described by Eq. (2).

$$Fe(III)_{(bioavailable)} + e^-_{(bacteria)} \rightarrow Fe(II)species \quad (2)$$

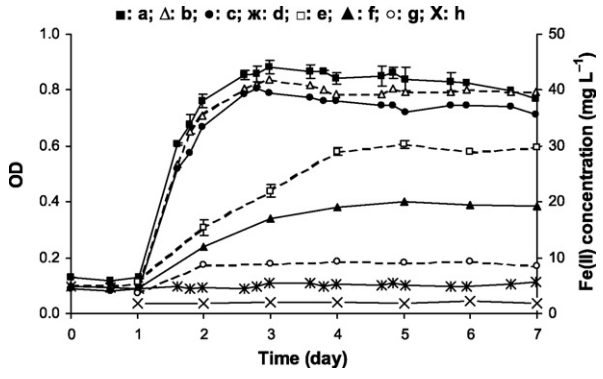

Fig. 3. Variation in bacterial growth and Fe(II) concentration with initial Fe(III) concentration in chemically defined growth medium. Bacterial growth curve in medium containing: (a) MLL and 50 mg L^{-1} Fe(III); (b) MLL and 25 mg L^{-1} Fe(III); (c) MLL and 10 mg L^{-1} Fe(III); (d) sterilised MLL and 50 mg L^{-1} Fe(III). Measured Fe(II) concentration in medium containing: (e) MLL and 50 mg L^{-1} Fe(III); (f) MLL and 25 mg L^{-1} Fe(III); (g) MLL and 10 mg L^{-1} Fe(III); (h) sterilised MLL and 50 mg L^{-1} Fe(III). Concentration of MLL and sterilised MLL: 10% (v/v); growth medium: 80% (v/v). OD, Optical density. Results in Profiles (a and e) are means and S.D.'s from duplicates.

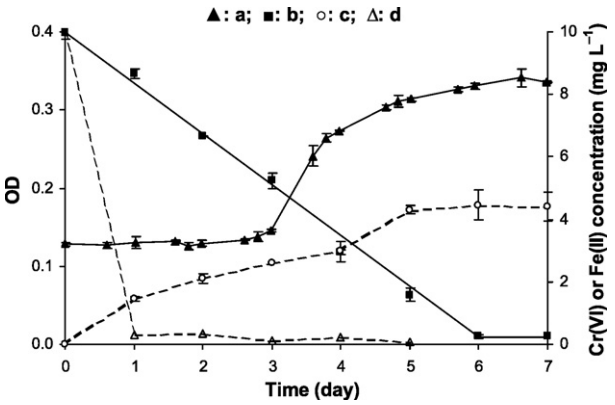

Fig. 4. Variation in concentrations of Cr(VI) and Fe(II) with bacterial growth in chemically defined growth medium containing 10% MLL, 10 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III). (a) Bacterial growth curve; (b) Cr(VI) concentration; (c) Fe(II) concentration; (d) Cr(VI) concentration in a medium containing sterilised MLL(10%, v/v), 10 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(II). OD, Optical density. Results in Profiles (a–c) are means and S.D.'s from duplicates.

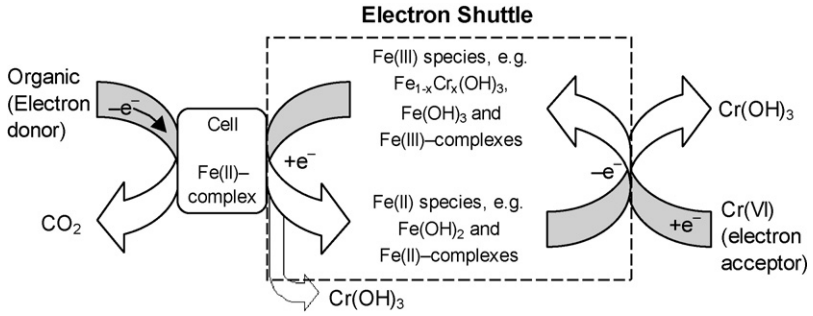

Fig. 5. Postulated mechanism for Cr(VI) reduction in MLL using Fe(II)/Fe(III) as an electron shuttle.

