CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN 978-88-95608- 56-3; **ISSN** 2283-9216

Bacterial Cr(VI) Reduction with Internal Carbon Recirculation Using Freshwater Algae as Primary Producers

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Chromium exits naturally in two oxidation states: hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)). Cr(VI) is carcinogenic and mutagenic to living organisms and Cr(III) is 100 times less toxic than Cr(VI). Conventional treatment of Cr(VI) involves the reduction of Cr(VI) to Cr(III), followed by the precipitation of Cr(III) as chromium hydroxide [$Cr(OH)_3(s)$]. Several species of bacteria have been shown to reduce Cr(VI) to Cr(III) either as metabolic necessity or as detoxification strategy for survival. Biological reduction processes can be engineered to reduce cost. In this study, a well-studied locally isolated culture of bacteria is utilised to reduce Cr(VI) to Cr(III) while growing on carbon sources produced as algal metabolites. The algae are also tested in their ability to adsorb the reduced Cr(VI), thereby reducing the total chromium in solution. Locally isolated bacteria combined with algae achieved 100 % removal of Cr(VI) in 24 hours without additional carbon sources, whereas bacteria with added glucose also achieved 100% removal in less than 7 hours. The algae were not able to adsorb the reduced Cr(VI), but was instead used as a carbon source for Cr(VI) reduction. Without carbon sources the bacteria could only reduce 29 % of the Cr(VI) and up to 53 % in cell free spent algae media which contained external metabolites produced by algae. Utilizing carbon sources produced by algae would be more practical to implement in the real world than adding glucose. This demonstrates the potential of combining locally isolated Cr(VI) reducing bacteria and green algae to decontaminate Cr(VI) polluted sites in South Africa.

1. Introduction

Cr is used in a variety of metallurgical, refractory and chemical processes, as well as in industrial activities such as the refining of ore, the productions of production of steel and alloys, metal plating and tanneries (Gomes et al., 2012). Cr is one of the top 20 contaminants on the Superfund priority list of hazardous substances (Gomes et al., 2012). In groundwater Cr typically occurs either in its trivalent oxidation state as Cr(III), or in its hexavalent oxidation state as Cr(VI) (Dermatas et al., 2012). Of the two oxidation states that are considered Cr(III) is the predominant form in most minerals, and occurs most commonly in acidic and reducing conditions, Cr(III) is insoluble at pH levels above 5, is immobile, and has a relatively low toxicity - especially when compared with Cr(VI) - and readily precipitates as $Cr(OH)_3$ (Molokwane and Chirwa, 2009) In trace amounts Cr(III) is considered to be essential for the proper functioning of living organisms (Gomes et al., 2012). Cr(VI) occurs in alkaline and oxidizing conditions, is very soluble and mobile, is a human carcinogen, and is toxic to plants, animals, humans and microorganisms (Dermatas et al., 2012). Cr(VI) presents a high risk of groundwater contamination due to its high mobility in subsurface environments, and is considered a high priority pollutant in many countries (Di Palma et al., 2012). The reduction of Cr(VI) to Cr(III) is more desirable than outright removal because Cr(III) is an essential nutrient to many living organisms. Furthermore, if the goal is the complete removal of Cr, the reduction of Cr(VI) to Cr(III) is a desirable first step in the removal process, due to the fact that Cr(III) is has a lower mobility, lower toxicity, and a lower bioavailability (Di Palma et al., 2015). This has caused increased interest in technologies such as the remediation of Cr(VI) through reduction by zero-valent iron, and the bioremediation of Cr(VI) through the use of bacteria facilitate biological reduction.

Bioremediation is an alternative to physical and chemical processes and incorporates biological transformation of toxic metals under natural pH and redox conditions. Microorganisms exposed to toxic compounds such as Cr(VI) ions developed diverse resistance mechanisms to tolerate the toxicity. The resistance mechanisms involve specific biochemical pathways that can alter chemical properties of toxic metal (Igboamalu and Chirwa, 2016). Bacteria species that can tolerate Cr(VI) toxicity, and can reduce Cr(VI) to Cr(III), are called chromium reducing bacteria (CRB).

