In situ bioremediation of hexavalent chromium by permeable reactive barrier using wastewater sludge bacteria

by

BUYISILE KHOLISA

Thesis submitted in fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY IN CHEMICAL ENGINEERING

In The

FACULTY OF ENGINEERING, BUILT ENVIRONMENT AND INFORMATION TECHNOLOGY

UNIVERSITY OF PRETORIA

21 November 2022

ABSTRACT

In situ bioremediation of hexavalent chromium by permeable reactive barrier using wastewater sludge bacteria

By

Buyisile Kholisa

Supervisor: Professor EMN Chirwa

Department: Chemical Engineering

Degree: Philosophiae Doctor (Chemical Engineering)

Environmental pollution is a global problem that affects both developed and developing countries by contaminating soil and water, threatening biodiversity, ecosystems, and human health. South Africa holds the largest chrome ore reserves in the world, and it is one of the largest producers of ferrochrome. During steel and chromate production, enormous quantities of ferrochrome wastes are generated and discarded in dumps. This waste has been shown to contain significantly higher levels of Cr(VI) than the maximum acceptable risk concentration that is allowed for waste disposal in South Africa, which becomes a serious concern for soil and groundwater pollution.

There are various conventional technologies available for minimizing the environmental impact of Cr(VI), including chemical reduction, ion exchange, electrochemical treatment, membrane separation, etc. However, most of these technologies are often ineffective and very expensive, especially for low concentrations of metals. Additionally, the use of chemical reagents produces an enormous amount of hazardous sludge that requires further

treatment. The bioreduction of toxic Cr(VI) to less toxic Cr(III) using microbial organisms is considered a valuable, promising, and cost-effective approach for Cr(VI) remediation.

In this study, using batch and continuous flow bioreactor systems, the efficiency of the indigenous culture of bacteria from the local wastewater treatment plant located near the contaminated site was evaluated for Cr(VI) reduction potential.

The Cr(VI) reduction capability and efficiency of the isolated bacteria were investigated under a range of operational conditions, i.e., pH, temperature and Cr(VI) loading in a batch system. The culture showed great efficiency in reduction capability, with 100% removal in less than 4 h at a nominal loading concentration of 50 mg Cr(VI)/L. The culture showed resilience by achieving total removal at concentrations as high as 400 mg Cr(VI)/L. The consortia exhibited considerable Cr(VI) removal efficiency in the pH range from 2 to 11, with 100% removal being achieved at a pH value of 7 at a 37 ± 1 °C incubation temperature. The ability of the mixed bacterial consortium to treat Cr(VI) may be explored further in a continuous flow process for practical application.

The effectiveness of bioremediation of Cr(VI) contaminated water using biological permeable reactive barrier technology was evaluated through bench-scale studies. Successful Cr(VI) reduction was achieved with 95.9% removal over the 90 days operational period of the BPRB system. When glucose was used as the carbon source, a drastic decline in effluent pH from 6.91 to below 5.5 was observed in the effluent. The decrease in pH values was ascribed to the oxidation of glucose forming several types of organic acids by different Bacillus species and other bacterial species which result in a subsequent drop in medium pH. However, it did not influence the overall reactor performance. These results could also be effective in optimizing and improving the operation and performance of in situ bioremediation of Cr(VI) at target sites. Cr(VI) reduction kinetic parameters in both batch

and continuous-flow systems were estimated using a modified non-competitive inhibition model with a computer program for simulation of the aquatic system (AQUASIM 2.0).

Further studies are required to understand the interaction of bacteria with other heavy metals that co-exist with Cr(VI) in the environment and also to evaluate the effect of operating the BPRB under various HRTs while occasionally backwashing or dislodging the accumulated precipitate from the system. Finally, experiments should be conducted with real contaminated groundwater to study the effect of different chemical compositions and conditions of contaminated water on the Cr(VI) removal efficiency by bacteria and the hydraulic behaviour of the used mixtures.

Key Words: Cr(VI) reduction; bioremediation; Chromium reducing bacteria; heavy metal, hexavalent chromium, permeable bioreactive barrier, microbial culture

DECLARATION

I, Buyisile Kholisa, hereby declare that this research dissertation is my own work. It is being submitted for the PhD Degree at the University of Pretoria. It has not been submitted before for any degree or examination at any other University. Furthermore, it represents my own opinions and not necessarily those of the University of Pretoria.

_____ _____

Signed **Date**

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my supervisor, Prof Evans Chirwa, for his excellent guidance and technical expertise, which set high standards for my PhD work. Every research student in our team could attest to his exceptional mentorship, exciting individuality, resilient work ethic and fun-loving charisma even in pressing situations. I will forever be grateful for all the efforts he took to assist in this study. Finally, I would also like to thank him for funding my PhD research work and for several conferences and training exposures granted to me during this study.

I would like to express my heartfelt gratitude to colleagues in the research group at the Water Utilization and Environmental Division of the Department of Chemical Engineering, University of Pretoria, for their assistance, friendship and interest in my progress, and to Mrs Alette Devega and Mrs Elmarie Otto for their prompt assistance. I have learned so much from all of you.

I would also like to express my deepest appreciation to Ms Zimkitha Jini for supporting and loving me throughout this journey.

My sincere gratitude also goes to Dr Malibongwe Manono and Dr Lukhanyo Mekuto for their invaluable advice which contributed effectively to this study.

The financial assistance of the National Research Foundation (NRF) towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the NRF.

DEDICATION

I wish to dedicate this thesis to my late mother ***Ntombizandile Kholisa****, who could not witness and be with me at end of this road and to my lovely daughter* ***Analo Nosandise****.*

To my family and close friends for their encouragement and support.

To all those having difficult times with their studies

"One day you will say you did it!!!"

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AAS	Atomic adsorption spectrophotometer
AC	Activated Carbon
APHA	American public health agency
BDF	Backward differentiation formula
BIC	Bushveld igneous complex
BLAST	Basic logical alignment search tool
BPRB	Bacterial Permeable Reactive Barriers
CFU	Colony forming unit
CPRB	Continuous permeable reactive barrier
Cr	Chromium
Cr(III)	Trivalent chromium
Cr(VI)	Hexavalent chromium
DAE	Differential algebraic equation
DPC	Diphenyl carbohydrazide
DTPA	Diethylenetriamine pentaacetate
EDDS	Ethylenediaminedisuccinic acid
EDTA	Ethylenediaminetetraacetic acid
FGS	funnel-and-gate system
HRT	Hydraulic retention time
LB	Luria-Bertani
MSM	Mineral salt medium

MTBE	methyl-tert-butyl-ether
NF	Nanofiltration
NTA	Nitrilotriacetic acid
ODE	Ordinary differential equation
ORC	Oxygen-releasing compounds
PC	Plate count
PRB	Permeable reactive barriers
RO	Reverse Osmosis
TCE	tetrachloroethylene
UF	Ultrafiltration
WHO	World Health Organisation
ZVI	Zero-Valent Iron

RESEARCH OUTPUTS

Journal Publications

Kholisa, B., Matsena, M.T & Chirwa, E.M.N. 2022. In situ bioreduction of hexavalent chromium contaminated water using a microbial culture barrier. *Chemical Engineering and Technology*. Under review

Kholisa, B., Matsena, M.T & Chirwa, E.M.N. 2022. Biotreatment of hexavalent chromium in a rectangular column using Municipal Wastewater Sludge as a bioreactive barrier. *Chemical Engineering Transactions*, 94: 1405-1410.

Chirwa E., Molokwane P., Chihambakwe P., Kholisa B., Mtimunye P., Matsena M.T. 2022. Microbial Cr(VI) Reduction by Indigenous Activated Sludge Bacilli and Pure Culture of Escherichia Coli ATCC 3345. *Chemical Engineering Transactions*, 94: 1417-1422.

Kholisa, B., Matsena, M. & Chirwa, E.M.N. 2021. Evaluation of Cr(VI) reduction using indigenous bacterial consortium isolated from a municipal wastewater sludge: Batch and kinetic studies. *Catalysts*, 11(9).

