Microbial reduction of hexavalent chromium by landfill leachate

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Abstract

The reduction of hexavalent chromium (Cr(VI)) in municipal landfill leachates (MLL) and a non-putrescible landfill leachate (NPLL) was investigated. Complete Cr(VI) reduction was achieved within 17 days in a MLL when spiked with $100\,mg\,l^{-1}$ Cr(VI) or less. In the same period, negligible Cr(VI) reduction was observed in NPLL. In MLL, Cr(VI) reduction was demonstrated to be a function of initial Cr(VI) concentration and bacterial biomass and organic matter concentrations. The bacteria were observed to tolerate $250\,mg\,l^{-1}$ Cr(VI) in MLL and had an optimal growth activity at pH 7.4 in a growth medium. The MLL also possessed an ability to sequentially reduce Cr(VI) over three consecutive spiking cycles.
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1. Introduction

Hexavalent chromium (Cr(VI)) has many industrial uses. It has high water solubility, is the most toxic of the chromium species and is a known carcinogen. Alternately, trivalent chromium (Cr(III)) is less soluble in water and a micronutrient. Reducing Cr(VI) to Cr(III) is therefore beneficial in eliminating the toxicity of Cr(VI) in the environment. Conventional Cr(VI) removal is by chemical reduction, ion exchange or adsorption [1]. Recently, the research has focused on bioremediation of Cr(VI) by microorganisms such as bacteria. In contrast to the conventional methods, bioremediation is cost-effective and can be used for in situ remediation of Cr(VI) wastes.

Since the first report on Cr(VI) reduction by bacteria [2], a number of bacterial species have been identified as capable of reducing Cr(VI). *Streptomyces griseus* strain is capable of reducing a $50\,mg\,l^{-1}$ Cr(VI) standard solution within 72 h [3]. *Arthrobacter* sp. and *Bacillus* sp., isolated from a contaminated long-term tannery waste soil, have been shown to reduce Cr(VI) concentrations up to $50\,mg\,l^{-1}$ [4]. Chromium-resistant bacteria from Cr(VI) contaminated soils are also reported to reduce Cr(VI) concentrations up to $1500\,mg\,l^{-1}$ [5]. Cr(VI) was found to be reduced by *Shewanella oneidensis* [6] and a metal reducing bacteria, *Shewanella alga* (BrY-MT) ATCC 55627 [7]. Sulfate-reducing bacteria from marine sediment were capable of almost completely reducing 0.6 mM Cr(VI) in a culture medium within 168 h [8].

Many factors influence microbial Cr(VI) reduction [4]. For example, the presence of aerobic or anaerobic conditions [8–10] or the availability of energy sources such as sulfur (for *Acidithiobacillus thiooxidans*) [11] and glucose (for *P. fluorescens* LB300) [12], or other suitable organic materials. In an activated sludge process, Cr(VI) reduction was primarily affected by the initial concentration of organic substrate, which acted as an electron donor [13]. Natural organic matter (NOM) may also play an important role in microbial Cr(VI) reduction [14]. In soil containing high levels of NOM, Cr(VI) reduction was dramatically more significant, indicating the NOM served as a suitable reductant [15]. The addition of organic carbon to soil has been shown to accelerate Cr(VI) reduction [16].

In landfill environments, microbial processes biodegrade the organic material and contribute to landfill leachate generation. Besides high contents of humic substances, landfill leachates contain a wide variety of inorganic and other organic contaminants [17,18]. Moreover, landfill leachates also contain a high biomass of diverse bacteria [19,20]. Our recent studies have shown landfill leachates possess an ability to reduce Cr(VI) [21].

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The aims of this work were to investigate Cr(VI) reduction in landfill leachate and identify whether correlations exist between Cr(VI) reduction and concentrations of Cr(VI), bacterial biomass and organic matter. The effect of pH on bacterial growth was also investigated.

2. Materials and methods

2.1. Materials

2.1.1. Landfill leachate

Landfill leachates were sampled from two separate municipal landfills (MLL(A) and MLL(B)) and a non-putrescible landfill (NPLL), located in regions surrounding Sydney, Australia. All landfills have been in operation for up to 20 years. The two municipal landfills accommodate both municipal and non-putrescible wastes, whereas the non-putrescible landfill only accepts non-putrescible wastes such as construction and demolition wastes, wood and industrial wastes. MLL(A) and MLL(B) were collected from pipes leading to leachate ponds in the landfills. NPLL was collected using a bailer from a well leading to an underground leachate pipe. Leachate samples from each individual landfill were collected and stored in multiple 25 l containers. The leachate characteristics from each sample container were determined and averaged for that landfill (Table 1). The results indicate both MLL(A) and MLL(B) contain high levels of total organic carbon (TOC), BOD and COD and NPLL has a high conductivity. The high BOD in MLL(B) suggests MLL(B) is younger than MLL(A) [17]. Preliminary microbial tests indicated the presence of a variety of

bacteria and a high bacterial biomass concentration in each leachate.