A microorganism in most cases requires an organic carbon source, as either an energy source or as an electron donor, to be able to reduce Cr(VI). In the past the carbon source was provided in forms similar to glucose and Luria-Bertani (LB) (Zhiguo et al., 2009 and Molokwane and Chirwa, 2009). Recently Molokwane and Chirwa (2010) used saw dust as a carbon source for Cr(VI) reduction. The cost of the carbon source is one of the most discussed drawbacks that can hinder the commercial application of this pathway (Vidotti et al., 2014). The possibility of using algae and algae metabolites is yet still unexplored.

Microalgae are photoautotrophic microorganisms that can produce energy by using sunlight, water, and CO_2 (phototrophic metabolism) (Visca et al., 2017). Thus, algae use CO_2 from the atmosphere as its main carbon source. It has been established by Dvoretsky et al. (2017) that the cultural liquid of microalgae can be used as the basis of the nutrient medium in the cultivation of the Lactobacillus casei B-3241 bacteria. This separated cell culture liquid was found to contain ammonium salts, amino acids, B vitamins, cobalt, copper, manganese, molybdenum, iron, zinc, iodine, and other trace elements. (Dvoretsky et al., 2017) Chromium ruptures algae cell walls, causing cells to leak lipids, proteins, carbohydrates, and primary metabolites such as sugars, sugar alcohols, amino acids, and organic acids. These ruptured cell walls produce nutrients that surrounding bacteria can use (Cicci et al., 2017).

A consortia of algae and bacteria can work in a synergistic manner to detoxify pollutants. Algal photosynthesis produces O_2 which the aerobic bacteria requires, and the bacteria provides CO_2 and other stimulatory means to support the photoautotrophic algae. The bioremediation processes, by means of algae-bacteria consortia, have the potential to be a self-sustaining system, which is cheaper compared to conventional remediation technologies, which have several disadvantages such as high costs and the production of secondary pollutants. Even though heavy metals are potent inhibitors of photosynthesis, as they can replace or block the prosthetic metal atoms in the active sites of certain enzymes, bacteria can reduce significant concentrations of aqueous cations, which affect the speciation, distribution and mobility of those cations (Fu et al., 2016).

The aim of this study is to determine the extent of Cr(VI) reduction by locally isolated CRB and if these CRBs are able to utilise the algae and algal metabolites as a carbon source during the reduction process. The algae are also tested in their ability to absorb the total chromium in the system.

2. Section headings

2.1 Algae Cultivation

Chlamydomonas reinhardtii algal species were purchased from the Culture Collection of Algae and Protozoa (CCAP). The algae strain was cultured axenically in the modified recipe of 3-fold Nitrogen, Bold Basal Media with Vitamins (3N-BBM+V). Cultures were grown in 1000 mL Erlenmeyer flasks under the required algal light conditions (Osram L 36W/77 Floura) at 20-23 °C (Birungi and Chirwa, 2017). After 14 days the algae cells were harvested, and then centrifuged for 10 min at 6 000 rpm at 4 °C. The supernatant was further centrifuged multiple times until it was completely clear and cell free. The cell free spent media was then autoclaved and the solution pH was adjusted to 7.

2.2 Bacteria Cultivation

The Brits Wastewater Treatment Plant occasionally receives loadings of sodium dichromate from nearby chrome refineries. Due to these occasional sodium dichromate loadings the microorganisms found in the sludge samples had developed a resistance to Cr(VI) over a long period of exposure (Igboamalu and Chirwa, 2016). These sludge samples were used as inoculum to culture the CRB. Approximately 5 g of the inoculum was added to 400 ml of enriched media, which was then spiked with Cr(VI), and incubated at 30 °C for 12 hours in a continuous shaker (Labotech, Gauteng, South Africa). Bacteria strains were isolated from the enriched media by serial dilutions and the use of the spread method (Molokwane et al., 2008).