Kholisa, B. & Chirwa, E.M.N. 2021. Bioreduction of Cr(VI) Using Bacterial Consortia Isolated from a Municipal Wastewater Sludge Experiencing Cr(VI) Loading from an Abandoned Chrome Foundry. *Chemical Engineering Transactions*, 88: 115–120.

Conference Proceedings

Kholisa, B., Matsena, M.T & Chirwa, E.M.N. 2022. 'In situ bioreduction of hexavalent chromium contaminated water using a microbial culture barrier', 26th International Congress of Chemical and Process Engineering CHISA 2022. Conference proceeding 2022, Prague, Czech Republic, 21 August – 25 August.

Kholisa, B. & Chirwa, E.M.N. 2021. 'Bioreduction of Cr(VI) Using Bacterial Consortia Isolated from a Municipal Wastewater Sludge Experiencing Cr(VI) Loading from an Abandoned Chrome Foundry', PRES21: 24th Conference on Process Integration for Energy Saving and Pollution Reduction, Press conference proceeding 2021, Brno, Czech Republic, 31 October – 3 November.

Chapter 1 INTRODUCTION

1.1 BACKGROUND

Chromium (Cr), is one of the main heavy metals that causes pollution in groundwater; soils; surface waters and aquatic sediments, and is extensively used in industrial activities like metal electroplating, textile dyeing, and, tanneries, etc. (Lyu et al., 2017). It is a steel-grey, lustrous, hard and brittle metal, which exists by nature in the form of ores. Cr has various oxidation states, however, it is mainly present in the trivalent [Cr(III)] and hexavalent [Cr(VI)] being the most stable (Troiano et al., 2013). Cr(VI) state is known to be extremely toxic to biological and ecological systems. As a result, Cr(VI) concentration in drinking water may not exceed 0.05 mg/L as per the Environmental Protection Agency (USA) standard (Baral & Engelken, 2002; Tekerlekopoulou et al., 2013; Fernandez et al., 2018), while Cr(III) remains as an important nutrient for plants and wildlife metabolism (Poljsak et al., 2010; Frois et al., 2011). Exceeding the tolerable limit for Cr(VI) concentration causes cancer in humans and aquatic wildlife, and at significantly higher concentrations it is extremely toxic (Jaishankar et al., 2014). Cr(VI) is released into the environment naturally or by the discharge of effluent from different processing industries such as chromite ore processing, leather tanning, electroplating, steel production, wood preservation, wood pulp processing and textile and so on (Tekerlekopoulou et al., 2013).

In the South African context, the Cr(VI) contaminated locations, the majority of them, the problem is intensified by the presence of abandoned and closed mining or processing operations. Due to the threat posed by Cr(VI) on humans and aquatic wildlife, the removal of this metal must be applied effectively and without causing an impact on the environment.

The most widely used methods for environmental remediation of Cr(VI) in practice are conventional physicochemical processes such as ion exchange, adsorption on activated carbon, reverse osmosis, electrochemical process, and precipitation among others (Witek-Krowiak, 2013). Although these technologies can decrease the adverse metal impact, their major drawbacks include the production of toxic waste sludge, high-energy demands or inefficient removal (Seh-Bardan et al., 2012). Therefore, the search for innovative, cheaper, environmentally friendly and more effective techniques has become important for the removal of toxic Cr(VI) ions from polluted areas.

Reduction of Cr(VI) using microbial organisms has been proposed as an alternative technique of remediation. The microbial strategy could offer in situ and on-site bioremediation strategies and use in permeable reactive barriers. A number of microorganisms such as fungi, yeast, bacteria, and algae are found in waters and soil environments receiving industrial effluents (Chai et al., 2009; Yadav et al., 2017). These microorganisms have developed adaptation strategies and capabilities to defend themselves against Cr(VI) toxication by transforming the more toxic Cr(VI) into less toxic Cr(III) (Megharaj et al., 2003). Therefore, biological reduction serves as a sustainable alternative technology for Cr(VI) bioremediation (Ackerley et al., 2004; Kabir et al., 2018). The most important benefits of biological Cr(VI) decontamination are the lower costs, significantly smaller footprint on the environment (Kabir et al., 2018) and, it could be implemented in situ within the interior of the dumpsite or the polluted environment (Bansal et al., 2017).

1.2 AIM OF THE STUDY

The current study was aimed at exploring and evaluating the prospect of Cr(VI) contamination control in groundwater aquifers at contaminated sites using natural microbial processes. The proposed study offers an environmentally friendly, cost-effective and self-

sustained biological method to curb the spread of chromium at the contaminated sites. To accomplish the main aim of this study, different experimental tasks were conducted on Cr(VI) reduction process:

Objectives

- To evaluate the performance of consortium bacteria culture and kinetics of Cr(VI) reduction in batch reactors over a wide range of initial Cr(VI) concentrations.
- To demonstrate the feasibility of a biological permeable reactive barrier application over a range of Cr(VI) feed concentrations.
- To investigate the microbial culture diversity changes during Cr(VI) bioremediation in a biological permeable reactive barrier system.
- To develop a predictive model for Cr(VI) movement across the biological permeable reactive barrier system using numerical modelling tools.

1.3 THESIS OUTLINE

The outline of this dissertation is listed as follows:

Chapter 1 describes the background information and the objective of this thesis.

Chapter 2 reviews current and previous studies on chromium.

Chapter 3 describes the materials and methods used in this study.

Chapter 4 presents experimental results and interpretation in a batch reactor.

Chapter 5 describes Cr(VI) reduction in a biological permeable reactive barrier reactor.

Chapter 6 describes the kinetic Cr(VI) reduction modelling of the batch system.

Chapter 7 describes kinetic Cr(VI) reduction modelling of a continuous-flow bioreactor system.

Chapter 8 presents the conclusion of the thesis and the future work required.

1.4 SIGNIFICANCE OF THIS RESEARCH

South Africa holds the largest chrome ore reserves in the world, and it is one of the largest producers of ferrochrome. During steel and chromate production, enormous quantities of ferrochrome wastes are generated and discarded in dumps. This waste has been shown to contain significantly higher levels of Cr(VI) than the maximum acceptable risk concentration that is allowed for waste disposal in South Africa, which becomes a serious concern for soil and groundwater pollution (Coetzee et al., 2018). In situ biological permeable reactive barriers (BPRBs) systems application for the removal of Cr(VI) in groundwater has been extensively studied at the laboratory scale with various degrees of success or feasibility. However, the demonstration of the feasibility of such a system at the pilot-scale level is still limited.

Thorough laboratory assays are of paramount importance to ensure a smooth transition of findings at lab-scale to in situ demonstrations of this technology.

In this view, to transition from lab-scale to pilot-scale, bench-scale studies are required to bridge this gap. This process could contribute to the protection of natural water resources from toxic Cr(VI) waste arising from ferrochrome processing facilities.

Chapter 2 **LITERATURE REVIEW**

2.1 INTRODUCTION

Cr is a transition metal that is the sixth most abundant among the transition metals and the twenty-first most abundant metal in the earth's crust (Haynes, 2016). This metal has an atomic number of 24 and an atomic weight of 51.996 g/mol and appears as a grey-white, hard, and brittle metal with a crystal-like structure. It was first discovered by the French chemist Nicholas-Louis Vauquelin in the Siberian red lead ore (crocoite) during the late 1700s. Vauquelin named the element chroma from the Greek word χρωμα, which means colour due to the intense colours found in its compounds (Mohan & Pittman, 2006). Cr is characterised by high relative densities of 7.15 at room temperature and 6.3 at melting point. In addition, Cr has a high melting point of 1907 °C and a boiling point of 2671 °C (Haynes, 2016). Furthermore, Cr does not exist in its pure elemental form geologically but is rather found in complex mineral forms.

