Kinetic Study of Cr(VI) Reduction in an Indigenous Mixed Culture of Bacteria in the Presence of As(III)

Tony E. Igboamalu, Evans M.N. Chirwa*

Organic compounds can serve as electron donors during Cr(VI) reduction by live microbial cultures. However, Cr(VI) reduction with concomitant oxidation of metalloids such as arsenic has not been studied. In this study, an indigenous mixed culture of bacteria collected from the Brits Wastewater Treatment Plant (North West Province, South Africa) was used to biologically reduce Cr(VI) to Cr(III) while utilising electrons derived from the oxidation of As(III) to As(V). Both processes, i.e., reduction of Cr(VI) oxidation of As(III), are desirable since both the hexavalent form of chromium and the trivalent state of arsenic are acutely toxic at high concentrations and carcinogenic under low and subchronic conditions. In experiments conducted under aerobic conditions, near complete reduction of Cr(VI) was achieved under initial Cr(VI) concentrations up to 70 mg/L and a fixed As(III) concentration of 20 mg/L. However, Cr(VI) reduction was inhibited at Cr(VI) concentrations equal to and above 100 mg/L. Further experiments conducted at Cr(VI) concentration of 70 mg/L and varying As(III) concentration from 5-70 mg/L showed that Cr(VI) reduction rate increased with increasing As(III) concentration from 5-40 mg/L. However, 20% drop in Cr(VI) reduction efficiency was observed at concentrations higher than 40 mg/L. A similar trend of Cr(VI) reduction was observed in a continuous reactor operated under an optimum pH of 7.0±0.2, and dissolved oxygen concentration (DO = 0.6±0.1 mg/L) during steady state operation. These results show that arsenic served as an electron donor for the beneficial reduction of Cr(VI) to Cr(III) using a mixed culture of Cr(VI) reducing bacteria.

1. Introduction

Chromium (Cr) and arsenic (As) containing wastewater is routinely discharged from mineral processing operations especially from the gold and platinum mining industries. In most sources, Cr(VI) and As(III) are discharged as co-pollutants. In nature they are found in a range of oxidation states. The mobility, toxicity and environmental fate of Cr and As species dependent on their oxidation states and speciation. Cr and As are also found in livestock pesticides, wood preservatives and petrochemical waste sludge. Cr(VI) is mainly discharged as effluent from textile production, leather tanning, electroplating, paint and pigment manufacturing and wood processing industries. Arsenic from natural sources, on the other hand, is released from arsenate laden sediments by arsenate respiring bacteria which utilise As(V) as an energy source (Stolz et al., 2006). The As(V) oxidised is mobilised as As(III) resulting in contamination of natural water bodies (Dastidar and, Wang, 2010). Both metals are known to be carcinogenic and mutagenic to living organism at low exposure conditions, and acutely toxic at high concentrations (Federal Register, 2004). Biological transformation of toxic metals may be considered as an alternative to physical and chemical processes, as it can be achieved under natural pH and redox conditions, and therefore less environmental intrusive. Detoxification of Cr(VI) together with As(III) by microorganism has not been studied extensively. Previously, it was established that microorganisms exposed to toxic metals/metalloid ions developed diverse resistance mechanisms to tolerate the toxicity of toxic metal ions (Bachate et al., 2013). The resistance mechanisms involve specific biochemical pathways that can alter chemical properties of toxic metal ions, resulting in their detoxification (Silver and Phung, 2005). For example, conversion of Cr(VI) and As(III) was observed in ice samples by a bacterium Bacillus firmus (Bachate et al., 2013). Simultaneous conversion of Cr(VI) and As(III) to Cr(III) and As(V) under acidic condition was also reported earlier by Wang et al. (2013). These studies did not really explain the underlying biotransformation reaction. Our previous studies entirely
explored the theory behind cellular conversion of Cr(VI) to less toxic Cr(III), using As(III) as an electron donor (Igboamalu and Chirwa, 2014). In the latter study, the thermodynamic relationship between Cr(VI) reduction to Cr(III) and As(III) oxidation to As(V) was represented by equation 1 (below):

\[
3\text{HAs}^{3+} + 2\text{HCr}^{5+} + \text{O}_2 + \text{H}_2\text{O} \xrightarrow{\text{microbes}} 3\text{HAs}^{5+} + 2\text{Cr}^{3+} + 3\text{HO}^- + (\Delta G^\circ = -256 \text{ kJ/mol}) \tag{1}
\]

The above equation shows that Cr(VI) reduction involves an unstable intermediate product [Cr(V)] by accepting 1e\(^-\) from cellular NADH before accepting 2e\(^-\) from As(III) for final conversion to Cr(III) (Cervantes et al., 2001).