2.1.2. Cr(VI) solutions and Luria–Bertani (LB) medium

Cr(VI) stock solutions (1000 and 5000 mg l^{-1}) were prepared by dissolving potassium dichromate in deionised water. LB medium was prepared by dissolving 10 g tryptone, 5 g yeast extract and 10 g sodium chloride in 1 l deionised water and sterilised prior to use.

2.2. Methods

2.2.1. Leachate preparation

Landfill leachates were filtered through a filter paper (Whatman 541, UK) for removal of particulate matter and stored at 4 °C. Sterilised leachates were prepared by autoclaving at 121 °C for 15 min. The leachates were warmed to room temperature (22 °C) for 24 h prior to use.

2.2.2. Cr(VI) reduction in MLL and NPLL

In order to demonstrate Cr(VI) reducing ability of landfill leachates, 100 mg l^{-1} Cr(VI) was chosen in this study. For investigation the effects of leachate type and sterilisation on Cr(VI) reduction, MLL(A), MLL(B) and NPLL (25 ml) were spiked with 1000 mg l^{-1} Cr(VI) solution (10 ml) and diluted to 100 ml with deionised water. This provided samples comprising 25% (v/v) leachate and 100 mg l^{-1} Cr(VI). A similar procedure was used to prepare a sample containing 75% (v/v) MLL(B) and 100 mg l^{-1} Cr(VI). Control samples comprising 100 mg l^{-1} Cr(VI) and 25% (v/v) sterilised MLL(A), 25 mg l^{-1} Cr(VI) and 75% (v/v) sterilised MLL(B) and 100 mg l^{-1} Cr(VI) and 25% (v/v) sterilised NPLL were also prepared for this study.

2.2.3. Effects of Cr(VI) and pH on bacterial growth

Due to the naturally dark leachate colour, direct measurement of bacterial growth in MLL was difficult. Instead of using MLL, the effects of Cr(VI) and pH on bacterial growth and the correlation between bacterial growth and Cr(VI) reduction were carried out in LB media. In order to inoculate LB medium with bacteria in MLL(B), a series of LB media (85 ml) were spiked with MLL(B) (10 ml) and various volumes (0–5 ml) of Cr(VI) solutions (1000 or 5000 mg l^{-1}). These media were then made up to 100 ml with deionised water to provide LB media comprising 10% (v/v) MLL(B), 5% (v/v) deionised water and various concentrations of Cr(VI) (0–250 mg l^{-1}). The pH of a set of LB media containing 10% MLL(B) was adjusted from 2 to 12 with 5.0 M hydrochloric acid or 5.0 M sodium hydroxide to study the effect of pH on bacterial growth.

2.2.4. Effect of Cr(VI) concentration

Investigations into the effect of initial Cr(VI) concentration on Cr(VI) reduction were undertaken with samples comprising 75% (v/v) MLL(B) at various Cr(VI) concentrations (25–250 mg l^{-1}). These were prepared by spiking a 5000 mg l^{-1} Cr(VI) solution in MLL(B) and diluting with deionised water.

Table 1
Landfill leachate characteristics[a]

Analyte	MLL(A)	MLL(B)	NPLL
Calcium	92	140	180
Chromium	0.3	<0.1	<0.1
Iron (total)	14	6.5	22
Iron (II)	N/A[b]	5.2	N/A
Potassium	1400	630	840
Sodium	1700	2300	5000
Chloride	2900	2100	N/A
Sulfate	32	2.1	5.4
Sulfide	N/A	2	N/A
Ammonia–N	2200	570	610
Total Kjeldahl nitrogen	2300	830	680
Total phosphorus	10	6.1	0.48
Total organic carbon (TOC)	890	1600	270
Total carbon	2900	4600	740
BOD$_5$	840	2600	50
COD	3850	4900	930
Conductivity (ms cm^{-1})	24	16	33
Heterotrophic count (CFU ml^{-1}, 21 °C for 3 days)	4000	110000	75000
pH	7.8	7.7	7.4

MLL corresponds to municipal landfill leachates; NPLL corresponds to non-putrescible landfill leachate.