Cr can exist in different valence states that vary between Cr^{2-} and Cr^{6+} (Ashley et al., 2003; Coetzee et al., 2018; Mishra & Bharagava, 2016). Even though Cr occurs predominantly as Cr^{2+}; Cr^{3+}; and Cr^{6+} oxidation states, nevertheless the Cr^{2+} is not stable and oxidizes to Cr(III) (Mohan & Pittman, 2006; Coetzee et al., 2018). Cr(III) and Cr(VI) species are the most stable oxidation states. Cr in nature occurs largely in the Cr(III) form mostly as crocoite ($PbCrO_4$) and chromite ($FeOCr_2O_3$) in serpentine and granitic rocks, and coal (Kotaś & Stasicka, 2000).

There are more than fifty different chromium naturally occurring ores that have been identified thus far, including the following abundant types:

- Barbertonite: $Mg_6Cr_2(CO_3)(OH)_{16}.4H_2O$
- Brezinaite: Cr_3S_4
- Chromite: $(Mg,Fe^{2+})(Cr,Al,Fe^{3+})_2O_4$
- Chromatite: $CaCrO_4$
- Nichromite: $(Ni,CoFe^{2+})(Cr,Fe^{3+},Al,)_2O_4$

At present, almost all the production of Cr for commercial purposes is mined and extracted from chromite ore and with the largest deposits found in South Africa (Schulte, 2018). South Africa possesses approximately 72% of the world's useable chromite ore reserves (Cramer et al., 2004; Murthy et al., 2011; Beukes et al., 2012) and these reserves are predominantly found in the Bushveld igneous complex (BIC). Other countries with viable reserves of chromium ore include but are not only limited to Russia, Finland, Zimbabwe, the Philippines, India, Kazakhstan, Brazil and Turkey as shown in Figure 2-1.

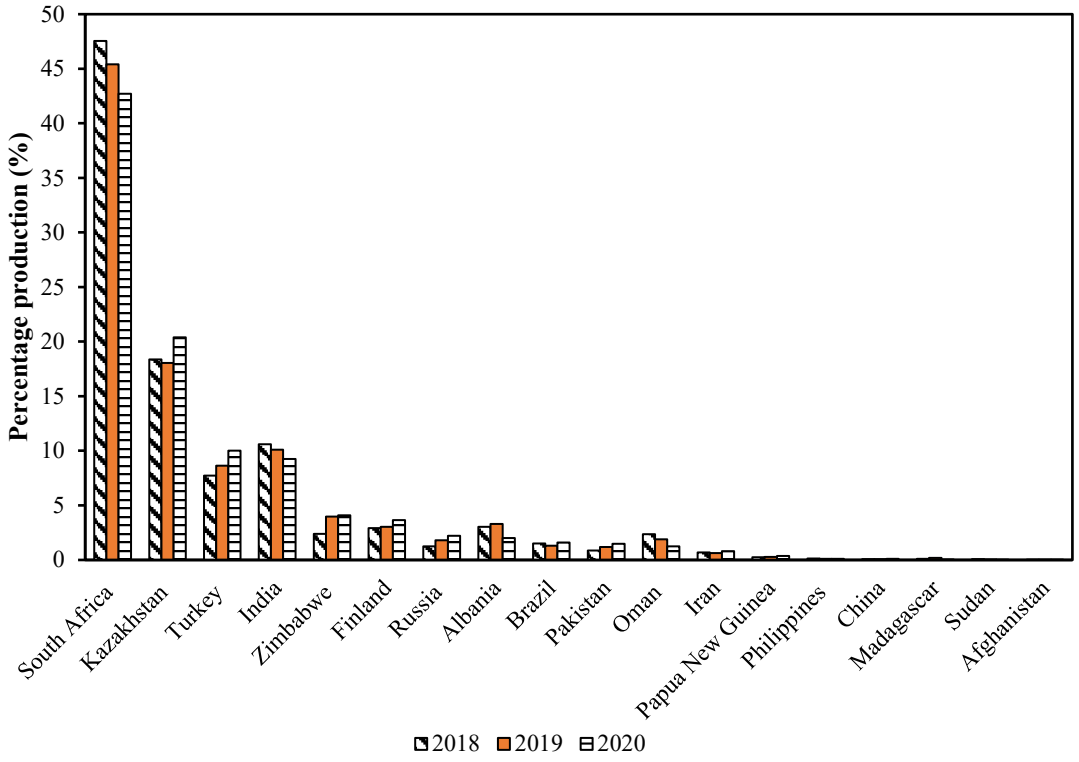


Figure 2-1: Worldwide production percentage of chromite ore in the year 2018, 2019 and 2020 (Idoine et al., 2022)**.**

2.2 USES OF CHROMIUM

Cr has been extensively used in metallurgical, chemical, and refractory industries. According to Luo and Li (2012) and Sardohan et al. (2010), the more common uses of chromium in the world at present are as follows:

- Chromium is fairly hard and is corrosion resilient. For that reason, chromium is plated to give steel a polished silvery mirror coating.
- Chromium magnetic compounds are used in the production of magnetic tape for audial cassettes and high-quality aural tapes.
- Due to the high heat resistance of chromium, alloys of iron, nickel and chromium handle high temperatures very well and are used in gas turbines and jet engines.

- Coloured pigments and dyes are made from various chromium compounds.
- Chromium (VI) salts are used as a wood preservative.
- Additive in stainless steel alloys, which are used in various applications.

Bielicka et al. (2005) pointed out that about 90% of the extracted chromium ore is used in metallurgical processes for the production of steel, and stainless-steel nonferrous alloys. While the remaining 10% is shared equally by chemical (leather tanning, plating, wood preservation and pigment) and refractory (iron & steel, cement, glass, ceramics and machinery) industries as shown in Figure 2-2.

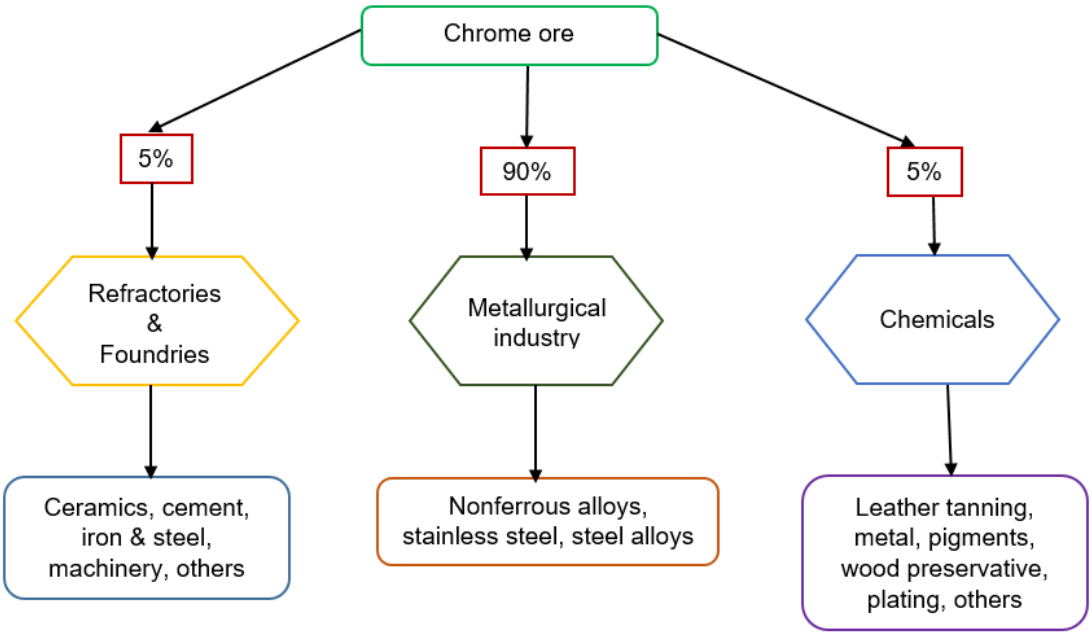


Figure 2-2: Distribution of chromium usage in different industries

The extensive usage of chromium by industrial activities has led to the production of large amounts of chromium wastes, which are often very difficult to deal with (Dhal et al., 2013; Dey & Paul, 2013). These chromium-containing wastes are discharged into the environment without any proper treatment and therefore are serving as a significant risk to the

environment and public and soil health (Çeribasi & Yetis, 2001; Coetzee et al., 2018; Huang et al., 2021). Furthermore, leakage of these wastes as a result of inadequate management and impaired storage containers also adds to the increase of chromium in the environment. As a consequence, nearly all contamination of chromium in the natural schemes comes from human activities (Krishna & Philip, 2005; Wani et al., 2019; Kholisa et al., 2021).