The bacteria strain identification was based on the ± 700 bp partial sequence of the 16S rRNA gene of the organisms. The sequences were compared against the GenBank of the National Center for Biotechnology in the United States of America using a basic BLAST search. The isolated bacteria were identified as Escherichia coli, Bacillus thermoamylovorans and Citrobacter sedlakii. The different species of bacteria were used as a reconstituted consortium in experiments. The bacteria were stored as soon as possible after isolation in a -70 °C freezer to preserve the bacteria's chromium reducing capabilities. In a 1 000 mL Erlenmeyer flask containing 400 mL LB broth, cultures were grown aerobically for 24 hours. Cells were

collected, and centrifuged for 10 min at 6 000 rpm at 4 °C. The supernatant was decanted and the remaining pellet was washed three times in a sterile saline solution (0.85 % NaCL) (Igboamalu and Chirwa, 2016).

2.3 Analytical method

Cr(VI) was measured using the UV/Vis spectrophotometer (WPA, light wave II, Labotech, South Africa) at a wavelength of λ = 540 nm (10 mm light path). The appearance of a purple colour after acidification with 1N N_2SO_4 and adding 1,5-diphenyl carbazide indicates the presence of Cr(VI) in the sample (APHA, 2005). The intensity of the purple colour is related to the Cr(VI) concentration. However, total Cr was determined in the Varian AA–1275 Series Flame Atomic Adsorption Spectrophotometer (AAS) at 359.9 nm wavelength (Varian, Palo Alto, CA (USA)) equipped with a 3 mA and chromium hollow cathode lamp. Cr(III) was determined as the difference between total Cr and Cr(VI) concentration.

2.4 Cr(VI) removal in a batch experiment

The harvested cells from both the bacteria and algae cultures were re-suspended in 50 mL sterilised Erlenmeyer flasks containing BBM (pH of 7.5) and Cr(VI) to give a desired concentration. Varying initial concentration of Cr(VI) between 30 and 100 mg/L were used in the experiments. All the experiments were conducted at 30±2 °C over time at 120 rpm on the orbital shaker (Labotec, Gauteng, South Africa). The samples taken at predetermined intervals were centrifuged using a 2 mL Eppendorf tube at 6,000 rpm for 10 min in a Minispin® Microcentrifuge (Eppendorf, Hambury, Germany) and the supernatant was used for Cr(VI) reduction analysis.

3. Results and discussion

Three main sets of experiments and two controls were performed. The first set of control experiments used only bacteria without any carbon source, and the second set of control experiments used only algae (as potential biosorbent). The main batch of experiments included a set of bacteria and algae combined experiments, a set of bacteria in cell free spent algae media experiments, and a set of bacteria with glucose experiments. In all the relevant experiments the same biomass concentration for bacteria and algae were used, i.e. 6060 mg/L bacteria and 1000 mg/L algae.

3.1 Cr(VI) removal

Figure 1 shows the results of the first set of control experiments, where only bacteria was used in a Cr(VI) solution without a carbon source. Even at a low initial Cr(VI) concentration the bacteria were only able to reduce 25 % of Cr(VI) after 24 hours. This demonstrates the importance of a carbon source during Cr(VI) reduction. Figure 2 shows the results of the control where only algae and chromium were present. The Cr(VI) concentration initially had a spiked increase within the first 3 hours. It is theorised that the Cr(III) in the solution was converted to Cr(VI) via an unknown mechanism. This phenomenon only occurred in the presence of algae cells. A recent study has revaluated the toxicity of Cr(III) to algae and have found that Cr(III) can also be toxic to certain microorganisms (Vignati et al., 2010). The spike could be attributed to a response of the algae towards Cr(III) in the solution, or a change in oxidation conditions. Although this is not conclusive evidence. After the initial spike, the Cr(VI) concentration returns to the initial value, and stays stable for the duration of the experiments. This suggest that biosorption of Cr(VI) by algae cells was not significant.