[a] Leachates collected in multiple sample containers on only one occasion from each landfill. Values given are averages of all containers from each particular landfill. All values are in mg l^{-1} except where indicated.

[b] N/A: not analysed.

2.2.5. Effect of bacterial biomass and TOC

Samples containing $50 \, mg \, l^{-1}$ Cr(VI), 0–90% (v/v) MLL(B) and 0–90% (v/v) sterilised MLL(B) were used to investigate the effect of bacterial biomass concentration on Cr(VI) reduction. The bacterial biomass concentration of each sample is proportional to the percentage of MLL(B) present. Sterilised MLL(B) was used as the make-up component to maintain the same TOC level ($1440 \, mg \, l^{-1}$) in each sample.

To investigate the effect of TOC on Cr(VI) reduction, a set of samples was prepared comprising $50 \, mg \, l^{-1}$ Cr(VI), 50% (v/v) MLL(B) and 0–40% (v/v) sterilised MLL(B). TOC concentration increased from 800 to $1440 \, mg \, l^{-1}$ with the increase in sterilised MLL(B) content. For comparative purposes, D-glucose was used as an alternate carbon source to prepare a set of samples comprising $50 \, mg \, l^{-1}$ Cr(VI), 50% (v/v) MLL(B) and $5–2000 \, mg \, l^{-1}$ glucose (TOC in the range $800–1600 \, mg \, l^{-1}$).

2.2.6. Cyclic Cr(VI) reduction

Sequential Cr(VI) spiking was employed to investigate the capability of MLL(B) for repeatedly reducing Cr(VI). Initially, a sample containing $100 \, mg \, l^{-1}$ Cr(VI) and 75% (v/v) MLL(B) was prepared (defined as first phase). On day 30, following complete Cr(VI) reduction in the first phase, 5.0 ml $1000 \, mg \, l^{-1}$ Cr(VI) and a 10 ml sample from the first phase were diluted to 100 ml with sterilised MLL(B) (defined as second phase). On day 55, following complete Cr(VI) reduction in the second phase, a third phase sample was prepared in the same manner as the second phase sample and Cr(VI) reduction monitored.

2.2.7. Analytical methods

For each Cr(VI) reduction experiment, a set of 2.0 ml aliquots were kept in the dark in airtight tubes without headspace, at room temperature (22 °C), with continual shaking (250 rpm). The zero headspace was designed to mimic the anaerobic conditions encountered in landfill leachates. At selected time intervals, a sample was analysed for Cr(VI) concentration and discarded. An aliquot (15 ml) of each LB medium sample was also prepared in a larger airtight tube to continually monitor the growth of bacteria.

Cr(VI) concentration was determined by the diphenylcarbazide method [22] using UV–vis spectrophotometry (CARY 400, Varian, Australia). Bacterial growth and biomass concentration were monitored by optical density (OD) readings, measured at 600 nm [4] using spectrophotometry (DR/2000, Hach, USA). Redox potential was measured by a pH meter (Model 420Aplus, Thermo Orion, USA) using an ORP Triode Electrode (Model 91–79, Thermo Orion, USA) in a closed container without headspace. Redox potential was measured relative to the hydrogen redox potential.

3. Results and discussion

3.1. Cr(VI) reduction in MLL and NPLL

The effects of leachate type (MLL and NPLL), leachate source (MLL(A) and MLL(B)), leachate content and leachate sterilisation on Cr(VI) reduction are provided in Fig. 1. The fig-
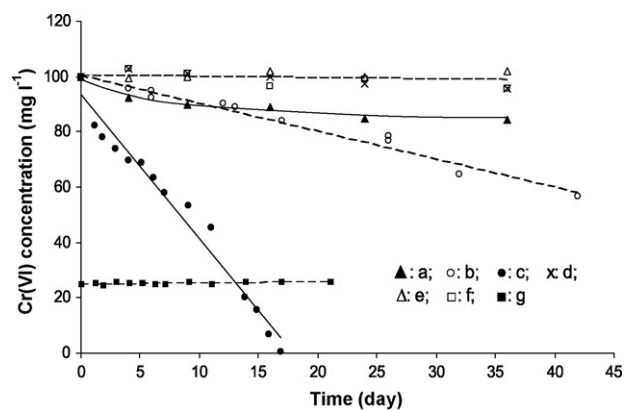

