Simultaneous Cr(VI) Reduction and Phenol Degradation in a Trickle Bed Bioreactor: Shock Loading Response

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Toxic heavy metals such as Cr(VI) are discharged together with toxic organic compounds in effluent streams from industry. Treatment strategies applied for a specific metallic pollutant may not work due to dual toxicity imposed by the organic component. In this study, Cr(VI) reduction was achieved by a mixed culture of Cr(VI) reducing bacteria isolated from activated sludge from a local wastewater treatment plant. The culture was started in a mixture of phenol and glucose to acclimate the bacteria to phenol toxicity and then supplied with phenol as a sole carbon. A trickle-bed reactor system was operated under varying influent Cr(VI) and concentration. Catastrophic failure was observed under a Cr(VI) concentration of 20 mg/L and incipient failure was observed in the acclimated system operated under an influent Cr(VI) concentration of 30 mg/L at operating for 502 h. System recovery was facilitated by lowering the influent Cr(VI) to threshold toxicity levels for the culture (10 mg/L). The reactor achieved 58% Cr(VI) and 86% phenol removal under shock the highest shock loading conditions of 30 mg/L. The trickle-bed system showed resilience and the ability to recover autonomously without the need to re-inoculate the reactor. The catalyst in this system (bacteria) has the potential of being self-regulatory and naturally regenerative.

1. Introduction

Chromium (VI), known as hexavalent chromium poses a serious biological threat due to its common ecological occurrence and carcinogenic properties. Hexavalent chromium intermediates are believed to be involved in the deletion of cellular constituents although the mechanism by which this occurs is still unknown (Lui et al., 2001). Its presence in the environment can be ascribed to its use in industrial applications such as chrome plating and leather tanning as well as the manufacture of dyes, paints and pharmaceuticals. Cr(VI) is often introduced into the environment through direct releases into water systems. It may leach into groundwater aquifers where it can accumulate to dangerous concentrations. It is possible to reduce Cr(VI) to Cr(III) which is much less toxic.

Current methods for Cr(VI) reduction are expensive and are poorly utilized (if at all) by most of the establishments responsible for Cr(VI) production. The more economical alternative being the dilution of Cr(VI) to such an extent that the effluent concentrations

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fall below the limit of 0.05 mg/L set by national safety standards. The problem with this is that Cr(VI) dilution from the point of origin does not prevent chromium from accumulating in the environment. Biochemical reduction of Cr(VI) involves an enzymatic catalysed process in which bacteria reduce Cr(VI) to Cr(III). Compared to expensive chemical treatment this process offers a relatively inexpensive and environmentally friendly solution if it can be implemented in a large scale continuous manner. A trickle bed bioreactor was proposed as a trial model for simultaneous Cr(VI) and phenol reduction. The purpose of the investigation is to assess reactor performance with respect to the following:
The effect of abrupt changes in Cr(VI) and phenol feed concentrations on Cr(VI) and phenol conversions.
- The reactor recovery period that is required to reach new steady state operation upon initiation of shock loading treatment.
- The fluctuation in Cr(VI) and phenol reduction capabilities of the reactor throughout the entire period of reactor operation.
- Cr(VI) reduction performance when phenol is the sole carbon source present in the feed.

Attached biomass in a trickle bed system is expected to promote the growth of biofilm which provides protection of useful microorganisms against toxicity through mass transport resistance. Bacteria grown in suspension is known to be highly susceptible to toxic compounds such as Cr(VI) and phenol (Nkhalambayausi-Chirwa and Wang, 2001).

Phenol was representative of organic compounds in wastewater. Initially, glucose was added as an additional carbon source to promote rapid bacterial growth. Operational temperature varied within the limits of 26°C to 30°C. Effluent pH was not measured since the mineral buffer solution acted as a pH stabilizer. Due to time constraints mass transfer considerations within the reactor was not investigated (Coenye et al., 1999).

2. Materials and Methods

2.1 Culture conditions
The bacterial co-culture inoculated into the reactor comprised of the phenol reducing species *Pseudomonas Putida* and a mixed culture of chromium reducing species containing *Bacillus cereus* strain 213 16S, *Bacillus thuringiensis* 16S, *Bacillus sp. ZZ2* 16S, *Bacillus cereus* ATCC 10987, *Bacillus thuringiensis* str. Al Hakam, *Bacillus mycoides* strain BGSC 6A13 16S and *Bacillus thuringiensis* serovar finitimus Strain BGSC 4B2 16S. This consortium of Cr(VI) reducing bacteria was first isolated and characterized by Molokwane et al. (2008). The different species were characterized by 16S rRNA fingerprinting.

2.2 Reactor configuration
The reactor comprised of an 8.9 L cylindrical vessel having a diameter of 15 cm (Figure 1). The vessel was filled to approximately 80% of its volume with coarse stones having a mean diameter of 9 mm. A plastic disc with 5 mm holes drilled into it served as support for the packing media. Liquid feed entered at the top of the column while aeration was supplied from the bottom (counter current flow). To improve liquid
distribution over the packing media a perforated flow distribution head was inserted in the feed line at the top of the vessel. Air inlet and outlet piping was inserted at the bottom and top of the column respectively. The effluent from the column was drawn off at the bottom through a funnel that was attached to the reactor. Two peristaltic pumps were employed. One pump provided the required reflux flow rate for the internal flow loop. The second pump delivered new feed to the system from the feed container.