In Figure 3 the results are shown for the experiments where algae cells were used as a potential carbon source for the bacteria. The Cr(VI) was only completely removed after 24 hours. From Figure 2 it can be concluded that biosorption of Cr(VI) was not significant. It is more likely that bacteria were able to utilise metabolites produced by the algae, or even the algae cells themselves, as a source of carbon during the experiments. The slow reaction rate could be contributed to the slow rapturing of the algae cell walls by Cr(VI) or the slow release of metabolites from the algae cells, and the possibility that different metabolites are utilised at different rates. It is clear that the reduction of Cr(VI) is inhibited, and it is possible that multiple side reactions are also taking place, along with a small degree of biosorption.

Figure 4 displays Cr(VI) concentration in a system containing bacteria and the cell free spent algae media. This test was done to determine if algal metabolites in the spent media can be used as a carbon source. After 24 hours 53% of the Cr(VI) was removed, with an initial concentration of 100 mg/L, compared to just 29 % Cr(VI) removal by bacteria without a carbon source shown in Figure 1. At an initial concentration of 30 mg/L Cr(VI), 88 % removal was achieved. This indicates that the bacteria were able to use the algal metabolites to some extent. However, the algal metabolites were not sufficient to allow the bacteria to completely reduce the Cr(VI) in 24 hours. In Figure 5 it is shown that with glucose as a carbon source the bacteria cells were able to completely reduce Cr(VI) from an initial concentration of 100 mg/L in less than 7 hours, whereas in the

bacteria algae system complete reduction took 24 hours. An initial glucose concentration of 100 mg/L was used.

Figure 6 shows a comparison of the percentage removal for each system at 100 mg/L initial Cr(VI) concentration. The bacteria glucose system has a faster Cr(VI) removal rate than the bacteria algae system, but both systems achieve 100 % removal within 24 hours. The different form of the bacteria glucose and bacteria algae curves suggest that different processes are taking place.

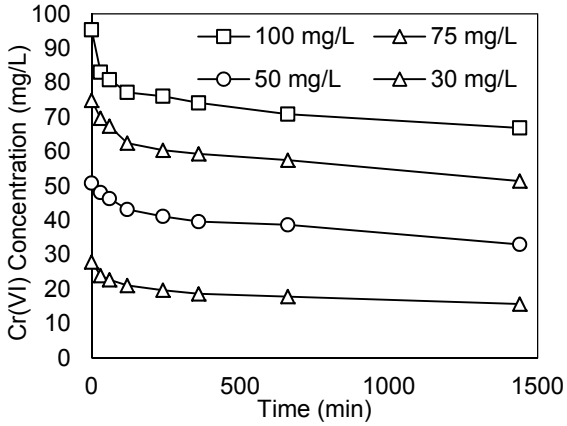

Figure 1: Cr(VI) concentration in a system containing only bacteria that serves as a control.

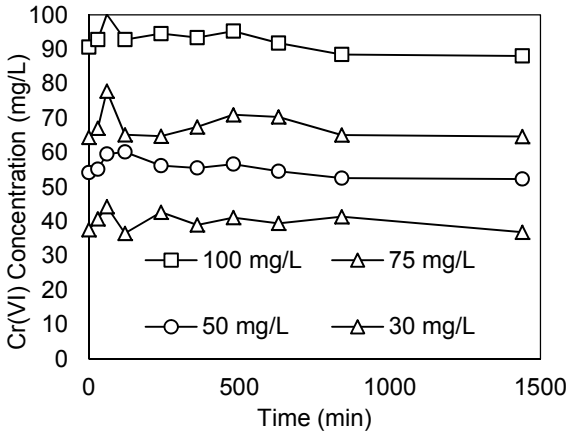

Figure 2: Cr(VI) concentration in a system containing only algae that also serves as a control.