2.3 CHROMIUM ECOTOXICOLOGICAL EFFECTS

Anthropogenic activities are responsible for discharging large quantities of chromium compounds into the environment predominantly as Cr(VI) (V Mishra et al., 2010; Saha et al., 2011; Barrera-Díaz et al., 2012; Pradhan et al., 2017). The concentration of Cr(III) and Cr(VI) in industrial waste ranges from 10 to 100 mg/L (Dhal et al., 2010). Since discharging industrial waste into the environment has significantly increased the chromium concentration in soil, this is typically associated with groundwater contamination (Bhuvaneshwari et al., 2011).

Hexavalent and trivalent chromium compounds have contrasting effects on health and environmental risks. Cr(III) in trace amounts is a necessary nutrient for mammals and lack of, may result in a vicious impact on the metabolism of fatty acids, cholesterol and glucose (Viamajala et al., 2003; Rossouw, 2009). However, consumption of large amounts of Cr(III) may also lead to health problems for example lung cancer (Alloway, 2013; Zhitkovich et al., 1996).

On the other hand, Cr(VI) compound is toxic, mutagenic and carcinogenic to humans and animals and is correlated to the alteration of plant morphology and decreased plant growth (Mount & Hockett, 2000; Ackerley et al., 2004; Turpeinen et al., 2004; Mishra & Bharagava, 2016). The toxicity of chromium (VI) in the form of chromate on living organisms is

predominantly due to its ability to easily diffuse through cells barrier via sulphate transport pathways as it shares structural similarity with SO_4^{2-} (Pattanapipitpaisal et al., 2002; Hosseini & Sarab, 2007). Once the chromate is in the cell, it modifies the DNA transcription process which can lead to the digestive tract and lung cancer within the living cell (Bhuvaneshwari et al., 2011).

Effects of short-term exposure to chromium (VI) in humans and other mammals include eye and respiratory irritation, sensitisation or sneezing but exposure to high levels can cause liver and kidney damage, anaemia, and ulcers in the nasal septum (Bhaumik et al., 2014; Khedr et al., 2014). Chronic exposure results in decreased reproduction health and birth defects such as abortions and premature births in mammals (Losi et al., 1994). These complications are accompanied by the death of organisms (Zayed & Terry, 2003). Consequently, the World Health Organisation (WHO) has set the limit for maximum tolerable chromium (VI) concentration in drinking water to 0.05 mg/L and 0.1 mg/L on inland surfaces (Baral & Engelken, 2002; Ashraf et al., 2017). Thus, most studies have focused on the removal of Cr(VI) from the environment.

2.4 CHROMIUM ENVIRONMENTAL CHEMISTRY

Chromium can be found in all corners of the environment such as soil, water and air. Chromium contamination that occurs in natural systems is generally due to human activities. The main causes are leakage, inappropriate storage, or inadequate discarding procedures of chromium wastes (Rock et al., 2001; Lyu et al., 2017). Due to the fact that most Cr(VI) compounds are soluble in water, soil contamination can ultimately result in water contamination (Ashley et al., 2003). This could occur as a result of Cr(VI) compounds leaching from soils.

2.4.1 Chromium in soil

As pointed out above that the existence of Cr(VI) compounds contained in soil originates from human activities or as a result of oxidation of Cr(III) pollution (Ashraf et al., 2017; Xia et al., 2019). Cr(VI) is a strong oxidising agent and commonly exists only in oxygenated forms such as chromate (CrO_4^{2-}) or dichromate ($Cr_7O_4^{2-}$) oxyanions which are soluble and pH dependent (Lai & Lo, 2008; Mishra & Bharagava, 2016).

In neutral to alkaline soils above the pH value of 6.4, Cr(VI) exists primarily as chromate ions (CrO_4^{2-}), as either water-soluble (Na_2CrO_4) or moderately soluble ($CaCrO_4$, $BaCrO_4$, and $PbCrO_4$) (Kotaś & Stasicka, 2000). However, under acidic environments at pH values lower than 6.4, it occurs predominantly as hydrogen-chromate ions ($HCrO_4^-$) (Barrera-Díaz et al., 2012; Dhal et al., 2013; Jobby et al., 2018). The CrO_4^{2-} and $HCrO_4^-$ species are the most mobile Cr forms in soil, and can be easily transported by plants into the deeper layers of the soil, triggering contamination of surface and groundwater (Kotaś & Stasicka, 2000).

There are two main Cr(III) constituents that occur within the soil, that is, oxidizable soluble species or Cr(III) forms that are attached to the soil particles and then become immobile. Cr(III) in the environment occurs predominately in cationic forms unlike Cr(VI) which exist as an oxyanion. The dominant form of Cr(III) at pH less than 4 is $Cr(H_2O)_6^{3+}$ while its hydrolysis products ($CrOH^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_3$, $Cr(OH)_4^-$ dominate at a pH range of 4 to 8 (Landrot et al., 2012). It reacts readily with oxygen, hydroxide, sulphate, and organic matter to form insoluble chelates, or it is absorbed by soil colloids to form precipitates.

Both the reduction and oxidation of Cr(VI) and Cr(III) can be thermodynamically favourable in soil (Makino et al., 1998). However, the reduction and oxidation reactions of chromium

in soils are highly dependent on the structure of the soil and the soil redox conditions (Kožuh et al., 2000).

2.4.2 Chromium in water

In an aquatic environment, the redox process plays a key role in the movement, transport, and fate of organic and inorganic chemical constituents. Cr(VI) species that are mostly found in the water are $Cr_2O_7^{2-}$, CrO_4^{2-}, H_2CrO_4, and $HCrO_4^-$. Similarly, Cr(III) species found in solution most often are Cr^{3+}, $Cr(OH)^{2+}$, CrO^+, $HCrO_2$ and CrO_2^- (Barrera-Díaz et al., 2012; Pradhan et al., 2017).

Generally, at low pH and E_h values, the occurrence of Cr(III) species is favoured, while high pH and E_h values favour the Cr(VI) forms (Dhal et al., 2013), as shown by the E_h-pH diagram in Figure 2-3. As can be seen in Figure 2-4 the Cr(IV) exist as an anion species under natural conditions; in acidic environments at pH values lower than 6.4, it occurs predominantly as hydrogen-chromate ions ($HCrO_4^-$) whereas, in alkaline environments above the pH value of 6.4, it exists primarily as chromate ion (CrO_4^{2-}) (Barrera-Díaz et al., 2012; Dhal et al., 2013; Jobby et al., 2018). These Cr(VI) oxyanion species are generally mobile in most neutral to alkaline systems. The equilibria of the Cr(VI) oxygenated species favour extremely high solubility and the proportion of each ion in solution depends on pH and total chromium concentration (Mohan & Pittman, 2006) as shown in Figure 2-4. As can be seen from Figure 2-4, Cr(VI) exists as chromic acid (H_2CrO_4) which is a strong oxidising agent under strongly acidic conditions at pH levels less than 1. $HCrO_4^-$ ions are predominant at the pH range of 1 to 6 and Cr(VI) concentrations below 1 g/L. However, above the 1 g/L Cr(VI) concentrations, the $HCrO_4^-$ ions polymerize to form $Cr_2O_7^{2-}$ ions under an acidic

environment. Whereas CrO_4^{2-} species predominates at pH levels above 6 (Sharma, 2002; Mohan & Pittman, 2006).

