Chromate detoxification using combination of ChromeBac™ system and immobilized chromate reductase beads

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Incomplete removal of chromium from discharge effluent of chromium-based industries is a serious problem due to its deleterious effect. ChromeBac™ is a locally developed Cr(VI) reduction system carried out by aerobic Cr(VI) resistant bacteria followed by chemical precipitation. The locally isolated Cr(VI) resistant-reducing Acinetobacter haemolyticus EF369508 was immobilized onto carrier materials inside a 50 L bioreactor. Mixture of 10% (v/v) liquid pineapple waste and neutralized Cr(VI) solutions (30–60 mg L−1) was fed into the bioreactor at 0.11 m3 h−1. Around 90% of the initial Cr(VI) was reduced after 24 h of contact inside the bioreactor. Residual Cr(VI) was then further reduced between 1.0 and 1.5 mg L−1 by immobilized chromate reductase alginate-beads packed in a 10 L flow-through column, after 15 h of contact. Some important characteristics for the chromate reductase activities for A. haemolyticus are as follows; not NADH-dependent, associated with CFE with notable contribution from the membrane fraction, enhanced in the presence of glucose, optimal at pH 7.0, 30 °C, in the presence of 1 mM Co2+ (highest) with Michaelis–Menten constant, Km and maximum reaction rate, Vmax of 184.47 μM and 33.3 nmol/min/mg protein respectively. Ag+ and Hg2+ inhibited the enzyme activity. This study demonstrated the potential of using immobilized chromate reductase beads to further reduce residual Cr(VI) present in the effluent of a ChromeBac™ process, hence reducing the time for overall Cr(VI) treatment process.

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1. Introduction

The contamination of the water system by the toxic heavy metals such as chromium, lead, copper, zinc, cadmium, nickel, arsenic and mercury is gaining serious attention nowadays. Chromium, Cr originates from various anthropogenic sources such as alloy manufacturing, dyes and pigments, electroplating, metal finishing, petroleum refining, leather tanning, wood preservation and corrosion inhibitor in conventional and nuclear power plants (Elangovan et al., 2010). Although Cr is able to exist in several oxidation states (−2 to +6), the most stable and common forms are Cr(III) and Cr(VI) species. Cr(VI), is the most toxic form of Cr, usually associated with oxygen as chromate (CrO4 2−) or dichromate (Cr2O7 2−) ions. In contrast, Cr(III) in the form of oxides, hydroxides or sulfates, is much less toxic, less mobile, less soluble under neutral pH and unable to cross cell membranes. The high solubility of Cr(VI) makes it a very toxic and carcinogenic element, which represents a serious threat to human health, living resources and ecological system as they considered as persistent and bioaccumulative. Therefore, it is compulsory to remove Cr(VI) from water/wastewater prior to discharging into the environment. Conventional methods for Cr(VI) removal involves physico-chemical techniques which are highly expensive, inappropriate at low Cr(VI) concentration, high reagent consumption, energy requirements and generation of toxic sludge (Ahmad et al., 2015). Therefore, development of an effective system for Cr(VI) bioremediation is highly desirable.

Microbial reduction of Cr(VI) is considered as an alternative
remediation technique for Cr(VI) contamination due to its lower cost and less sludge production. Various studies were reported on the ability of microbial species to carry out both the Cr(VI) reduction are operated in batch mode (Elangovan et al., 2010). However, this system is not fully effective (compared to continuous or fixed film bioreactor system) with an eventual loss of active biomass. This was mainly due to metal toxicity and the stage of biofilm development which has not fully matured. Several improvements are needed both at the enzymatic as well as cellular levels for bacteria to work efficiently as agents for Cr(VI) remediation. The use of cell-free enzymes has advantages over whole cells. Cell-free enzymes are not affected by growth inhibitors, toxins, predators of microbial growth or microbial competition in the environment. Moreover, cell-free enzymes do not require transport mechanisms that may impair microbial uptake of chromate. Furthermore, cell-free enzymes can be immobilized for pollutant removal in reactors. The enzyme can be re-used and no need for post-reaction treatment of biomass (Camargo et al., 2003).