The relationship between Cr(VI) reduction, Fe(II) production and bacterial growth for media containing 10% (v/v) MLL, 10 mg L^{-1} Cr(VI) and 50 mg L^{-1} Fe(III) is described in Fig. 4. Profile 4(b) indicates a linear decrease in Cr(VI) concentration over 6 days whereby all the Cr(VI) has been reduced. The bacterial biomass (Profile 4(a)) remains static over the first 3 days and then increases to a higher level where it again stabilises after a further 3 days. The difference in bacterial growth lag times between Figs. 3 and 4 is likely due to the absence and presence of Cr(VI), respectively. In Fig. 4 the Cr(VI) may impart some toxicity on the bacterial consortium in the decreased OD values compared with Fig. 3. Bacterial growth appears to occur when the Cr(VI) concentration decreases below 5 mg L^{-1} in this instance. An increase in the Fe(II) concentration (Profile 4(c)) indicates that although there is no growth in the bacteria they remain capable of reducing the Fe(III) to Fe(II). The Fe(II) concentration increases in a similar manner as was observed in Fig. 3 until day 4 where a 'jump' in the Fe(II) concentration is seen. This increase corresponds to the increase in bacteria levels with a days lag in effect. Beyond the fifth day the Fe(II) concentration stabilises at around 4.4 mg L^{-1}. Profile 4(d) illustrates complete chemical Cr(VI) reduction achieved before day 1, in agreement with rapid chemical reduction in MLL (Profile 1(f)). These results demonstrate bacterial activity results in reducing Fe(III) to Fe(II), which is responsible for Cr(VI) reduction in the system.

3.4. The Cr(VI) reduction mechanism

Figs. 1–4 have indicated bacteria and Fe play a role in Cr(VI) reduction in MLL. Wielinga et al. [15] and Hansel et al. [16,20] reported complete Cr(VI) reduction by ferric hydroxide with *Shewanella alga* strain BrY in an artificial nutrient medium using lactate as electron donor. Their results suggested bacteria reduced Fe(III) to Fe(II), which was used to reduce Cr(VI) to Cr(III) and subsequently re-oxidised Fe(II) to Fe(III). They proposed Fe was cycled in the system and behaved as a catalyst to constantly transfer electrons from organic material to Cr(VI). In this study, while the leachate is supplemented by growth medium during experiments, it introduces humic materials, organic and inorganic components and a bacterial consortium which may introduce additional complexities to the electron transferring processes. Such complexity has already been alluded in hydrolysis of Fe(III) and Fe(II), bioavailable Fe(III) and the slow release of Fe(II) from the bacteria in the system. Ultimately, the Cr(VI) is likely to be converted to $Cr(OH)_3$, possibly through formation and dissolution of a mixed Fe(III)–Cr(III) hydroxide with the general formula $Fe_{1-x}Cr_x(OH)_3$ ($0 < x < 1$) (Eq. (3)) [9]. In this study, precipitates were observed to form during Cr(VI) reduction which is consistent with the formation of mixed Fe(III)–Cr(III) hydroxide in the system. This pathway is supported by the findings of Hansel et al. [16] who observed an enrichment in Cr relative to Fe with time in the mixed hydroxide, with the final product approaching pure $Cr(OH)_3 \cdot nH_2O$. Incorporating Eqs. (1)–(3), the complexation of Fe(III)/Fe(II) species and their bioavailability, the pathways for Cr(VI) reduction in MLL are postulated as the electron shuttle mechanism in Fig. 5.