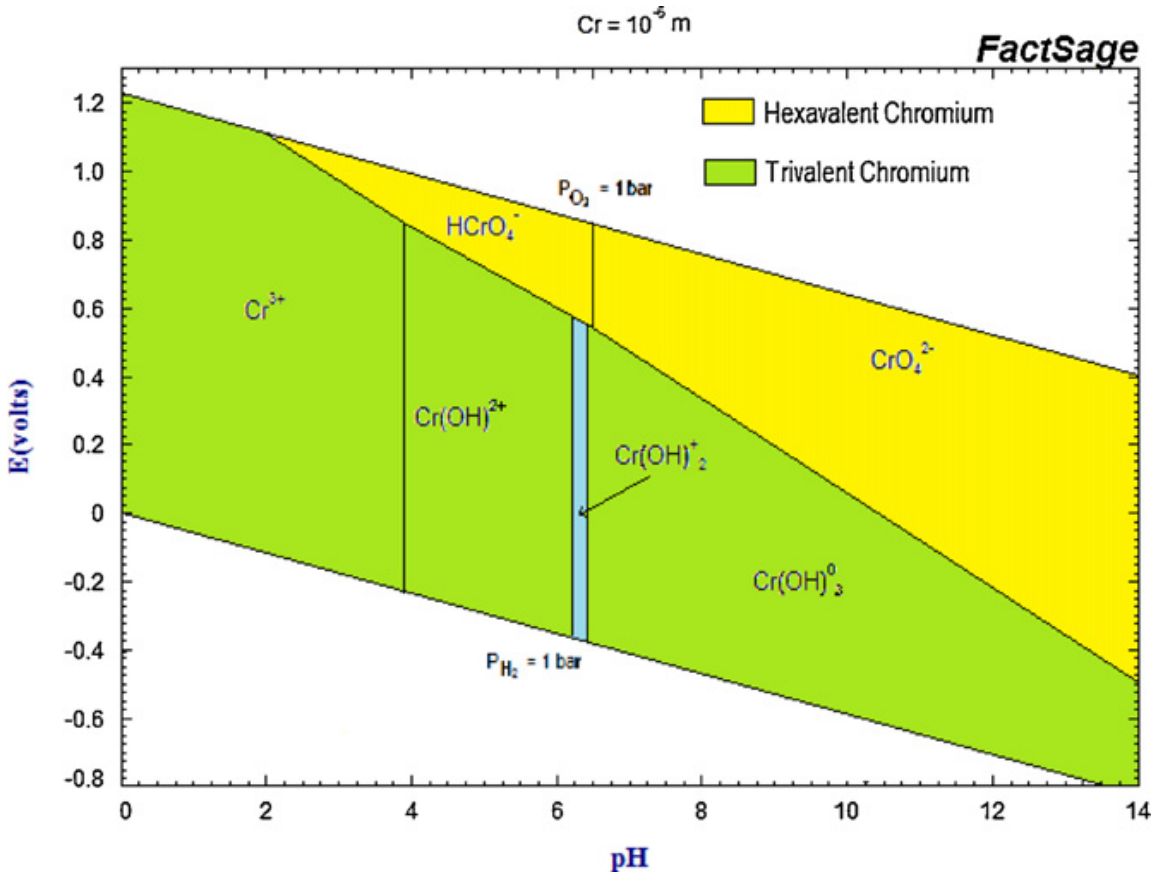


Figure 2-3: Eh-pH diagram for the system Cr-O_2-H_2O, for the concentration of Cr = 10^{-6} mol/kg at solid/liquid boundaries (Dhal et al., 2013)

On the other hand, Cr(III) tends to form Cr(III) hydroxo-complexes in aqueous mediums, which are expected to be the dominant Cr(III) species in environmental water. The dominant Cr(III) species occurring in groundwater is also a function of the pH. Cr(III) under acidic environments is very stable but in an alkaline medium is easily oxidized to Cr(VI) (Pradhan et al., 2017). Figure 2-3 also illustrates the dominant aqueous species and the redox stability for Cr(III). In water, cationic Cr(III) species are considered moderately nontoxic, and at pH levels above 5.5 virtually precipitate as insoluble $Cr(OH)_3$ under natural redox conditions.

Simple forms of Cr(III) species at pH values less than 3.5 are predominantly present. However, above the pH level of 3.5 Cr(III) hydrolysis into different species $CrOH^{2+}$, $Cr(OH)^{2+}$, $Cr(OH)_3^{0}$ and $Cr(OH)_4^{-}$ (Dhal et al., 2013)

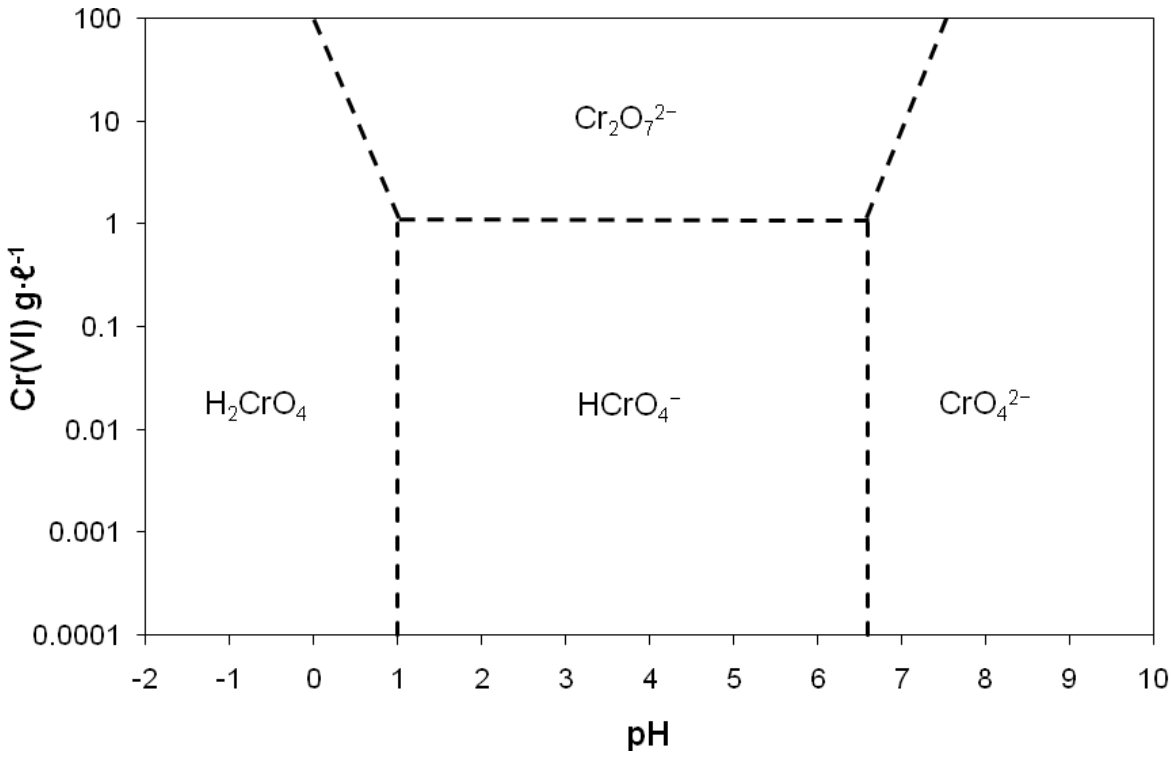


Figure 2-4: The dependence of different Cr(VI) species in the aquatic environment on Cr(VI) concentration and pH (Mohan & Pittman, 2006)

2.5 REMEDIATION TECHNIQUES FOR CHROMIUM-CONTAMINATED ENVIRONMENTS

Minimising the risk of public and aquatic life exposure to chromium-contaminated environments is of paramount importance. In order to reduce this risk of exposure, there are several techniques for the remediation of heavy metals contaminated groundwater and soil that can be employed to mitigate this problem, including physical, chemical, or biological processes. These technologies have some definite significance for instance: (i) complete or substantial destruction/degradation of the pollutants, (ii) extraction of pollutants for further

treatment or disposal, (iii) stabilization of pollutants in forms less mobile or toxic, (iv) separation of non-contaminated materials and their recycling from polluted materials that require further treatment and (v) containment of the polluted material to restrict exposure of the wider environment (Hashim et al., 2011).

