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In Situ Bioreduction of Hexavalent Chromium-Contaminated Water Using a Microbial Culture Barrier

Chromium (Cr) has been extensively used in many industrial applications. Inappropriate disposal of effluent has led to increased Cr concentrations in the environment. As a result, Cr(VI) has been classified under strict control measures by most national and international lists of highly toxic materials. Bioremediation of Cr(VI)-contaminated effluents appears to be a more economical and environment-friendly treatment method. This study investigates Cr(VI) removal in a bench-scale bioreactor using municipal dried sludge as a permeable bioreactive barrier. The 20-cm-thick permeable bioreactive barrier with 30 % sludge and 70 % sand was able to achieve 95 % Cr(VI) removal during 90 operational days, demonstrating the effectiveness of the biological permeable reactive barrier system in treating Cr(VI)-containing process effluent streams.

Keywords: Bioreduction, Chromium, Microbial cultures, Permeable bioreactive barriers

Received: September 04, 2022; *revised:* March 15, 2023; *accepted:* April 13, 2023

DOI: 10.1002/ceat.202200421

1 Introduction

Over the past few decades, there has been a significant increase in the application of heavy metals in various industrial activities. This led to soil, water source, and groundwater pollution, resulting in a serious threat to human health, the environment, and ecosystems [1]. Chromium (Cr) has been extensively used in many industrial applications (such as wood preservation, leather tanning, and textile dyeing) and is known to be one of the main toxic heavy metals [2]. Inappropriate disposal of effluent from these industrial activities has led to increased Cr(VI) concentrations in the environment, which triggered an alarming situation from the environmental safety perspective [3]. Cr exists in the form of chromium metal, hexavalent chromium (Cr(VI)), and trivalent chromium (Cr(III)). Cr(III) is less toxic than Cr(VI) and usually precipitates as a hydroxide at neutral pH, whereas Cr(VI) is considered highly toxic, with both mutagenic and carcinogenic properties, and it is soluble in water [4]. Hence, Cr(VI) is hazardous to both ecosystems and human health. As a result, Cr(VI) has been classified under strict control measures by most national and international lists of highly toxic materials.

Various treatment methods such as chemical precipitation, oxidation, reduction, reverse osmosis, and membrane technology are being applied for the removal of toxic Cr(VI). However, these procedures are expensive in terms of energy usage, treatment, and sludge disposal [5].

Bioremediation of Cr(VI)-contaminated environments using microorganisms appears to be a more economical and environment-friendly treatment method. The ability of bacteria to reduce Cr(VI) has been reported to depend on numerous environmental parameters that affect the transformation of Cr(VI), such as the pH and the temperature [6, 7]. However, many of these studies were done in batch systems and would offer challenges on the large scale. Continuous-flow systems such as a biological permeable reactive barrier (BPRB), on the other hand, have the potential of continuously treating large volumes of Cr(VI)-contaminated water. This study investigates the applicability of Cr(VI) reduction using a mixed culture of bacteria as a permeable reactive barrier in a bench-scale bioreactor system.

2 Materials and Methods

2.1 Sample Collection

Dried sludge samples were collected from the Brits Wastewater Treatment Works sand drying beds (North West Province, South Africa). The treatment works periodically receives high Cr(VI) levels from an abandoned sodium dichromate-processing facility. Therefore, the bacteria in the sludge were expected to be acclimatized to high Cr(VI) exposure conditions. The samples were stored in sterile containers at 4 °C for further use.

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2.2 Microbial Characterization

Phylogenetic cell characterization was performed on individual colonies of bacteria grown aerobically from a sludge sample. LB agar was used for colony development. Agar plates were inoculated with 1-mL samples, and the colonies were subcultured using differential techniques (exhibiting colors and morphologies) and incubated at 37 °C for 24 h. In preparation for the 16S rRNA sequence identification, the colonies were first classified based on their morphology. Genomic DNA was extracted from the pure cultures using a DNeasy tissue kit (QIAGEN Ltd., West Sussex, UK) as per the manufacturer's instructions. The 16S rRNA genes of the isolates were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using the primers pA and pH1; primer pA corresponds to position 8–27, primer pH1 to position 1541–1522 of the 16S gene. The internal primer pD, corresponding to position 519–536 of the 16S gene, was used for sequencing. The resulting sequences were matched to known bacteria in the GenBank using a basic BLAST search of the National Center for Biotechnology Information.