Chromate reductase facilitates the reduction of Cr(VI) to Cr(III) either in aerobic or anaerobic conditions. Aerobic Cr(VI) reduction is generally associated with a soluble fraction that utilizes NADH as an electron donor. Conversely, anaerobic Cr(VI) reduction is mediated by membrane bound cytochrome b, c and d, or cytoplasmic membrane proteins (Bae et al., 2005). To date, the use of purified, partially purified or crude enzymes in bioremediation of Cr(VI) from contaminated wastewater treatment has not been reported. The present study highlights the incorporation of an immobilized chromate reductase beads in the existing ChromBioB™ system in order to reduce the overall Cr(VI) treatment time.

2. Materials and methods

2.1. Design and operation of the pilot plant

The operation of the 50 L pilot plant of the ChromBioB™ system can be summarized as follows (Ahmad et al., 2009, 2015); in the 100 L mixing tank, Cr(VI) solution (30–60 mL L−1) was mixed with liquid pineapple waste (LPW) to a final LPW concentration between 1 and 20% (v/v). The pH of the mixture was adjusted to 7.0 ± 0.5 using controller-regulated dosing of 12.5% (v/v) NaOH prior to transferring into the 150 L holding tank. Upon reaching the capacity of the holding tank, the neutralized and LPW amended Cr(VI) solution entered the 50 L bioreactor gravitationally in a down flow mode at 0.11 m3 h−1. After 24 h of continuous treatment inside the 50 L bioreactor, residual Cr(VI) was passed through a 10 L overflow to allow initial development of the biofilm on the RWS. Then, 50 L of solution mixture consisting of 30 mL Cr(VI) and 10% (v/v) LPW was introduced into the column. Sampling of the effluent fraction was carried out every hour for the first 4 h followed by 3 h gap for subsequent sampling. Each of the samples was determined for the Cr(VI) and COD values. The immobilized chromate reductase beads were prepared as follows; 10 L solution of a 24 h-grown culture broth for A. haemolyticus (1 g cell wet weight: 5 mL buffer) were placed in an ice bath and disrupted using a Sonics Vibra Cell 500 Ultrasonic Probe (amplitude - 9%, 50 W, pulse - 9 s, 1 s off-mode, 35 min). The sonicated fraction containing intracellular chromate reductase was mixed with 3% (v/v) sodium alginate solution at a ratio of 4:1 (v/v). The mixture was then homogenized and extruded drop-wise into a beaker (with stirring) containing 100 mL of 0.2 M CaCl2 (110.99 g/mol) solution. beads produced have an average diameter of 0.3–0.4 cm. The beads were left in the 0.2 M CaCl2 solution to cure for 0.5–3 h prior followed by distilled water washing until the spent-wash solution reached pH 7.

2.3. Chromate reductase assay and protein estimation

Chromate reductase activity assay was carried out using modified procedures from Elangovan et al., (2010) as follows; Cr(VI) solution (48 µM) was added into a series of 1.5 mL Eppendorf tubes containing a mixture of 0.8 mL of 100 mM potassium phosphate buffer and 0.2 mL aliquots of sub-cellular fractions. The incubation mixture was then incubated for 6 h at 30 °C. One unit enzyme activity for chromate reductase was defined as the amount of enzyme that reduces 1 pmol of Cr(VI) per min at 30 °C specific activity as unit of chromate reductase activity per mg protein in the CFE. Total protein was estimated using the Bradford’s method using bovine serum albumin as standard (Bradford, 1976).
2.4. Characterization of chromate reductase activity in CFE

2.4.1. Effect of pH, temperature and electron donors on crude enzyme activity and stability

The optimum pH for the chromate reductase activity was determined by transferring 0.2 mL of CFE at different initial pH values (5.8, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0) into a series of 1.5 mL Eppendorf tubes containing 1.0 mL mixture of buffer and 48 μM Cr(VI). The mixtures were then incubated at 30 °C for 6 h and the residual enzyme activity was measured in terms of Cr(VI) reduction. The CFE solution at different pH was also tested for stability test via storage at 4 °C for two weeks prior to the determination of the chromate reductase activity. Similar experimental setup was used to evaluate the effect of temperature on chromate reductase activity where the CFE – Cr(VI) mixtures were incubated at 25, 30, 35 and 40 °C for 6 h. The crude enzyme activity was ceased via cooling in an ice bath where the residual enzyme activity was expressed in terms of Cr(VI) reduction. The effect of electron donors on the chromate reductase activity of CFE were conducted as the following electron donors; NADH (7416.4 g/mol), sodium pyruvate (110.04 g/mol), glucose (α –) = glucose monohydrate; 198.17 g/mol), acetate (sodium acetate trihydrate; 136.08 g/mol), citrate (trisodium citrate (pH 7) were incubated for 6 h, 200 rpm and 30 °C of 1.5 mL Eppendorf tubes containing a 1.0 mL mixture of 1 mM of A. haemolyticus Pb(NO3)2, 331.21 g/mol; CuCl2.2H2O, 170.48 g/mol; CdCl2.2H2O, 360.32 g/mol). Reaction mixtures (final volume of 1.0 mL) consisting of 0.2 mL of CFE, 1 mM of electron donor and 48 μM of Cr(VI) in buffer (pH 7) were incubated for 6 h, 200 rpm and 30 °C followed by determination of residual Cr(VI). The heat treated CFE (representing non-enzymatic cells) was acted as control.