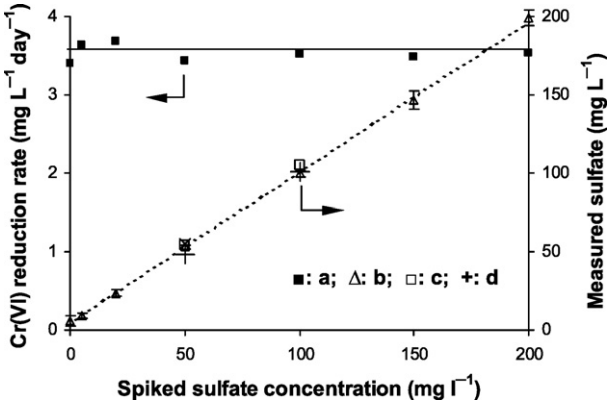

Fig. 6. The concentration of measured sulfate and the relationship between the rate of Cr(VI) reduction and the initial spiked sulfate concentration in MLL. Cr(VI) reduction rates in (a) MLL containing Cr(VI) and 0–200 mg L^{-1} sulfate. Measured sulfate concentrations in: (b) MLL containing Cr(VI) and 0–200 mg L^{-1} sulfate; (c) sterilised MLL containing Cr(VI) and 50 and 100 mg L^{-1} sulfate; (d) MLL containing 50 and 100 mg L^{-1} sulfate. MLL concentration: 75% (v/v); initial Cr(VI) concentration: 50 mg L^{-1}. Results in Profile (b) are means and S.D.'s of sulfate concentrations between days 0 and 11.

$$x\mathrm{Cr(VI)} + (1-x)\mathrm{Fe(II)} + 3\mathrm{H_2O} \rightarrow \mathrm{Fe_{1-x}Cr_x(OH)_3} + 3\mathrm{H^+} \quad (3)$$

3.5. Influence of sulfate on Cr(VI) reduction

Fig. 6 displays the sulfate concentration and the relationship between the rate of Cr(VI) reduction (between days 0 and 11) and the initial spiked sulfate concentration in MLL. The figure illustrates the variation in Cr(VI) reduction rate is small (3.5 ± 0.1 mg L^{-1} day^{-1}) irrespective of the initial sulfate concentration. Furthermore, there is no change in sulfate concentration, regardless of time, in the presence or absence of Cr(VI) and with the sterilisation of MLL. No evidence of microbial sulfate reduction indicates sulfate is not involved in Cr(VI) reduction in this system. Smith and Gadd [17] reported sulfate-reducing bacteria can reduce Cr(VI) by using sulfate/sulfide as an electron shuttle, which implies active sulfate-reducing bacteria are not present in this MLL or their activities are restrained by Cr(VI) or Fe species in our system.

4. Conclusions

Bacteria, Fe(III) and Fe(II) were found to be crucial components for Cr(VI) reduction in MLL. Cr(VI) reduction occurred via an electron shuttle process where Fe(III) was microbially reduced to Fe(II)

with the Fe(II) then chemically reducing Cr(VI) to Cr(III). MLL, as a media for the bacterial reduction of Cr(VI), introduces a number of complexities to the electron shuttle process as it comprises humic materials, organic and inorganic compounds and bacteria. These components and the pH of MLL influence aspects such as bioavailability of the Fe(III) for bacterial reduction and availability of Fe(II) for chemical Cr(VI) reduction and impact on the Cr(VI) reduction rate. Consequently, this study supports the idea of an electron shuttle mechanism as one manner by which Cr(VI) can be bacterially reduced. Moreover, it clearly highlights the need to consider local environmental characteristics for regulating Cr(VI) waste disposal, as well as for bioremediation or treating Cr(VI)-contaminated industrial effluents if Cr(VI)-reducing bacteria are to be used. Results from this study further suggest the effects of Cr(VI) waste on the environment can be attenuated by the natural presence of iron and iron-reducing bacteria. No evidence of microbial sulfate reduction was observed indicating sulfate did not participate in Cr(VI) reduction in this system.

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