2.3 Bioreactor Setup

Two horizontal flow tanks with the dimensions of 820 mm × 170 mm × 200 mm ($L \times B \times H$) were constructed using 5-mm-thick transparent Perspex sheets (Evonik Rohm GmbH, Essen, Germany), as shown in Fig. 1. The reactor consists of five compartments: the influent and effluent reservoirs (100 mm × 170 mm × 200 mm), the sand (230 mm × 170 mm × 200 mm), and the biobarrier (150 mm × 170 mm × 200 mm). The sand compartments were filled with thoroughly washed pure river sand with a granular size ranging from 0.6 to 1.5 mm. To simulate the biobarrier conditions, the middle compartment was filled with a mixture of dried sludge and sand with a mass ratio of 30 %:70 %. The compartment dividers were perforated to ensure evenly distributed flow. The reactor was operated as plug-flow system with four sampling ports along the length.

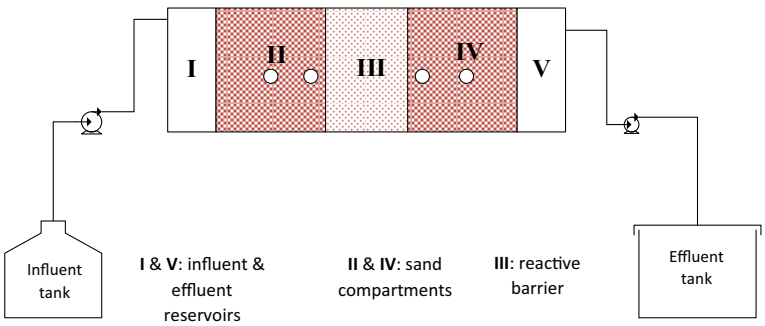

Figure 1. Bench-scale setup of a permeable reactive barrier system.

2.4 Reactor Startup

Two reactors were operated under a constant flow rate of 200 mL h^{-1}. Before starting the experiments, the two reactors were saturated with distilled water for 14 days. The second reactor was filled only with sand quartz, to serve as a control. The influent solution of 40 mg L^{-1} Cr(VI) (initial pH = 6.8) was pumped into the reactors by using a peristaltic pump and a liquid detention time of about 8 h in the biobarrier for 30 days; then it was increased to 65 mg L^{-1}. After 60 days of operation, 5 g L^{-1} glucose was added into the system while keeping the Cr(VI) concentration at 65 mg L^{-1} for another 30 days. Samples of the influent and effluent were collected periodically for Cr(VI) and pH analysis. The operation of the reactors was without any supplementary organic carbon sources and minerals, except for those already found in the sludge.

2.5 Determination of Cr(VI) and Total Chromium

Measurement of Cr(VI) was carried out by sampling 2 mL of sample across each reactor at regular time intervals with a disposable syringe. The sample was then centrifuged for 10 min at 6000 rpm (2820*g*), using a Minispin® Microcentrifuge (Eppendorf, Hamburg, Germany) to remove the suspended solids. The sample was then analyzed in a UV-vis spectrophotometer (WPA, Light Wave II, and Labotech, South Africa) at the wavelength of 540 nm, using the diphenylcarbazide (DPC) method as described by Kholisa and Chirwa [8]. The total chromium in each sample was measured using a Varian AA – 1275 Series flame atomic adsorption spectrophotometer (AAS) (Varian, Palo Alto, CA, USA) at a wavelength of 359.9 nm.

3 Results and Discussion

3.1 Microcosm Performance

Under constant hydraulic loading, the performance of each microcosm reactor (control and BPRB) was assessed using the influent and the effluent Cr(VI) concentrations. The overall performance of both reactors is summarized in Tab. 1. The BPRB reactor inoculated with a mixed culture of bacterial from sludge performed best with near-complete Cr(VI) removal up to 65 mg L^{-1} under a variety of conditions. This indicates the ability of native species from polluted environments to tolerate and reduce Cr(VI) in the system at various feed Cr(VI) concentrations in the system with or without an external carbon source supply. The control reactor (cell free), on the other hand, exhibited insignificant Cr(VI) removal during the first 30 days of operation tested, and afterwards the operation was terminated. The insignificant Cr(VI) removal observed in the control reactor is associated with the absence of Cr(VI)-reducing bacteria in the reactor.

Table 1. Overall performance of the BPRB and control reactors under various conditions.