2.4.2. Effect of metal ions, respiratory inhibitor and protein denaturant

The chromate reductase activity in CFE of A. haemolyticus was characterized for the effect of metal ions using 1 mM solutions of either monovalent (AgNO3, 169.87 g/mol; NaCl, 58.44 g/mol; KCl, 74.56 g/mol), divalent (CaSO4.2H2O, 172.17 g/mol; MgSO4.7H2O, 246.48 g/mol; NiCl2.6H2O, 237.73 g/mol; ZnCl2, 136.29 g/mol; Pb(NO3)2, 331.21 g/mol; CuCl2.2H2O, 170.48 g/mol; CdCl2.2H2O, 228.34 g/mol; CoCl2.6H2O, 237.93 g/mol; BaCl2.2H2O, 244.27 g/mol; HgCl2, 271.50 g/mol) or trivalent ions (FeCl3, 162.21 g/mol). The effect of protein denaturant (1 mM EDTA, 372.24 g/mol) and respiratory inhibitor (1 mM NaN3, 65.01 g/mol) was also studied. Reaction mixtures (final volumes of 1.0 mL) consisted of 0.2 mL of CFE, 1 mM of either metal ions, protein denaturant or respiratory inhibitor and 48 μM of Cr(VI) in 100 mM potassium phosphate buffer (pH 7) were prepared. It was then incubated at 30 °C for 6 h prior to analysis where the Kd was determined using 1–14 mM Ag⁺.

2.4.3. Time course and kinetic studies

The kinetics and time course study of Cr(VI) reduction by CFE of A. haemolyticus was evaluated by adding 0.2 mL of CFE into a series of 1.5 mL Eppendorf tubes containing a 1.0 mL mixture of 1 mM of Cr(VI) and 100 mM potassium phosphate buffer. The mixtures were then incubated at 30 °C between 3 and 360 min followed by determination of the residual Cr(VI). The reaction kinetic parameters, Km (Michaelis–Menten constant) and Vmax (maximum velocity of reaction) were determined using the Lineweaver–Burke equation (Eq. (2)) which is derived by simply taking the reciprocal of the Michaelis–Menten equation, Eq. (1) where vo is the initial velocity and S is the substrate concentration (Boyer, 2006):

\[ V_o = \frac{(V_{\text{max}}[S])}{(K_m + [S])} \]

\[ 1/V_o = K_m/V_{\text{max}} \times 1/[S] + 1/V_{\text{max}} \]