2.5.1 Physical-Chemical Treatment

The physical-chemical treatment process for chromium and other heavy metals involves physical extraction of conventional methods such as pump and treat, ion exchange, electrochemical precipitation, and adsorption amongst others (Dhal et al., 2013; Fernandez et al., 2018).

2.5.1.1 *Pump and treat*

The pump and treat technique is one of the commonly employed techniques for the remediation of chromium-polluted groundwater environment. Through pumping, the contaminated groundwater is removed from the aquifer and transported to a treatment plant above the ground where it can be treated and then released back into the aquifer (Higgins & Olson, 2009). Since hexavalent chromium is highly soluble and not easily adsorbed onto sediment surfaces, the pump and treat method has been considered an ideal option for such cases.

However, remediation of groundwater contaminated by chromium using the pump and treat technique has not always yielded adequate results due to intrinsic limitations such as diffusion-restricted rates of extraction and exponentially deteriorating reaction to treatment. Another drawback of this method is the failure to attend to the pollution source in the vadose zone and it also lowers the water table as result leaving contamination in the new vadose zone. It was concluded by Mackay and Cherry (1989), that instead of using the pump and

treat as a technique for permanent remediation of aquifers, it would best work as a management tool to avert the pollution spread by hydraulic manipulation of the aquifer.

2.5.1.2 *Ion exchange*

Ion exchange generally refers to a process in which charged ions from an ion-exchange material are exchanged with charged ions from a solution (Sahu et al., 2009), and the charged ions on the surface of the ion-exchange material are displaced by those in the solution (Dąbrowski et al., 2004; Fu & Wang, 2011). The operating principle of an ion exchanger is such that a hexavalent chromium-polluted solution passes through a column packed with resins and exchange ions with the chromium solution and then the chromium-free solution comes out at the other end of the column. Once the resin capacity is reached, then the column is backwashed using acid or alkali to remove the trapped chromium and regenerate the resins' efficiency. Synthetic organic exchanger resins are the commonly used matrices for the ion exchange process.

These ion exchange resins consist of carboxyl and sulphonic functional acid groups which enables the physio-chemical reaction as follows (Fu & Wang, 2011):

$$nRCOOH + M^{n+} \rightleftharpoons nRCOO^{-}{:}M^{n+} + nH^{+} \qquad 2\text{-}1$$

$$nRSO_3H + M^{n+} \rightleftharpoons nRSO_3^{-}{:}M^{n+} + nH^{+} \qquad 2\text{-}2$$

where $nRCOOH$ and $nRSO_3H$ represent the carboxyl and sulphonic acid functional cationic exchange resins respectively. M represents the metal being removed, while n is the metal valence state.

In the case of hexavalent chromium removal, an anionic type resin is required as demonstrated by the following reaction (Dąbrowski et al., 2004):

$$2ROH + CrO_4^{2-} \rightleftharpoons R_2CrO_4 + 2OH^- \qquad 2\text{-}3$$

where ROH is the anionic exchange resin, and it can be regenerated using NaOH as follows:

$$R_2CrO_4 + 2NaOH \rightleftharpoons 2ROH + Na_2CrO_4 \qquad 2\text{-}4$$

Several researchers have investigated the use of ion exchange as a method of hexavalent chromium removal. Shi et al. (2009) studied three anion exchange resins for the removal of Cr(VI) from electroplating industry wastewater. Different experimental conditions were set out, including Cr(VI) concentrations, initial pH, resin amounts, contact time and temperatures. Their results showed that the ion-exchange process was pH-dependent, with maximum Cr(VI) removal in the pH range of 1–5 for an initial concentration of 100 mg/L of Cr(VI). The process achieved a 99.4% Cr(VI) removal under optimal conditions. Rapti et al. (2016), studied the potential use of an anion exchange composite material based on a protonated amine-functionalized metal-organic as a hexavalent chromium remediation technique. Under various experimental conditions, they observed that the material has an exceptional capability to rapidly and selectively sorb Cr(VI) in the presence of several competitive ions. Nam et al. (2018), evaluated an amine-functionalized acrylic ion exchange fibre for Cr(VI) removal using flow-through experiments modelling and real wastewater. It was observed that during the five regeneration cycles, Cr(VI) adsorption capacity retained was 95.96% of the initial capacity. Liu et al. (2020), studied Cr(VI) removal from water using cetylpyridinium chloride (CPC)-modified montmorillonite as an ion exchanger. The

results showed a maximum adsorption capacity of 43.84 mg/g at low to neutral pH values. Han et al. (2020), examined the performance of co-monomer polymer anion exchange resin for Cr(VI) removal. They reported that after four operation cycles, the adsorption capacity of EDE-D301 remained at 93%.

Although the ion exchange technique is efficient in the removal of hexavalent chromium, the shortcoming of these ion exchange resins is that are very selective for certain ions (Lin & Kiang, 2003). Furthermore, the resins are very sensitive to the pH of the solution and suspended solids have to be removed by other methods prior ion exchange process. Also, this method is unable to handle metal solutions of high concentration as the resins are easily fouled by organics and other solids in wastewater. On top of these limitations, the costs of operating an ion exchange treatment process at full-scale are high (Cheung & Gu, 2007).

2.5.1.3 *Membrane filtration*

The membrane filtration technique has received much attention in wastewater as it can assist with the removal of dissolved inorganic pollutants such as heavy metals, suspended solids and organic compounds (Pugazhenthi et al., 2005). Various membrane filtration methods such as ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) can be used depending on the size of the particles to be removed (Doke & Yadav, 2014; Björkegren et al., 2015). The polluted water is passed through the semipermeable membrane under the application of hydraulic pressure. The membrane is designed such that it rejects the pollutants of interest (Baharuddin et al., 2014).

Numerous types of membranes such as polymeric, inorganic, and liquid membranes can be used for Cr(VI) removal. Saucedo-Rivalcoba et al. (2011), investigated the feasibility of using a polyurethane–keratin hybrid membrane for the removal of Cr(VI). They tested their filtering system at low contact time in continuous flux, and they achieved a maximum

removal of 38% at neutral pH. Bhowal et al. (2012), studied the continuous removal of Cr(VI) by emulsion liquid membrane in a modified spray column. They observed that as the emulsion flow rate increased, the percentage of Cr(VI) extraction increased up to 54.3%. Tang et al. (2012), prepared porous stainless steel supported magnetite crystalline membranes for the removal of Cr(VI) from aqueous solutions. In their study, they could achieve 92.5% removal, indicating a promising application to remediate Cr(VI) from an aqueous solution. Doke and Yadav (2014), studied a Titania microfiltration membrane prepared from polymeric sol-gel derived TiO_2 powder for removal of Cr(VI). The normal flow microfiltration process was operated at lower pressure (1 bar), which showed 99% efficiency for removal of Cr(VI) from aqueous solution using a cationic surfactant, cetylpyridinium chloride at CPC/Cr ratio of 2.5 and initial chromate concentration of 100 mg/L. Björkegren et al. (2015), investigated the extraction efficiency of Cr(VI) from water using a vegetable oil-based emulsion liquid membrane technique. Their results demonstrated that this formulation, using vegetable palm oil as a diluent, is useful for the removal of hexavalent chromium with an efficiency of over 99%. Wei et al. (2019), studied a negatively charged nanofiltration membrane for its hexavalent chromium removal performance. The membrane was fabricated through an interfacial polymerization reaction. They performed the filtration experiments at various Cr(VI) concentrations ranging from 10 mg/L to 500 mg/L, while the pH ranged between 4 to 9. Their results showed that Cr(VI) rejection increased with the increasing pH, while it decreased with increasing Cr(VI) concentration. Dognani et al. (2019), studied the effective of chromium removal from water by polyaniline-coated electro spun adsorbent membrane. It was found that the polyaniline coating greatly enhanced the chromium removal efficiency with the maximum adsorption capacity being 15.08 mg/g at a pH of 4.5. Furthermore, the polyaniline coated membrane showed greater the recyclability capacity achieving chromium efficiency of over 70% after

5 cycles of usage. Zolfaghari & Kargar (2019), evaluated the hybrid system performance of nanofiltration and microfiltration process for Cr(VI) removal. A maximum Cr(VI) removal efficiency of 99% was achieved with the hybrid system under various operating conditions. However, this study only evaluated a low Cr(VI) concentration not exceeding 0.1 mg/L. Bandehali et al. (2020), examined the application of a thin film-PEI nanofiltration membrane for chromium removal. The membrane achieved 81% removal of chromium and experienced membrane fouling during the process. Li et al. (2021) showed that with a flexible and free-standing pristine polypyrrole membranes, Cr(VI) removal could be achieved.