Fig. 1. The varation of Cr(VI) concentration with time in: (a) 25% (v/v) MLL(A) containing $100 \, mg \, l^{-1}$ Cr(VI); (b) 25% (v/v) MLL(B) containing $100 \, mg \, l^{-1}$ Cr(VI); (c) 75% (v/v) MLL(B) containing $100 \, mg \, l^{-1}$ Cr(VI); (d) 25% (v/v) NPLL containing $100 \, mg \, l^{-1}$ Cr(VI); (e) 25% (v/v) sterilised MLL(A) containing $100 \, mg \, l^{-1}$ Cr(VI); (f) 25% (v/v) sterilised NPLL containing $100 \, mg \, l^{-1}$ Cr(VI); and (g) 75% (v/v) sterilised MLL(B) containing $25 \, mg \, l^{-1}$ Cr(VI).

ure shows that on day 17 approximately $12 \, mg \, l^{-1}$ Cr(VI) was reduced in MLL(A) (25%, v/v), $15 \, mg \, l^{-1}$ Cr(VI) was reduced in MLL(B) (25%, v/v) and complete Cr(VI) reduction was observed in MLL(B) (75%, v/v). At the same time, no Cr(VI) reduction was observed in sterilised MLL(A) or MLL(B), indicating Cr(VI) reduction occurs in MLL and the reduction is related to the presence of bacteria. Fig. 1 also shows the Cr(VI) reduction profiles vary for MLL(A) and MLL(B). In MLL(A), initially the Cr(VI) is reduced however, the reduction has ceased by day 17, whereby approximately $15 \, mg \, l^{-1}$ of Cr(VI) has been reduced. In the case of MLL(B), the reduction occurs at a constant rate and is a function of the percentage of leachate present in the system. For 25% (v/v) MLL(B) the rate is $1.0 \, mg \, l^{-1} \, day^{-1}$, whereas for 75% (v/v) MLL(B) the rate is $5.2 \, mg \, l^{-1} \, day^{-1}$, indicating Cr(VI) reduction is also related to the content of MLL. This is discussed further in Section 3.3.2. The differences in reducing abilities of each leachate may be attributed to differences in bacteria species, bacterial biomass concentration or organic type and content in MLL, as well as landfill conditions such as waste content and landfill age.

In contrast to the MLLs, a negligible amount of Cr(VI) was reduced by NPLL. This is despite a bacterial presence in NPLL, implying that Cr(VI) reduction in landfill leachates is related to the waste type of the landfill. The lack of Cr(VI) reduction by NPLL may be attributed to the presence of dissimilar bacterial species and/or its lower organic content (Table 1). No Cr(VI) reduction was observed in sterilised NPLL.

The effect of bacteria on the reducing ability of MLL(B) was further investigated by measuring redox potential in a closed system. Redox potentials were −0.11 V for 100% (v/v) sterilised MLL(B), −0.37 V for sterilised MLL(B) spiked with 33% (v/v) MLL(B), and −0.31 V for 100% (v/v) MLL(B). These results show the reducing ability of MLL(B) is related to the presence of bacteria and may be a result of anaerobic respiration of bacteria in MLL(B). Moreover, the redox potential of MLL(B) (−0.31 V) indicates it has a reducing ability, which may lead to direct Cr(VI) reduction by the leachate when
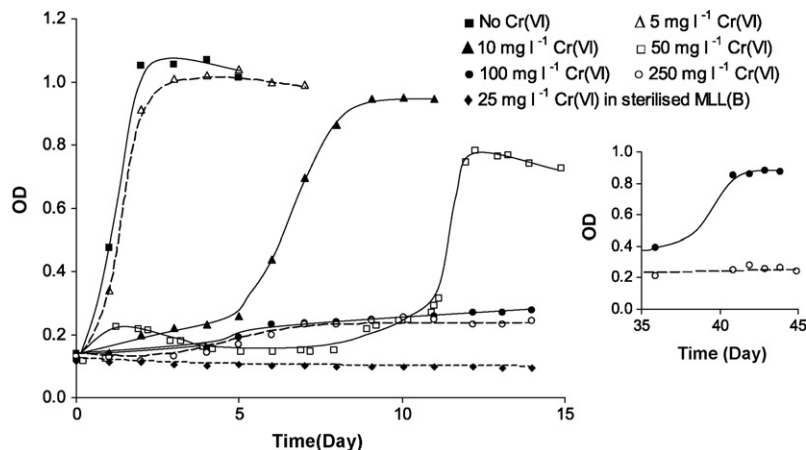
Fig. 2. Effect of Cr(VI) concentration on bacterial growth in LB media containing 10% (v/v) MLL(B) and sterilised MLL(B). Optical density (OD) corresponds to bacterial biomass concentration. Inset shows OD values between days 35 and 45 for LB media containing 100 and 250 mg l^{-1} Cr(VI).

compared with the redox potential of aqueous Cr(VI) at pH 7 (0.6–0.9 V) [23].