3. Results and discussion

3.1. Operation of the pilot-plant

More than 90% of the initial Cr(VI) of 30 mgL⁻¹ was reduced (residual Cr(VI) of 2.41 ± 0.23 mgL⁻¹) after 24 h of contact with the bioreactor at 0.11 m³ h⁻¹. Upon contacting with the 10 L flow-through column containing biobeads, complete Cr(VI) reduction was achieved after 9 h of contact time. This is a very significant finding as it can reduce the time required for recirculation into the bioreactor as well as demonstrating the potential of using immobilized chromate reductase beads in on-site application. Similar situation was also observed at initial Cr(VI) of 60 mgL⁻¹ where after 24 h of recirculation inside the bioreactor, more than 90% of the Cr(VI) was reduced based on residual Cr(VI) concentration of 6.24 ± 0.21 mgL⁻¹ (Table 1). However, a longer contact time (24 h) with biobeads (relative to residual Cr(VI) obtained from initial Cr(VI) of 30 mgL⁻¹) was required to achieve complete Cr(VI) reduction. This situation is expected as enzymatic-based Cr(VI) reduction is known to proceed through first-order reaction. The COD concentration in the influent mixture containing either 30 or 60 mgL⁻¹ Cr(VI) solution and 10% (v/v) LPW also showed considerable reduction, which can be attributed to the metabolism of the carbon-rich compounds such as glucose and sucrose in LPW by the microbial population present in the biofilm together with A. haemolyticus. More than 70% of the initial COD concentration present in the influent mixture for 30 mgL⁻¹ Cr(VI) was reduced during the reduction of Cr(VI) with COD value decreasing from 2659.17 ± 100.97 mgL⁻¹ to 716.85 ± 20.38 mgL⁻¹. Around the same percentage (79%) was also recorded for reduction in COD concentration for initial Cr(VI) concentration of 60 mgL⁻¹ with COD values decreasing from 4662.45 ± 133.90 mgL⁻¹ to 995.15 ± 19.59 mgL⁻¹. It is worthy to note that, these residual COD concentrations were successfully reduced to less than 100 mgL⁻¹ i.e. the permissible discharge limit as stipulated in Standard B, Environmental Quality (Industrial Effluent) Regulation 2009 (Department of Environment (2009)), after the coagulation and flocculation process. Fig. 1a and b showed the profile for Cr(VI) and COD concentrations present in the effluent fraction of the 50 L bioreactor before treated using the 10 L biobeads-containing flow-through column.

3.2. Localization of chromate reductase activity

Chromate reductase activity of A. haemolyticus was detected in all three cell fractions assayed namely whole cells, membrane fraction and CFE. The involvement of intracellular enzymes in Cr(VI) reduction was strengthened from the absence of chromate reductase activity in the spent broth. Highest chromate reductase activity was determined in the whole cells (4.73 pmol/min/mg of protein, 20% Cr(VI) reduction) followed by membrane fraction.

Table 1

<table>
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<tr>
<th>Hour</th>
<th>Influuent Cr(VI) 30 mgL⁻¹</th>
<th>10% COD (%)</th>
<th>Influuent Cr(VI) 60 mgL⁻¹</th>
<th>10% COD (%)</th>
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</thead>
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<tr>
<td></td>
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<td>LPW</td>
<td>Cr(VI), mg L⁻¹</td>
<td>LPW</td>
</tr>
<tr>
<td>0</td>
<td>2.26 ± 0.01</td>
<td>713.13 ± 5.61</td>
<td>982.97 ± 6.77</td>
<td>6.24 ± 0.21</td>
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<tr>
<td>2</td>
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<td>677.05 ± 8.41</td>
<td>911.25 ± 2.19</td>
<td>5.49 ± 0.39</td>
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<tr>
<td>4</td>
<td>1.855 ± 0.18</td>
<td>604.06 ± 8.68</td>
<td>854.05 ± 5.44</td>
<td>4.77 ± 0.21</td>
</tr>
<tr>
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<td>551.08 ± 3.95</td>
<td>807.65 ± 6.45</td>
<td>4.06 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td>1.13 ± 0.08</td>
<td>529.5 ± 5.66</td>
<td>762.25 ± 12.37</td>
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<td>605.5 ± 9.19</td>
<td>3.95 ± 0.11</td>
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<td>486.66 ± 0.48</td>
<td>529.5 ± 3.53</td>
<td>1.085 ± 0.05</td>
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(3.67 pmol/min/mg of protein, 11.4% Cr(VI) reduction) and the crude CFE fraction (1.10 pmol/min/mg of protein, 2.4% Cr(VI) reduction). Specific reasons for the high chromate reductase activity determined in the membrane fraction for an obligate aerobe such as *A. haemolyticus* is currently being investigated as normally aerobic bacterial Cr(VI) reduction takes place in the cytoplasmic fraction i.e. CFE (Bae et al., 2005; Camargo et al., 2003), hence its selection for further characterization.