Membrane technology is a very promising technique for the removal of heavy metals. However, deterioration of membrane efficiency due to fouling hinders the improvement of this technique. As a result of fouling, the membrane has to be replaced frequently, thus increasing the operating costs of the process.

2.5.1.4 *Soil washing*

To decontaminate soil, several methods can be applied to restore the soil to its natural, contamination-free state. The soil washing technique entails the separation of pollutants from the soil matrix by solubilizing them in a washing solution (usually water, occasionally combined with solvents) and mechanical processes to scrub soils (Khalid et al., 2017; Liu et al., 2018). The soil washing process is an *ex-situ* remediation technique.

During the soil washing process, the soil is extracted from the contamination site and mixed with water or an appropriate solvent solution depending on the soil and type of metal. The soil and the washing solution are mixed thoroughly for a specified time. During this time the contaminants are either dissolved or suspended in the solution (Gitipour et al., 2011). The soil is then separated from the washing solution through a physical process such as sieving or hydrocyclone. The use of the physical process is to isolate the oversized soil particles

(sand gravel) (> 0.05 mm) from the fine soil (clay and silt) portion (<0.05 mm). The washed oversize fraction of soil is considered to be less contaminated (non-toxic) and can be used for a refill or returned to the original site. The solution which contains finer soil particles remains hazardous due to the presence of heavy metal contaminants. Therefore, the soil washing process is often combined with other technologies to further treat the spent washing solution (Moutsatsou et al., 2006).

The soil washing process heavily depends on washing solutions to remove heavy metals from the soil. The removal of contaminants from the soil with washing agents depends on the factors such as soil geochemistry, characteristics of the metal pollutants, reagent dosage and chemistry, and the process operating conditions (Dermont et al., 2008). These factors play a major role in the selection of an appropriate washing solution solvent. For example, one reagent might be effective in removing a certain metal pollutant, while contact with a different metal pollutant in the soil might lead to enhanced contamination in the soil environment. An ideal washing solution has to intensely enhance the mobility and solubility of heavy metal pollutants but interact weakly with soil constituents and must be biodegradable and nontoxic (Liu et al., 2018). A range of reagents that have been studied as washing solutions for effective removal of heavy metal pollutants includes acetic acid, citric acid, formic acid, fluorosilicic acid, hydrochloric acid, nitric acid, oxalic acid, phosphoric acid, sulphuric acid, EDTA, DTPA, NTA, EDDS, carbonate/bicarbonate, ammonium acetate, ammonium chloride, calcium chloride, dithionite, ferric chloride, isopropyl alcohol, sodium hydroxide, subcritical water (Moutsatsou et al., 2006; Fedje et al., 2013; Alghanmi et al., 2015; Zhu et al., 2015; Bilgin & Tulun, 2016; Yang et al., 2017). The effectiveness of the washing solutions on metal removal varies with the metal species and is also dependent on the soil characteristics such as texture, organic matter content, pH, and moisture content (Mulligan et al., 2001). In general, over an array of heavy metals and soils,

the highest washing efficiency was achieved with EDTA, hydrochloric acid, and subcritical water. It should be noted that these results are case-specific and were achieved by small-scale experiments.

Based on the environmental and economic point of view, soil washing has been demonstrated to be an effective alternative for landfilling and solidification/stabilization (Gitipour et al., 2011). Also, this technique can be operated as a closed treatment process allowing control for fugitive dust and volatile emissions. In addition, it has a high-efficiency removal of metal pollutants and the process is relatively quick (Liu et al., 2018). The major drawback of soil washing is that it is extremely disruptive to the soil environment. The technology is not suitable for soils with high clay content (Karthika et al., 2016).

2.5.1.5 *Electrokinetic remediation*

Electrokinetic remediation is an environmental technique that involves the transportation of pollutants through soils and groundwater under the influence of an electric field (Qian et al., 2014). This technique is relatively new and cost-effective for the remediation of heavy metals (Khalid et al., 2017). The electrokinetic remediation method is carried out by applying a low-voltage direct current to the cathode and anode electrodes embedded in the contaminated ground area. This generates an electric field between the electrodes, which triggers a movement of water, ions and small charged particles between the electrodes. During the electrokinetic process, cations migrate towards the negative electrode while anions move towards the positive electrode. The migration of species occurs through several mechanisms, which include electromigration, electroosmosis, and electrophoresis (Liu et al., 2018). However, electromigration is the most predominant transport mechanism while diffusion rates are insignificant (Niroumand et al., 2012).

- **Electromigration**

Electromigration is defined as movement induced by the electric field of dissolved ions and ionic complexes in the soil solution. As pointed out earlier that the charged ions migrate towards oppositely charged electrodes. Thus, cations migrate towards the negatively charged electrode (cathode) and anions are transported towards the negatively charged (anode) electrode. The migration rate of ions under applied electrical current gradient depends on the ionic species mobility involved and can be given by (Fonseca et al., 2012):

$$q_{ion} = \mu_i \nabla E \qquad 2\text{-}5$$

where: q_{ion} is the migration rate of ions ($m.s^{-1}$), μ_i is the ionic mobility in free solution ($m^2.V^{-1}.s^{-1}$) and ∇E is the electrical potential gradient ($V.m^{-1}$).

Ionic mobility can be defined as the velocity of the ion in the soil under the influence of a unit electrical potential gradient.

- **Electroosmosis**

Electroosmosis is the movement of water through a porous media under an applied electric field. Since soil particle surface is negatively charged, so when an electric current is applied and maintained to the soil, water molecules flow from the anode towards the cathode due to the electrical potential difference between the negatively charged soil surface and the soil solution (Gidudu & Nkhalambayausi Chirwa, 2020). This process is dependent on pore fluid concentration of ions, soil water content, type of soil, and ionic species types (Niroumand et al., 2012; Gidudu & Nkhalambayausi Chirwa, 2020). The water flow rate during the electroosmosis process depends on the balance between the electrical force on the fluid and the soil particle surface. Electroosmotic flow rates in the soil is described by an equation similar to Darcy's law (Asadi et al., 2013):

$$q_w = K_e \nabla E A \quad \text{2-6}$$

where q_w is the electroosmosis-induced flow rate of water (m^3/s), K_e is the coefficient of electroosmosis permeability (conductivity) of soil ($m^2.V^{-1}.s^{-1}$), ∇E is the electric field gradient ($V.m^{-1}$) and A is the cross-section of the treated volume (m^2).

❖ **Electrophoresis**

Electrophoresis is the transport of charged particles (colloidal, clay, organic particles) under the influence of an electric field (Luo & Keh, 2021). Like in the electromigration process, electrophoresis occurs, when an electrical gradient potential is applied across a colloidal suspension, positively charged particles and colloids will migrate toward the cathode and negatively charged particles will move toward the anode. The mass transport by the electrophoresis process is negligible in compact soil systems such as clay, compared to electromigration and electroosmosis due to the restrained movement of contaminants (Luo & Keh, 2021).