![Diagram of the trickle bed reactor configuration](image)

*Figure 1. Trickle bed reactor configuration featuring a column packed with 7-10 mm diameter pebbles and a counter-flow mode of operation.*

### 2.3 Measurement of Cr(VI)
Cr(VI) was measured using a UV/VIS spectrophotometer (WPA, Light Wave II, Labotech, South Africa) at a wavelength of 540 nm (10 mm path) after acidification of 0.2 mL samples with 1N H₂SO₄ and reaction with 1,5-diphenyl carbazide to produce a purple colour (APHA, 2005). The conversion of Cr(VI) to Cr(III) was validated by measuring total chromium at a wavelength of 359.9 nm using a Varian AA – 1275 Series Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, CA (USA)) equipped with a 3 mA chromium hollow cathode lamp.

### 2.4 Measurement of phenol
Phenol concentration was measured using the Waters Alliance 2695 High Performance Liquid Chromatograph (HPLC) (Meadows Instrumentation Inc, Illinois, USA) equipped with a 717 Plus Waters PAH C18 Symmetry Column (4.6 mm × 250 mm, 5μm stationary phase) and 996 Photodiode Array Detector. For sample injection, 5 mL
syringes attached with 0.45μm pore size filters were used to transfer 10μl of sample into the column. The mobile phase comprised of a 1% acetic acid in water : 1% acetic acid in acetonitrile. Data was interpreted by the Millennium® 2010 Chromatography Manager.

3. Results and discussion

3.1 Reactor operation during start-up phase
Reactor operation with a Cr(VI) phenol feed showed a characteristic failure from the on start indicating that the bacteria was susceptible to either of the compounds. Substitution of the phenol with a less toxic substrate resulted in the complete reduction of Cr(VI) in the reactor as feed solutions up to 30 mg/L (8 hours HRT). This result was expected as Cr(VI) reduction by this culture was previously demonstrated (Molokwane et al., 2008, 2009).

The main experiment comprised the study of the adaptive change in performance of the system after shock loading. In Figure 2, it is shown that Cr(VI) reduction improved with time when a certain favourable concentration was sustained. This was evident at the influent Cr(VI) concentrations of 10 and 50 mg/L. At the high influent concentrations of 20 mg/L and 30 mg/L, incipient failure was observed.

![Graph showing variation of Cr(VI) feed and effluent concentrations during operation.](image)

*Figure 2. Variation of Cr(VI) feed and effluent concentrations during operation.*
Phenol concentration was increased together with influent Cr(VI) at time 115 h and 502 h (Figure 3). This resulted in the catastrophic failure under the Cr(VI) feed concentration of 20 mg/L (hour 115-200) and incipient failure after hour 502. It is evident from these results that the impact of phenol toxicity on the culture was not as severe as the impact of Cr(VI) toxicity.

![Graph of Phenol Feed and Effluent Concentrations](image)

*Figure 3. Variation of phenol feed and effluent concentrations during operation.*

### 3.2 Shock loading response

The influence on the Cr(VI) reduction was more severe than the influence on the phenol reduction as can be seen from Figures 2 and 3. Effluent phenol concentration remained quite low throughout the period of operation. It was also noted that the performance of the bacteria was within the order of magnitude of the culture investigated by Nkhambayausi-Chirwa and Wang (2001) using a coculture of *P. putida* DMP-1 and *E. coli* ATCC 33456. In the latter study, the coculture achieved approximately 100% removal of Cr(VI) under a phenol feed of 800 mg/L, Cr(VI) feed of 26.5 mg/L, and 24 hours HRT. In this study, up to 60% was achieved under a similar influent feed while operating at a third of the previous HRT.

Reactor response during a short loading after operating for 502 hours was far more robust than in the earlier shock loading events at 100 h and 165 h. Both the Cr(VI) and phenol effluent concentrations revealed a slight increase after which their concentrations stabilized and started decreasing again. The reactor still maintained a Cr(VI) conversion of 58% only 42 hrs after shock loading treatment and a phenol conversion of 86% only
18 hrs after shock loading had been implemented. The flexibility of the reactor to accommodate sudden fluctuations in feed concentrations has noticeably improved since the first shock loading treatment.

The ability of the reactor to produce Cr(VI) and phenol conversions of at least 58 % and 86 % within approximately 50 hrs after the shock loading treatment, proves that Cr(VI) reduction in bioreactors is a viable and sensible endeavor.

4. Conclusion

The trickle bed reactor for treatment of toxic waste streams offers particular advantages as the bacteria may acclimate quickly due to controlled exposure of the microorganisms in the biofilm formed on the support media. The bioreactor was capable of producing Cr(VI) and phenol conversions of no less than 58 % and 86 % in only 50 hrs after shock treatment was executed. It is therefore concluded that with proper conditioning, a trickle bed bioreactor can be made to adapt in a timely manner to accommodate sudden fluctuations in feed conditions. The performance of the microorganisms was affected by pre-exposure to the waste stream, thus performance in later shock loadings improved over when compared to performance of the previous phases. This phenomenon confers great complexity during mathematical modeling of the biofilm process.

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References