3.2. Relationship between bacterial growth and Cr(VI) reduction

The bacteria present in MLL have been shown to promote Cr(VI) reduction. The effect of initial Cr(VI) concentration on bacterial growth and Cr(VI) reduction in LB media (containing 10% (v/v) MLL(B)) is provided in Fig. 2. The results show Cr(VI) does not significantly delay bacterial growth at concentrations below 5 mg l^{-1}. However, at Cr(VI) concentrations of 10 mg l^{-1} or greater, a lag time prior to the onset of bacterial growth is evident, increasing with increasing Cr(VI) concentration. This implies Cr(VI) is toxic to certain bacteria present in MLL(B), inhibiting their growth. Nevertheless, rapid growth phases of bacteria were observed after 5, 11 and approximately 40 days in media containing 10, 50 and 100 mg l^{-1} Cr(VI) (Fig. 2, inset), respectively, indicating the presence of Cr(VI)-resistant bacteria capable of tolerating Cr(VI) concentrations as high as 100 mg l^{-1}. However, the bacteria growth phase was absent in media containing 250 mg l^{-1} Cr(VI) (Fig. 2, inset). This indicates 250 mg l^{-1} Cr(VI) is toxic to the bacteria and the threshold inhibitory concentration of Cr(VI) may be within

the range of 100–250 mg l^{-1}. The inhibition concentration of Cr(VI) on the bacterial growth may be related to the growth medium, content of MLL, bacterial species and organic matter in the MLL.

Fig. 3A illustrates the relationship between bacterial growth and Cr(VI) reduction by 10% (v/v) MLL(B) in LB medium. Cr(VI) reduction commenced after spiking and the concentration decreased constantly with time to an undetectable value. There was no significant change in bacterial biomass concentration during this period. However, upon complete Cr(VI) reduction (day 11) the bacteria grew rapidly, reaching a maximum on day 12, indicating the rapid bacterial growth phase only appears after Cr(VI) is completely reduced. Therefore, it can be inferred the bacterial growth curves in Fig. 2 also reflect the time complete Cr(VI) reduction is achieved. Fig. 3B indicates that 100 mg l^{-1} Cr(VI) is completely reduced by around day 40, corresponding to the time of bacteria growth in Fig. 2 and supporting this observation. It is also apparent from Fig. 3B the bacteria are tolerant to initial Cr(VI) concentrations of 250 mg l^{-1}. This is considered further in the next section. The results here suggest the Cr(VI) inhibits bacterial growth, and therefore, the Cr(VI)-resistant bacteria create a reducing environment to detoxify Cr(VI) for their own growth. Similar to bacterial growth and Cr(VI) reduction in LB medium containing 250 mg l^{-1} Cr(VI), bacterial growth was
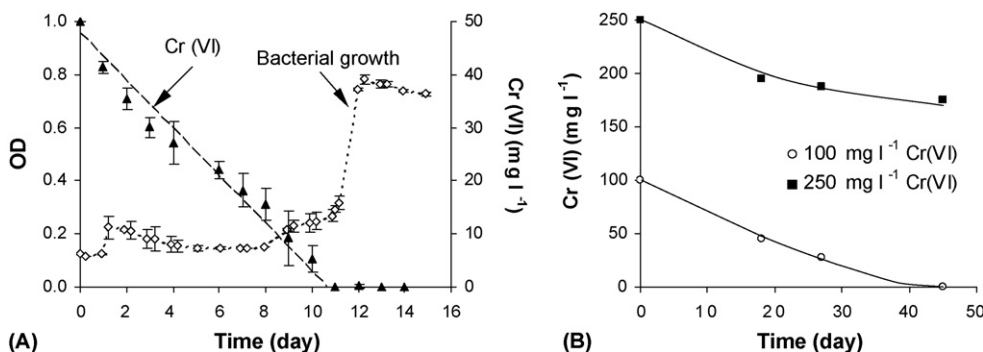