### 3.3. Characterization of chromate reductase activity in crude CFE

3.3.1. Effect of temperature, pH and electron donors

Maximum chromate reductase activity for crude CFE (8.2 pmol Cr(VI)/min/mg protein) was determined at 30 °C with Cr(VI) reduction percentage of 21% followed by 37 °C (6.3, 15.8%), 25 °C (2.2, 10.1%) and 40 °C (1.2, 5.9%) (data not shown). The chromate reductase activity was optimum at 30 °C probably due to the initial isolation temperature of *A. haemolyticus*. The optimum temperature of 30 °C agrees well with the use of this bacteria in bioremediation of Cr(VI) since no external energy is required to carry out the Cr(VI) reduction process. Heat killed CFE did not exhibit any chromate reductase activity. Wang et al., (1990) reported that no chromate reduction was observed at either 4 or 60 °C for CFE of *Enterobacter cloacae* while Camargo et al., (2003), Pal et al., (2005), Elangovan et al., (2006, 2010) and Desai et al., (2008a, 2008b) reported 30 °C being the optimum temperature for maximum Cr(VI) reduction by CFE of other bacterial species. As chromate reduction by CFE is enzyme-mediated, changes in pH will directly affect the degree of ionization of the enzyme, hence the enzyme activity. Maximum specific activity for crude CFE of *A. haemolyticus* was optimum at pH 7.0 with a value of 8.65 pmol/min/mg protein, representing 33.5% Cr(VI) reduction. The crude CFE also showed

![Fig. 1. Cr(VI) and mean COD concentrations in effluent fraction of the 50 L bioreactor for (a) influent Cr(VI) of 30 mgL⁻¹ (b) influent Cr(VI) of 60 mgL⁻¹; - Cr(VI), Δ - COD.](image-url)
good stability when stored at pH 7, 4 °C for two weeks (compared to other pH values that resulted in precipitation) with specific activity maintained at 6.92 pmol/min/mg protein (11.3% reduction). Similar findings were also reported for CFE of *Bacillus fusiformis* (Desai et al., 2008a), *Pseudomonas* sp. G1DM21 (Desai et al., 2008b) and *P. phragmitetus* LSSE-09 (Xu et al., 2012) where pH 7 was the optimum pH for maximum chromate reductase activity. In this study, glucose showed the potential as the most effective electron donor to enhance chromate reductase activity from crude CFE with maximum specific activity of 9.1 pmol/min/mg protein (23% Cr(VI) reduction) compared to other electron donors evaluated namely NADH, sodium pyruvate, acetate, citrate and lactate. This finding directly supported our previous report where liquid pineapple waste (rich in glucose/sucrose content) was determined as the most useful carbon source to sustain the growth of *A. haemolyticus* when used for Cr(VI) reduction (Zakaria et al., 2007). Glucose also resulted in the maximum Cr(VI) reduction by *Bacillus coagulans* (Philip et al., 1998), *Escherichia coli* (Bae et al., 2000) and *Bacillus* sp. (Liu et al., 2006). The effective role of glucose as electron donor in Cr(VI) reduction can be described by Eq. (3) (Chirwa and Wang, 1997).

\[
\text{C}_6\text{H}_12\text{O}_6 + 8\text{CrO}_4^{2-} + 34\text{H}^+ \rightarrow 8\text{Cr}^{3+} + 6\text{HCO}_3^- + 20\text{H}_2
\] (3)

Acetate and sodium pyruvate slightly stimulated the chromate reductase activity while NADH did not show any effect on Cr(VI) reduction. One notable finding is the negligible role of NADH towards Cr(VI) reduction by CFE from *A. haemolyticus*, which is not normal as most of the previous reports strongly indicated the effectiveness of NADH in enhancing Cr(VI) reduction (Camargo et al., 2003; Desai et al., 2008a; 2008b).

### 3.3.2. Effect of metal ions, metabolic inhibitor and protein denaturant

Amongst the metal ions tested, Co$^{2+}$ ions resulted in a 90% increment on the Cr(VI) reduction activity by crude CFE followed by Cu$^{2+}$ and Mg$^{2+}$ (around 39%). On the other hand, Na$^+$, K$^+$, Ca$^{2+}$, Fe$^{3+}$ and Ba$^{2+}$ did not exhibit any significant effect on the reductase activity while Ag$^+$, Hg$^{2+}$ and Ni$^{2+}$ displayed strong inhibition capacity (20% Cr(VI) reduction). K$_i$ value of 0.22 mM suggested that Ag$^+$ is a non-competitive inhibitor of Cr(VI) reduction as only the V$_{max}$ was affected. The K$_{m}$ value remained constant at various Ag$^+$ concentrations while V$_{max}$ (maximum velocity) decreased with increasing Ag$^+$ concentrations. This is the first report on the significant role of Co$^{2+}$ as co-factor to enhance Cr(VI) reduction by enzymic extracts from bacterial cells, hence its definite role remains to be investigated. Previous reports by other researchers on the role of Co$^{2+}$ in enhancing the relative activity of CFE by other bacteria such as *Bacillus sphaericus* AND 303 (Pal and Paul, 2004), *Pseudomonas* sp. G1DM21 (Desai et al., 2008b) and *Arthrobacter rhombi*-RE (Elangoavan et al., 2016), were not encouraging. Most of the reports on the involvement of metal ions to enhance chromate reductase activity by bacterial cells were for Cu$^{2+}$ (Camargo et al., 2003; Desai et al., 2008a; 2008b; Xu et al., 2012) which can be attributed to its ability to act as a protective agent for electron-transport, as a single electron redox center and as a shuttle for electrons between protein subunits (Camargo et al., 2003; Xu et al., 2012).