In the present study, Cr(VI) reduction in the presence of As(III), in indigenous mixed culture of bacteria collected from a Wastewater Treatment Plant in Brits (North West Province, South Africa), was explored to evaluate the inhibitory effect of As(III) on Cr(VI) reduction in a batch and continuous reactor. The performance of both reactors at different Cr(VI) and As(III) concentration was investigated.

2. Material and media

2.1 Culture and media

Dried sludge from sand drying beds at the Brits Wastewater Treatment Plant was used as inoculum for the mixed culture of bacteria used following the method outlined by Molokwane et al. (2008). The plant received periodic loadings of effluents containing sodium dichromate from nearby chrome refineries. Microorganisms at the sludge were expected to have developed resistance to Cr(VI) toxicity due to long-term exposure to Cr(VI).

2.2 Cr(VI) reduction in a batch experiment in the presence of As(III)

The harvested cells were re-suspended in 100 mL sterilised bottles containing mineral medium before adding Cr(VI) and As(III), to give a desired concentration. Experiments were conducted with varying initial concentration of Cr(VI) (50-500 mg/L) in a fixed concentration of As(III) at 20 mg/L and varying initial concentration of As(III) (5-70 mg/L) with a fixed concentration of Cr(VI) at 70 mg/L. All experiments were conducted at a near constant temperature of 30±0.2°C with continuous shaking at 120 rpm.

2.3 Cr(VI) reduction in a continuous flow reactor in the presence of As(III)

The continuous flow reactor (packed bed reactor) was constructed from a Pyrex glass column (internal diameter: 6.0±0.01 cm, height: 40±0.01 cm) packed with 4480, 5 mm spherical Pyrex glass beads (Fisher Scientific Co, Pittsburgh, PA) (Figure 1). The total external surface area of the glass beads available for cell attachment is 88000 mm\(^2\), in the packed bed reactor volume of 1131 cm\(^3\), and surface area of 28.3 cm\(^2\). Prior to assembling, the components of the pumps, control valves and the connecting tubing were autoclaved at 121°C for 15 min. Subsequently, the interior of the reactor was rinsed in 95% ethanol and dried. For a working reactor volume of 1131 cm\(^3\), distilled water was used to pre-calibrate peristaltic pumps used in order to achieve the desired volumetric flow rate. The reactor was operated in an up-flow mode to ensure near completely submerged condition. The reactor was designed to operate continuously at hydraulic retention time of 14 h, under volumetric feed flow rate of 0.013 cm\(^3\)/s. The reactor consists of sample ports of same diameter, and 2 L influent and effluent tanks as shown in Figure 1.

2.4 Analytical method

Cr(VI) was measured using the UV/Vis spectrophotometer (WPA, light wave II, Labotech, South Africa). The presence of Cr(VI) in the sample was visualized by the change of the colour after adding DPC (APHA, 2005). However, total Cr on the other hand was determined in the Varian AA–1275 Series Flame Atomic Absorption Spectrophotometer (AAS) (Varian, Palo Alto, CA (USA)) equipped with a 3 mA and chromium hollow cathode lamp at 359.9 nm wavelength.