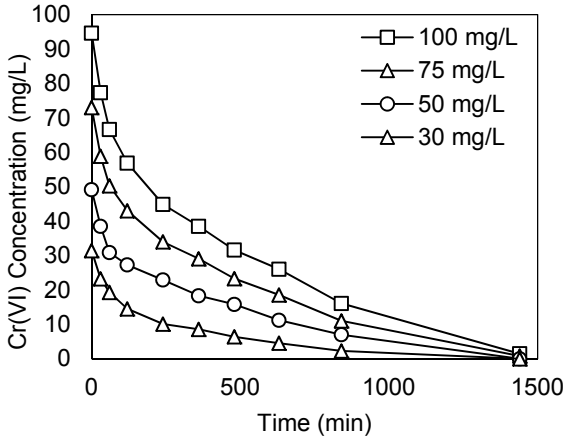

Figure 3: Cr(VI) concentration in bacteria and algae system without glucose.

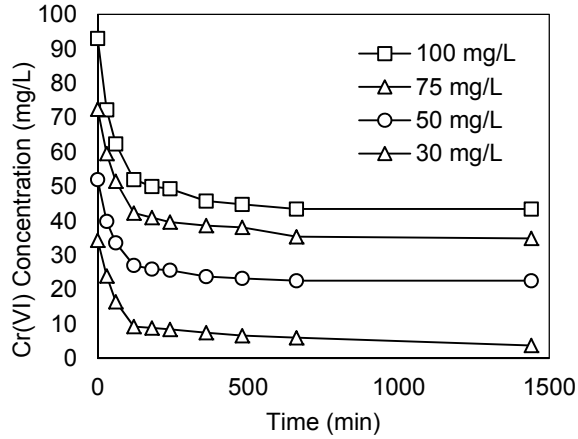

Figure 4: Cr(VI) concentration in bacteria and algae cell free spent media.

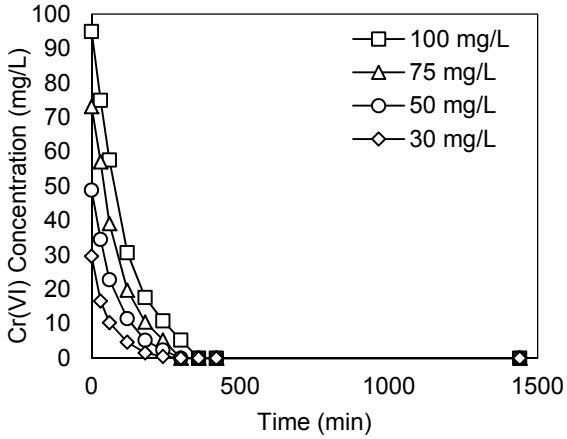

Figure 5: Cr(VI) concentration in a bacteria and glucose system

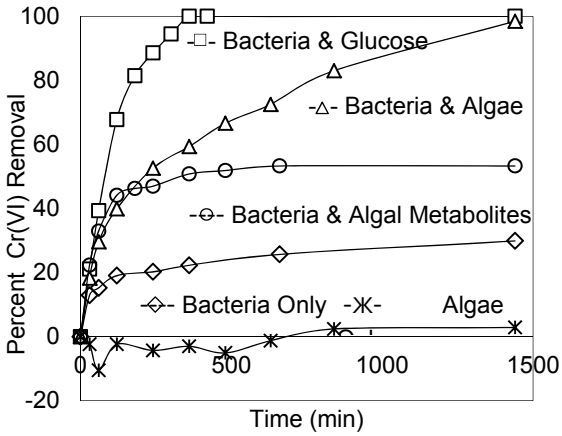

Figure 6: Percentage Cr(VI) removal for each system

3.2 Batch Kinetics

Chirwa and Wang, 2000 derived a model based on the Monod kinetic model which fitted the reduction of Cr(VI) by bacteria with glucose as the carbon source best. This mathematical model is shown in Eq(1).

$$-\frac{dC}{dt} = \frac{k_m C}{C + K_c}\left[X_0 - \frac{C_0 - C}{R_c}\right] \quad (1)$$

In Eq(1), km is the maximum specific Cr(VI) reduction rate (hr^{-1}), C_0 is the initial Cr(VI) concentration (mg/L), X_0 is the initial biomass concentration (mg/L), C is the Cr(VI) concentration (mg/L) at time, t, K_c is the half velocity concentration (mg/L) and R_c is the maximum Cr(VI) reducing capacity (mg/mg). The model fit results are shown in Figure 7 and the model parameters are shown in Table 1.