Reactor	Inlet concentration [mg L^{-1}]	Conditions	Effluent concentration [mg L^{-1}]	Removal efficiency [%]	Days of operation [d]
Control	46.03 ± 1.41	Control	43.21	6.11	30
BPRB	46.03 ± 1.41	No carbon source	0	100	30
	64.64 ± 1.63	No carbon source	4.21	93.49	30
	64.11 ± 1.14	Glucose	2.62	95.91	30

3.2 Cr(VI) Concentration Profile

Cr(VI) removal across the BPRB reactor was evaluated over the 90 days of operation using data collected from sampling ports placed across the reactor. Figs. 2a–c show no Cr(VI) removal in the sampling points before the barrier (port 1 and port 2) while high Cr(VI) removal is observed in the sampling ports after the barrier (port 3 and port 4) at the initial Cr(VI) feed concentrations of 45 and 65 mg L^{-1}, respectively. The insignificant Cr(VI) removal observed in sampling port 1 and port 2 was due to the fact that the compartment before the barrier was only filled with sand; hence, no Cr(VI) reduction occurred. It can be seen in Fig. 2a that, in port 3, no Cr(VI) was detected in the first 6 days. After day 6, an increase in Cr(VI) concentration was observed, which continued to increase up to 15 mg L^{-1} on day 10. Thereafter, the Cr(VI) concentration decreased and reached complete reduction on day 12. This was associated with microorganisms still acclimatizing to long Cr(VI)-stressed conditions. Complete Cr(VI) reduction was achieved in the barrier compartment as it can be seen that the Cr(VI) concentration in port 2 is approximately 45 mg L^{-1} while at ports 3 and 4 it is nearly 0 mg L^{-1}. After 30 days of operation, the feed Cr(VI) concentration was increased to 65 mg L^{-1}, as can be seen in Fig. 2b. The system reached steady state again on day 36, as the Cr(VI) concentrations at port 1 and port 2 were equal to the feed concentration. No Cr(VI) was detected in port 3 and port 4 until day 55 of operation. The Cr(VI) concentrations in these ports continued to increase, reaching 26 mg L^{-1} in port 3 and 16 mg L^{-1} in port 4. This increase in Cr(VI) concentration in port 3 and port 4 was attributed to depletion of the carbon source from the sludge. Microorganisms utilize a variety of organic carbon sources, as either an energy source or as an electron donor to facilitate Cr(VI) bioreduction [9]. Due to depletion of the carbon source and low Cr(VI) reduction, 5 g L^{-1} glucose was added to the reactor feed to provide the microorganisms with carbon and energy. After adding the glucose on day 60, the microorganisms completely reduce the Cr(VI) as observed on day 61, as shown in Fig. 2c. Cr(VI) was not detected until day 83. The Cr(VI) concentration continued to increase in both ports, reaching 29 mg L^{-1} in port 3 and 23 mg L^{-1} in port 4 on day 90. The deterioration of the Cr(VI) reduction was due to the decreasing pH (5.2) in the system. The decrease in pH values was ascribed to the oxidation of glucose forming several types of organic acids by different bacterial species, which resulted in a subsequent drop in the medium pH [10–13]. These findings demonstrate the significance of metal-cell interactions within the bioreactive permeable barrier matrix in reducing Cr(VI).

3.3 Bacterial Culture Composition

The changes in the microbial culture composition after 13 weeks of exposure to Cr(VI) were monitored by the 16S rRNA fingerprinting method. The results are presented in Tab. 2, and the predominant species under nutrient and oxygen stress conditions were the *Pseudomonas* groups: *P. fluorescens, P. shahriarae, P. hibiscicola, P. gessardi, P. geniculata*, and *Comamonas testosteroni* and *Stenotrophomonas maltophilia*, at an identity index of 100 %.

Table 2. Microbial characterization in the barrier after 90 days of Cr(VI) exposure.

Isolates	Blast results	Identity index [%]
Y1	*Pseudomonas fluorescens*	100
Y2	*Pseudomonas shahriarae*	100
Y3	*Comamonas testosteroni*	100
Y4	*Pseudomonas hibiscicola*	100
Y5	*Stenotrophomonas maltophilia*	100
Y6	*Pseudomonas gessardii*	100
Y7	*Pseudomonas geniculata*	100

4 Conclusion

The effectiveness of bioremediation of Cr(VI)-contaminated water using BPRB technology was evaluated through bench-scale studies. Successful Cr(VI) reduction was achieved over the 90-day operational period of the BPRB system. Therefore, it can be concluded that the indigenous bacteria obtained from a wastewater treatment plant were able to effectively treat Cr(VI) with or without any biostimulation. The results suggest that indigenous bacterial strains have potential application for Cr(VI) remediation in contaminated environments. These results could also be effective in optimizing and improving the operation and performance of in situ bioremediation of Cr(VI) at the target site. Further studies are required to understand the interaction of bacteria with other heavy metals that coexist with Cr(VI) in the environment and also to evaluate the effect of operating the BPRB under various hydraulic retention times (HRT) while occasionally backwashing or dislodging the accumulated precipitate from the system.