Ag$^+$ has been reported to inhibit the chromate reductase activity for *Pseudomonas putida* (Ishibashi et al., 1990) and *E. coli* ATCC 33456 (Shen and Wang, 1993) while Hg$^{2+}$ ions for *P. putida* (Ishibashi et al., 1990), *Bacillus* sp. (Elangoavan et al., 2006; Desai et al., 2008a), *Bacillus* sp. ES29 (Camargo et al., 2003) and *Pseudomonas* sp. G1DM21 (Desai et al., 2008b). Most common mode for chromate reductase inhibition by Hg$^{2+}$ was based on its role as disulfide reducers that result in denaturation of reductase protein. Furthermore, it is known to form a mercaptide bond with sulfhydryl groups of the enzyme molecule, thus inhibiting enzyme activity. Thus, the inhibitory effect by heavy metal ions is probably related to the oxidation of sulfhydryl groups on the surface of the enzyme molecule. The inhibitory effect of Hg$^{2+}$ was expected, because Hg$^{2+}$ binds to a variety of enzyme systems with a specific affinity for ligands containing the SH$^-$ systems.

A variety of metabolic inhibitors are known to interfere with electron transport at specific sites of the electron transport chain in microorganisms (Shen and Wang, 1993). Both of the metabolic inhibitors used in this study i.e. EDTA and azide (1 Mm) did not significantly affect the relative activity of CFE from *A. haemolyticus* with almost similar specific activity values and percentage of Cr(VI) reduced (13.5 pmol/min/mg protein, 27%) while control (8.7 pmol/min/mg protein, 22%). This clearly suggests that EDTA and azide does not interfere with specific sites of the electron transport chain in *A. haemolyticus*. Similar situation was reported for *E. coli* ATCC 33456 (Shen and Wang, 1993) and *Bacillus* sp. ES 29 (Camargo et al., 2003). However, sodium cyanide and sodium azide inhibited aerobic chromate reduction by *Bacillus subtilis* and inhibited more than 50% of membrane associated chromate reductase activity of *Shewanella putrefaciens* MR-1 (Elangoavan et al., 2010).

### 3.4. Kinetic and time course studies of chromate reductase activity

Cr(VI) reduction and specific activity of crude CFE increased with increasing Cr(VI) concentration up to 481 µM, beyond which the rate gradually decreased (Fig. 6). The chromate reductase activity of crude CFE showed strong dependence on substrate concentration with 90% reduction of 5–144 µM of Cr(VI) within 3 min of incubation. From the Lineweaver–Burke plots, the Km and V$_{max}$ values were calculated as 184.47 µM and 33.3 nmol/min/mg protein respectively. Detailed time course profiles for the Km (µM) of Cr(VI) and V$_{max}$ (nmol/min/mg protein) values for *A. haemolyticus* are shown in Table 2 while Table 3 compares the kinetic parameters obtained with other Cr(VI) reducing bacteria. Generally, the behavior of electron donors/cofactors varies with molecular structure and environmental conditions, which directly affect the Cr(VI) reduction rates and kinetic parameters (V$_{max}$ and K$_{m}$) of the bacterial chromate reductase.

### 4. Conclusion

This study demonstrates the potential of incorporating immobilized beads containing crude chromate reductase obtained from the Cr(VI) resistant-reducing *A. haemolyticus* into the existing ChromoBac™ system in order to reduce the overall Cr(VI)
treatment time. The outcome suggest that a higher Cr(VI) concent-
tration may be tolerated during application as no actively growing
cells were involved. Studies on chromate reductase purification and
elucidation on the role of Co^{2+} as co-factor during the Cr(VI)
reduction process are currently on-going.

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