Fig. 3. (A) Relationship between Cr(VI) concentration and bacterial growth in LB medium containing 50 mg l^{-1} Cr(VI) and 10% (v/v) MLL(B). The results were mean of triplicates. (B) Cr(VI) concentration in LB media containing 10% (v/v) MLL(B) and 100 and 250 mg l^{-1} Cr(VI).
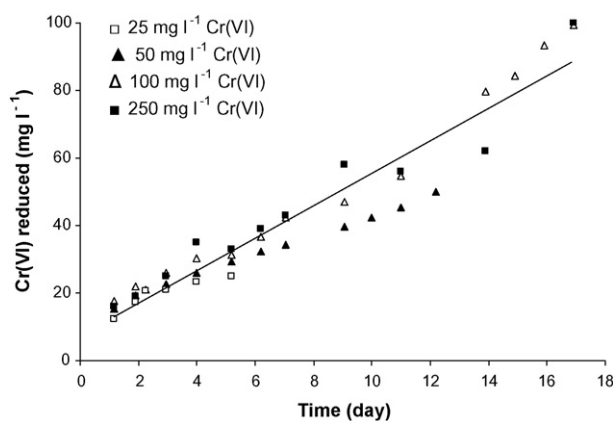
Fig. 4. Reduced Cr(VI) concentration as a function of time in 75% (v/v) MLL(B) initially spiked with various Cr(VI) concentrations.
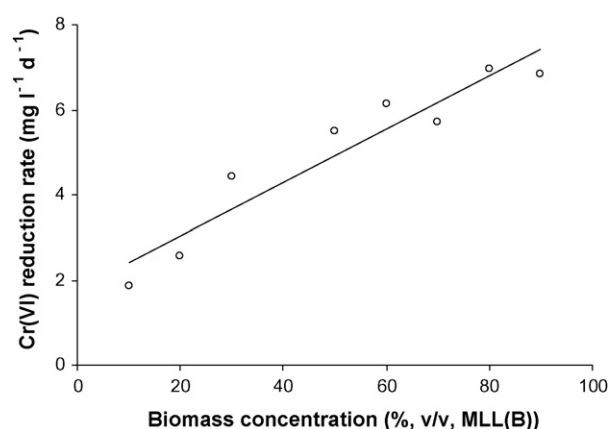

Fig. 5. Effect of bacterial biomass concentration on Cr(VI) reduction rate in MLL(B). Initial Cr(VI) concentration was $50 \, mg \, l^{-1}$. The initial bacterial biomass concentration is in proportional to the percentage (v/v) of MLL(B) in the sample.

inhibited and less than 20% of Cr(VI) was reduced by day 45 in a LB medium containing $500 \, mg \, l^{-1}$ Cr(VI). This indicates the bacterial species in MLL(B) is not suitable for treatment of wastes containing high level of Cr(VI) such as tannery effluents.

3.3. Factors affecting Cr(VI) reduction and bacteria growth

3.3.1. Effect of initial Cr(VI) concentration

The effect of initial Cr(VI) concentration on Cr(VI) reduction in 75% (v/v) MLL(B) for concentrations in the range $25–250 \, mg \, l^{-1}$ is given in Fig. 4. The figure shows complete Cr(VI) reduction is achieved within 5, 12 and 17 days for samples initially spiked with 25, 50 and $100 \, mg \, l^{-1}$ Cr(VI), respectively. The linear profiles indicate the reduction rates are zero order with respect to Cr(VI) for the concentrations considered. Comparing the rate values for each Cr(VI) concentration (Table 2) indicates at lower initial Cr(VI) concentrations the Cr(VI) reduction rate is slower. Furthermore, the reduction rates are similar at the 100 and $250 \, mg \, l^{-1}$ concentrations. This implies the reduction rate is limited by the Cr(VI) concentration at values below $100 \, mg \, l^{-1}$. This is in agreement with work by Laxman and More [3] and Stasinakis et al. [13] who have also reported variations in reduction rate with initial Cr(VI) concentration.

The Cr(VI) reduction rates obtained in this study are similar to sulfate-reducing bacteria from marine sediment ($4.5 \, mg \, l^{-1} \, day^{-1}$) [8], but slower than *Arthrobacter* sp. isolated from a contaminated soil (greater than $8 \, mg \, l^{-1} \, day^{-1}$) [4]. Fig. 4 also indicates the tolerance limit of the bacteria in MLL(B) to Cr(VI) is greater than $250 \, mg \, l^{-1}$. This tolerance lies within the reported limits of *Arthrobacter* sp. and *Bacillus* sp. ($100 \, mg \, l^{-1}$

Cr(VI) in a growth medium) [4], and chromium-resistant bacteria ($2500 \, mg \, l^{-1}$ Cr(VI) in LB medium) [5].