Table 1: Kinetic parameters for the bacteria glucose batch experiments.

Parameter	k_m (hr^{-1})	K_c (mg/L)	R_c (mg/mg)	χ^2
Value	0.084	627.28	0.6	16

The double exponential model (Deary et al., 2016) was found to most accurately model the Cr(VI) removal in the system where bacteria and algae were combined. The bulk effect of the complex physical and biological processes that takes place in this system can be approximated by a sequential two step reaction. The double exponential model is shown in Eq(2).

$$[Cr(VI)] = [Cr(VI)]_0 \Phi_a e^{-k_a t} + [Cr(VI)]_0 \Phi_b e^{-k_b t} \quad (2)$$

In Eq(2), k_a and k_b are the rates for the two stepwise processes and Φ_a and Φ_b are a corresponding fraction. Table 2 provide the kinetic parameters for Eq(2) and the fitted model are shown in Figure 8.

Table 1: Kinetic parameters for the algae bacteria batch experiments.

Parameter	Φ_a	Φ_b	k_a(min^{-1})	k_b (min^{-1})	R^2
30 mg/L	0.66	0.34	0.0022	0.043	0.986
50 mg/L	0.67	0.33	0.0022	0.043	0.994
75 mg/L	0.69	0.31	0.0017	0.034	0.995
100 mg/L	0.69	0.31	0.0017	0.034	0.992

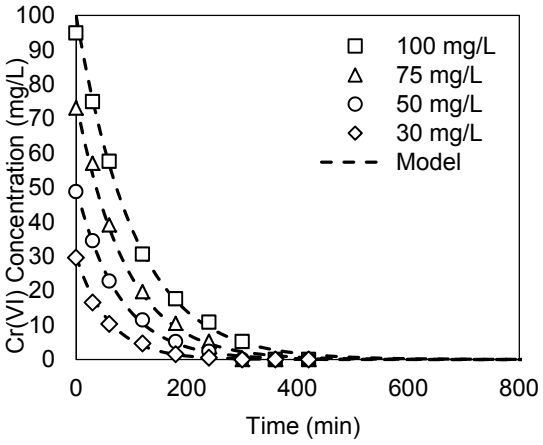

Figure 7: Monod model fitted to batch data from a bacteria glucose system.

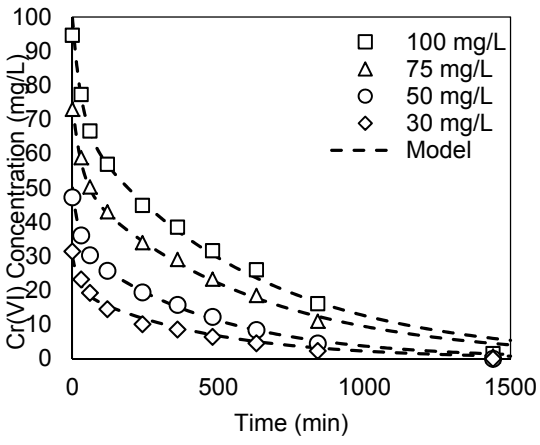

Figure 8: Monod model fitted to batch data from a bacteria glucose system.

4. Conclusions

Successful Cr(VI) removal was achieved in both bacteria-glucose, and algae-bacteria systems. This indicates the possibility that the bacteria were able to use a carbon source provided by the algae. Although the algae-bacteria system took three times longer than the bacteria-glucose system to completely remove Cr(VI), it presents the opportunity to eliminate the need to add glucose to the system. The overall removal rate of Cr(VI)

is approximated by Eq(2), although all of the processes involved in the algae-bacteria system have not yet been identified. The Practicality of using algae and bacteria combined in a continuous system must be evaluated, and if feasible pursued.

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