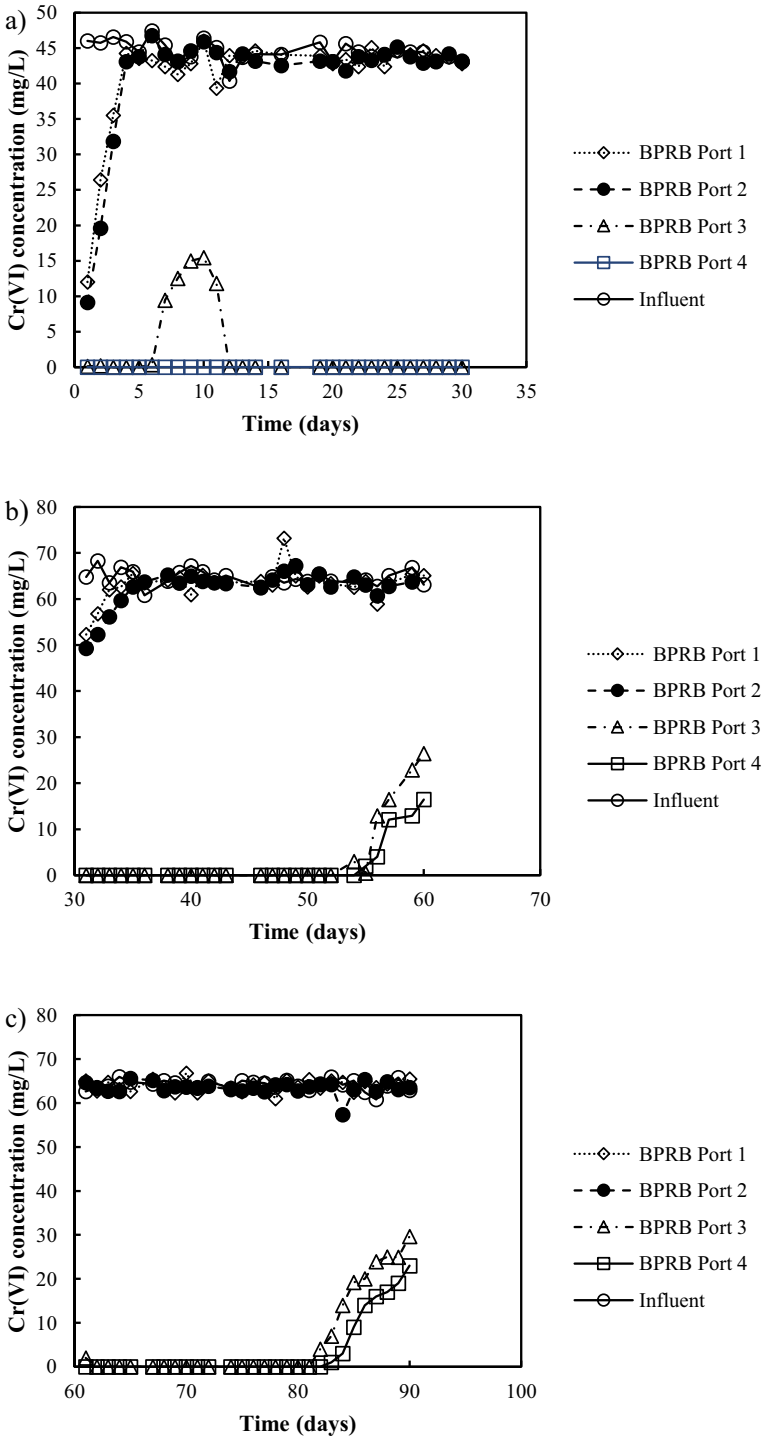

Figure 2. Cr(VI) concentration across the reactor at (a) 45 mg L^{-1}, (b) 65 mg L^{-1}, and (c) 65 mg L^{-1} and with or without an external carbon source.

Data Availability Statement

Data are available on request due to privacy or other restrictions.

Acknowledgment

The research was funded through the National Research Foundation (NRF) Incentive Funding for Rated Researchers, Grant No. IFR2010042900080 awarded to Prof. Evans M. N. Chirwa of the University of Pretoria. Buyisile Kholisa would like to acknowledge the National Research Foundation (NRF)

for granting him the Doctoral Scholarship, Grant No. MND190613447392.

The authors have declared no conflict of interest.

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