3.3.2. Effect of bacterial biomass and organic matter

Microbial activity of bacteria in MLL has been shown to reduce Cr(VI), therefore, it is likely factors which influence bacterial activity may also affect Cr(VI) reduction in MLL. Fig. 5 portrays the rate of reducing $50 \, mg \, l^{-1}$ Cr(VI) with respect to the initial bacterial biomass concentration. The figure shows Cr(VI) reduction rate has a positive linear correlation with the initial bacterial biomass concentration, indicating Cr(VI) reduction in MLL is a first order reaction with respect to the initial bacterial biomass concentration.

Fig. 6 shows the rate of reducing $50 \, mg \, l^{-1}$ Cr(VI) with respect to variation of TOC concentration in 50% (v/v) MLL(B). The figure indicates TOC is a controlling factor in Cr(VI) reduction, the extent of which being a function of the form of carbon available to the bacteria. Below TOC concentrations of $1100 \, mg \, l^{-1}$ for sterilised MLL(B) and $840 \, mg \, l^{-1}$ TOC for glucose the TOC controls the rate of Cr(VI) reduction in a linear manner. Above these concentrations the TOC no longer controls the rate of Cr(VI) reduction, with the reduction rate reaching a
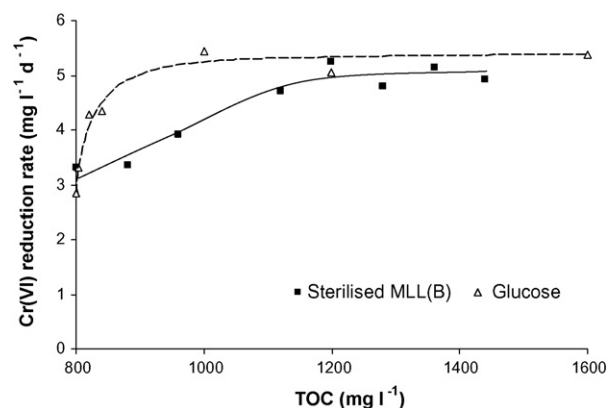
Table 2
Cr(VI) reduction rate in 75% (v/v) MLL(B)

Initial Cr(VI) concentration ($mg \, l^{-1}$)	Cr(VI) reduction rate[a] ($mg \, l^{-1} \, day^{-1}$)
25	2.8
50	2.9
100	5.2
250	4.7

[a] Slope of Cr(VI) reduction curve in Fig. 4.

Fig. 6. Effect of total organic carbon (TOC) concentration on Cr(VI) reduction rate in 50% (v/v) MLL(B). Initial Cr(VI) concentration was $50 \, mg \, l^{-1}$.

maximum value of approximately $4.8 \, mg \, l^{-1} \, day^{-1}$. This finding is in agreement with results by Eiler et al. [24] who reported a liner relationship between bacteria growth and dissolved organic carbon content and that oversupply of TOC is not beneficial to either bacterial activity or Cr(VI) reduction. Furthermore, glucose as a TOC source acts to accelerate the rate of Cr(VI) reduction compared to the TOC provided by MLL(B). This implies glucose is more bioavailable in this instance [25], providing a greater thermodynamic energy content than the humic organic matter in MLL(B). These results indicate Cr(VI) reduction rate in MLL(B) is affected not only by the concentrations of bacterial biomass and TOC, but also the bioavailability and thermodynamic energy content of the organic matter [26].

3.3.3. Effect of pH

Bacterial growth is strongly influenced by pH of the growth medium. Although municipal landfill leachate has a high buffer capacity [27], the pH may shift when it is applied to treat Cr(VI) wastes, such as Cr(VI) contaminated cementitious waste [21]. Thus, the tolerance limit of bacteria to pH is important for microbial Cr(VI) reduction in MLL. Fig. 7 illustrates the optimum pH for facilitating bacteria growth in LB media was 7.4. This coincides with the best pH (near neutral pH 7) for anaerobic process and lies close to the pH value (7.7) of the leachate (Table 1). Furthermore, the figure shows the bacteria can grow within the pH range 7–10, indicating the bacteria in MLL(B) are more prevalent under alkaline conditions. The variation of the maximum bacterial biomass concentration (OD) with pH indicates different bacterial species in MLL(B) may have different tolerance limits to pH. Although this study did not directly correlate pH with microbial Cr(VI) reduction, as the pH of MLL(B) is within the optimal pH values and the leachate is capable of microbially reducing Cr(VI), it is likely the bacteria present in the optimal pH range are responsible for Cr(VI) reduction.