3. Result and discussion

3.1 Cr(VI) reduction in the presence As(III) : Batch reactor performance

The results obtained from experiments conducted under varying initial Cr(VI) concentration (50-500 mg/L) showed that a reconstituted consortium or mixed culture achieved near complete Cr(VI) reduction at initial lower concentration 50-70 mg/L within 48 h of incubation in the presence of As(III). However, increasing Cr(VI) concentration up to 100 mg/L showed an incomplete Cr(VI) reduction (Figure 2). Above 350 mg/L Cr(VI) concentration, low or negligible reduction rate was observed. In addition, Cr(VI) reduction efficiency was evaluated in different batch experiments (C1, C2, C3, C4, C5, and C6).
Subsequently, the effect of different As(III) concentration on Cr(VI) reduction was also investigated. Cr(VI) concentration of 70 mg/L was investigated in a batch experiment at different As(III) concentration ranging from 5-70 mg/L. Despite the toxicity of Cr(VI) and As(III), a successful reduction was achieved at higher concentrations under anaerobic condition. Result showed that a near complete Cr(VI) reduction was achieved after 48 h of incubation, when the batch experiment was amended with As(III) concentration ranging from 5-40 mg/L at 70 mg/L Cr(VI). Consequently, after increasing As(III) concentration to 50 mg/L, incomplete Cr(VI) reduction was observed as shown in Figure 3. The incomplete Cr(VI) reduction observed was as a result of dual toxic effect of Cr(VI) and As(III) on microbial cell. Overall cumulative Cr(VI) reduction efficiency was evaluated in different batch experiments (B1, B2, B3, B4, B5, B6, B7, B8 and B9) . These batches represent different As(III) concentration ranging from 5-70 mg/L in the presence of 70mg/L of Cr(VI). Result showed that high reduction efficiency was achieved as As(III) concentration increases from 5-40 mg/L, and decreases above 50 mg/L.
3.2 Batch Kinetic Results

The batch data shown in Figures 2 and 3 was evaluated against the non-competitive inhibition model with cell deactivation that was derived earlier by Molokwane and Chirwa (2013) (Equation 2):

\[
\frac{dC}{dt} = \frac{\mu_{\text{max}} C}{k + \left(\frac{C}{C_0} - \frac{C}{K_c}\right) (C + K)}
\]

where, \(\mu_{\text{max}}\) = maximum specific Cr(VI) reduction rate (mg/L/h); \(C_0\) = initial Cr(VI) concentration (mg/L); \(X_0\) = initial biomass concentration (mg/L); \(C\) = Cr(VI) concentration (mg/L) at a time \(t\); \(K_c\) = maximum Cr(VI) reducing capacity (mg/mg); \(K\) = half velocity concentration (mg/L); \(\text{Cr}\) = Cr(VI) toxicity threshold concentration (mg/L); and \(k\) = limiting constant (mg/L). The model fit results shown in Figure 4 and Table 1 show a good fit of the model to the experimental data.

Figure 4: Batch performance evaluation Cr(VI) concentration (50-500) mg/L

Table 1. Biokinetic parameter for Cr(VI) reduction in the presence of As(III) in the batch assay

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K_c) (mg/mg)</td>
<td>0.998</td>
</tr>
<tr>
<td>(k) (mg/L)</td>
<td>4.317</td>
</tr>
<tr>
<td>(K) (mg/L)</td>
<td>732</td>
</tr>
<tr>
<td>(\mu_{\text{max}}) (h(^{-1}))</td>
<td>0.644</td>
</tr>
<tr>
<td>(\chi^2) (mg/L(^2))</td>
<td>230±50</td>
</tr>
</tbody>
</table>

3.3 Biomass characteristics

Phylogenetic characterization of cell was performed on individual colonies of bacteria isolated from Wastewater treatment Works Brits South Africa. The strains were identified by 16S rDNA sequencing, and it showed about 99.9 % sequence identity with Bacilli, Enterobacteria and staphylococcus (Igboamalu and Chiwa, 2014). Biofilm growth on the glass beads in the continuous flow reactor was examined under electron scan microscope. Morphological observation showed a dense population of microbial growth or biofilm growth on the glass beads as shown in Figure 5. However, the observed evidence of biofilm growth on the glass beads suggest that Cr(VI) feed content in the presence of As(III) was indeed reduced by biofilm attached on the glass beads.