3.4. Consecutive Cr(VI) reduction in MLL

The ability of MLL(B) to sequentially reduce Cr(VI) was assessed by the repeated addition of Cr(VI) to MLL(B) and sterilised MLL(B). Fig. 8 shows MLL(B) can completely reduce
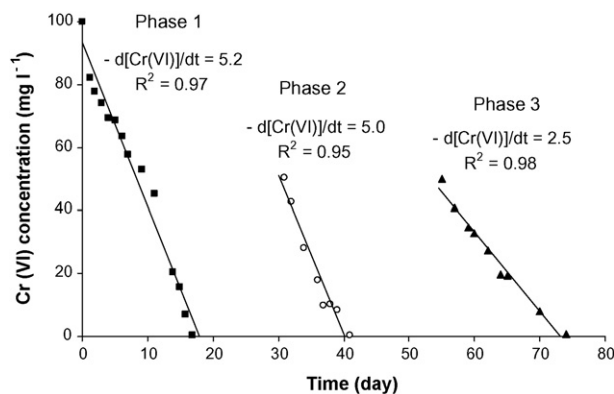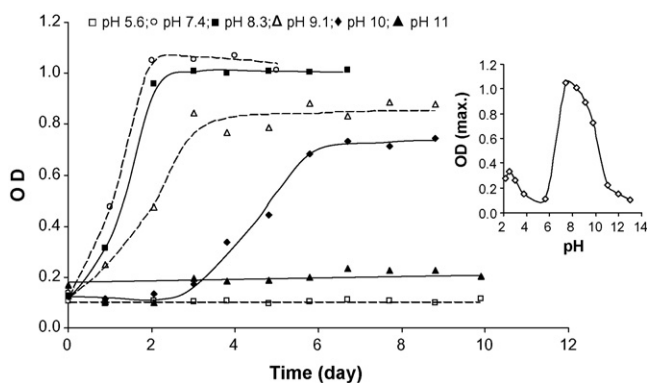
Fig. 7. Bacterial growth with respect to pH in LB media containing 10% (v/v) MLL(B). Inset shows optimum pH in LB media. No Cr(VI) was spiked in these samples.

Fig. 8. Change in Cr(VI) concentration with time following sequential phases of Cr(VI) spiking. Phase 1: $100 \, mg \, l^{-1}$ Cr(VI) in 75% (v/v) MLL(B); phase 2: $50 \, mg \, l^{-1}$ Cr(VI) in sterilised MLL(B) containing 10% (v/v) of first phase sample; phase 3: $50 \, mg \, l^{-1}$ Cr(VI) in sterilised MLL(B) containing 10% (v/v) of second phase sample.

Cr(VI) over three cycles in this system, indicating Cr(VI)-resistant bacteria in MLL(B) reduce Cr(VI) in consecutive cycles. The Cr(VI) reduction rates were approximately 5.2, 5.0 and $2.5 \, mg \, l^{-1} \, day^{-1}$ for phases 1, 2 and 3, respectively. The decrease in Cr(VI) reduction rate in phase 3 may be attributed to the accumulation of waste products and mutation of Cr(VI)-resistant bacteria, which could result in a decrease in Cr(VI)-resistant bacterial biomass concentration.

4. Conclusions

This study has shown Cr(VI) undergoes reduction in municipal landfill leachate, whilst no significant Cr(VI) reduction occurs in non-putrescible landfill leachate. Cr(VI) reduction in LB medium inoculated with bacteria and the lack of Cr(VI) reduction in sterilised MLL demonstrated the microbial activity of bacteria in MLL is responsible for Cr(VI) reduction. The bacteria in MLL remain tolerant to Cr(VI) concentrations as high as $250 \, mg \, l^{-1}$ and the bacterial growth rate may relate to the content of MLL, bacterial species, organic matter and exposed Cr(VI) concentration. The study also illustrated microbial Cr(VI) reduction is a first order reaction with respect to bacterial biomass concentration. The reduction rate is a function of the initial Cr(VI) concentration and type and concentration of organic matter. Sequential Cr(VI) spiking showed the bacteria in MLL are capable of repeatedly reducing Cr(VI).

Acknowledgements

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