3.4 Cr(VI) reduction in the presence of As(III) – Continuous flow Reactor Performance

Continuous biofilm reactor was investigated for Cr(VI) removal under anaerobic condition in the presence of As(III). The reactor was inoculated with a mixed culture of Cr(VI) reducing anaerobes prior to experimental start up. The reactor was operated continuously for a period of 120 days over a range of influent Cr(VI)
concentration of 20-100 mg/L, at hydraulic retention time of 14.4 h, volumetric flow rate (0.0131 m³/s) and temperature (30±2). Successful Cr(VI) reduction was achieved over 120 successive days of continuous operation. Figure 6 shows the influent and effluent Cr(VI) concentration of the reactor throughout the operational stages. Different stages (I, II, III, IV, V, VI, VII, VIII) marked on Figure 5 correspond to changes in inlet Cr(VI) concentration. However, the reactor steady states were assumed when Cr(VI) outlet concentrations remained constant for at least three hydraulic retention times. Result showed a successful Cr(VI) reduction at initial lower concentration ranging from 20-50 mg/L. About 95-97 % Cr(VI) removal efficiency was achieved in the first six stages of continuous operation.

Figure 5: SEM photographs of a crevice at different magnifications showing biofilm attachment on the glass beads

![Figure 5](image)

Figure 6 Cr(VI) reduction in a continuous flow (biofilm) reactor in the presence of As(III) at different Cr(VI) concentration of (20-100) mg/L.

Increasing the reactor loading up to 100 mg/L per 14.4 h hydraulic retention time, about 20 % decrease in removal efficiency was observed, given a removal efficiency of < 70 %. A system robust was achieved when the influent Cr(VI) concentration was increased up to 50 mg/L, with high reduction efficiency approximately 97 % after 60 days of continuous operation. This suggests that microbial growth or biomass increases as Cr(VI) concentration increases. Comparing Cr(VI) reduction efficiency at the first six stages of operation with seven and eight stages after 110 days of continuous operation, it could be establish that the efficiency of Cr(VI) reduction was inhibited, with 20 % drop in Cr(VI) reduction efficiency. This suggests that the reduction capacity of the cell is inhibited at this stage of operation. However, the observed inhibitory effect was attributed to the toxicity effect of Cr(VI) and As(III) to microbial cell.
Comparing Cr(VI) reduction efficiency in the presence of As(III), in the continuous reactor with reduction efficiency previously observed in the batch experiment. About 60-75 % Cr(VI) reduction efficiency was achieved in the continuous reactor at Cr(VI) concentration of 100 mg/L, whereas in batch system about 50 % Cr(VI) reduction efficiency was achieved. This suggests that continuous reactors performed better than batch reactors. The outperformance observed in the continuous flow packed bed biofilm reactor was attributed to biomass limitation in the batch reactor, while as in continuous biofilm reactor there was improved culture flexibility, allowing high specific biomass retention time (Nicolella et al., 2000).

Secondly, Culture adaption and mass transport resistance across the biofilm layer or cell exposure to toxicity also improved Cr(VI) reduction efficiency (Wang and Chirwa, 2001). Throughout the reactor operation, anaerobic condition was maintained at dissolved oxygen concentration (DO) levels ranging from 0.5-0.7 mg/L. In addition, the pH of the reactor was kept at 7±0.2, which was assumed as the optimum pH for Cr(VI) and As(III) conversion.

4. Conclusions

Successful Cr(VI) reduction was achieved in both batch and continuous biofilm flow reactor in the presence of As(III), thus gives a promising step towards simultaneous bioremediation of toxic waste from industrial and mining processes. Although As(III) concentration was not quantify in this study, but it was assumed that Cr(VI) reduction was linked to As(III) oxidation based on microbial catalysed redox reaction Equation 1. Further studies are required to evaluate the simultaneous Cr(VI) reduction and As(III) oxidation in the mixed culture of facultative anaerobes in a batch and continuous reactor in the presence of As(III).

Reference


Igboamalu T.E., Chirwa E.M.N., 2014, Cr$^{VI}$ reduction in an indigenous mixed culture of bacteria in the presence of As$^{III}$, Chemical Engineering Transactions, 39, 1237-1242 DOI: 10.3303/CET1439207.