❖ **Electrolysis of water**

When an electrical field is applied across a column of soil during electrokinetic remediation, some redox reactions occur at the boundaries of the electrodes. For example, in the presence of inert electrodes water oxidation occurs at the anode which releases H^+ ions while water is being reduced at the cathode which releases OH^- ions (Khan et al., 2018). This redox reaction is necessary to maintain the system's charge neutrality.

Anode reaction (oxidation):

$$2H_2O \longrightarrow 4H^+ + O_2(g) + 4e^- \quad \text{2-7}$$

Cathode reaction (reduction):

$$4H_2O + 4e^- \longrightarrow 2H_2(g) + 4OH^- \qquad 2\text{-}8$$

The oxygen and hydrogen produced during the electrolysis of water escape out of the soil. The generation of the OH^- ions causes a basic environment at the cathode and will move through the soil towards the anode via different transport mechanisms including electromigration due to electric field gradient, diffusion as a result of concentration difference, and electroosmosis due to movement of water through porous soil. Whereas the production of H^+ ions at the anode creates an acidic environment and moves towards the cathode through electromigration, electroosmosis and diffusion as well. Provided that the transportation of H^+ ions is not hindered by the soil buffering capacity, then in general terms, the acid front moves faster than the base front due to the high mobility of H^+ ions compared to OH^- ions and electroosmotic flow is largely in the direction of the cathode (Wu et al., 2016).

The pH during the electrokinetic remediation of the soil drops to about 2 at the anode while it rises to above 10 at the cathode. The acid and base production rate is a function of current density (Li et al., 2020). The electrokinetic process does not control the pH well, as a result, precipitated hydroxides are observed when pH change occurs at the minimum solubility of the metal ions. Therefore, controlling the pH and the electrolyte conditions inside the cathode and anode compartments is important for the optimization of the process. This can be done by adding buffer solutions in the electrode casings such as an alkaline solution for the anode and an acidic solution for the cathode compartment (Zhou et al., 2018). In addition to the pH control and electrolyte optimal conditions for an enhanced electrokinetic process, other measures such as increasing the treatment time, interchanging the electrodes for optimal

electrode configuration, increasing electric field gradient, varying the mode of the electric field application (from continuous to periodic), and anion/cation exchange membranes addition to the electrodes can improve the metal removal efficiency (Cameselle, 2015; Sakellariou & Papassiopi, 2018).

Like any other process, electrokinetic technology has its advantages as it is applicable for a broad range of pollutants and it can be used to clean up heavy metal pollutants in unsaturated soils; technically and cost competitive. However, the pollutants have to be solubilized by either a processing fluid or dilute acidic solution front in order for the electrokinetic process to take place. Also, if the pollutant migration path is long or the areas are of poor conductivity, remediation may be incomplete.

2.5.2 Biological treatment

Biological treatment is the remediation technique that involves the utilization of organisms, mainly microbes, to clean up polluted sites including soils, aquifers, sludges, residues and air (Soffritti et al., 2019; Xia et al., 2019). This process is also known as bioremediation and has been proposed to advance and/or substitute the conventional physicochemical methods for cleaning up polluted environments (Xue et al., 2017). Bioremediation is a fast-growing and changing area of environmental biotechnology, and its growing popularity is due to its low cost, higher effectiveness and efficiency than the traditional methods. Bioremediation can be applied both *in situ* and *ex-situ*. In the areas where contamination has already occurred, *in situ* bioremediation is favoured to prevent further pollutant migration (Krishna & Philip, 2005). *In situ* bioremediation is considered to be environmentally friendly as it causes minimal disturbance to the surrounding environments and as well reducing the risk related to pollutant migration (Krishna & Philip, 2005; Soffritti et al., 2019; Xia et al., 2019).

Many organic contaminants such as hydrocarbon compounds can be degraded by microorganisms in natural environments. However, unlike organic compounds, heavy metals cannot be destroyed and are not degradable but can only be transformed from a higher oxidation state (harmful) to a lower oxidation state (less toxic). For instance, more toxic Cr(VI) can be biologically reduced into Cr(III) which is less toxic and can be achieved by the use of microbes. In natural and synthetic environments, microorganisms have the ability to interact with toxic metals and in the process alters the physicochemical properties of the pollutants. These physicochemical properties include oxidation state, solubility, and sorption amongst others. Microorganisms have developed different mechanisms for detoxifying heavy metals and these include (i) biosorption (Hlihor et al., 2013; Yang et al., 2020; Kalola & Desai, 2020) which involves passive metal uptake to cell components, (ii) Bioaccumulation (Chojnacka, 2010) involves both active and passive metal uptake, (iii) bioprecipitation (Pagnanelli et al., 2012; Xu et al., 2018) involves reaction with inorganic ligands such as phosphate, and (iv) biotransformation (Chirwa et al., 2013; Bansal et al., 2017; Bansal et al., 2019) involves of changing the oxidation state of the metal.

2.5.2.1 *Biosorption*

Biosorption is a promising bioremediation process, which can be applied for the removal of heavy metals at low concentrations in water (Jobby et al., 2018; C. Zhang et al., 2020). It involves passive binding or uptake of heavy metal species (sorbate) onto the biomaterial (biosorbent). During the biosorption process, Cr(VI) contaminated water is brought into contact with either active or inactive biomass functional groups (hydroxyl, carboxyl, phosphate) on their surface wall. These charged functional groups on the surface layer interact with charged molecules or ions present in the Cr(VI) contaminated water, since there is direct contact between the cell surface layer and the external environment. As a result of

ion exchange reactions and complexation with charged functional groups, heavy metals are deposited in the cell surface layer (Ren et al., 2018). Extracellular polymeric substances (EPS) capable of immobilizing heavy metals may be produced by certain bacterial species (Comte et al., 2008).

The biosorption process is not dependent on metabolism, it is a rapid process, and is not a function of temperature, biosorbent are readily available and easily regenerated (Michalak et al., 2013). The benefits of using inactive biomass over active cells: addition of nutrients is not necessary; it is unaffected by harsh operating conditions and immune to toxicity; easily regeneration of biomass and allows for recovery of metals; and the biomass itself can be acquired more economically, as an industrial waste product (Michalak et al., 2013).

2.5.2.2 *Bioaccumulation*

Bioaccumulation is described as an active mode of accumulation of metal pollutants by living cells, which is governed by critical factors such as structural and biochemical properties, physiological and genetic adaptation, environmental modification of metal specification, availability and toxicity (Chojnacka, 2010; Diep et al., 2018). Therefore, the uptake of metal contaminants by living organisms through the transport of the pollutants into the cell and the accumulation of metals inside the cell is referred to as bioaccumulation (Timková et al., 2018). The most important thing about bioaccumulation is that the process is only facilitated by living organisms (Chojnacka, 2010; Timková et al., 2018). Diep and co-authors (2018), have cautioned that bioaccumulation and biosorption are not the same and therefore the use of these two mechanisms interchangeably is incorrect. The main disadvantage of the bioaccumulation process is cell growth inhibition at high metal concentrations. An understanding of how some microbes accumulate Cr(VI) is a determining factor in the advancement of the bioaccumulation process for Cr(VI)removal and recovery

from polluted sites. Ksheminska and co-authors (2008) state that the Cr(VI) species are structurally similar to SO_4^{2-} and PO_4^{2-} anions. As a result, Cr(VI) infiltrate the cells via anion channel which is non-selective and sulphate transporters which are oxidative state sensitive.

2.5.2.3 *Bioreduction*

The presence of hexavalent chromium in soil or underground water is toxic to the bacteria. However, some bacterial species have the ability to grow in high chromate concentrations and are usually recognised as resistant or tolerant to chromium (Murugavelh & Mohanty, 2012; Coetzee et al., 2018; Kholisa & Chirwa, 2021). The bioreduction of Cr(VI) is considered a decontamination mechanism due to the fact the Cr(III) form is less toxic and more stable than Cr(VI). Microorganisms which are able to survive the Cr(VI) toxicity can be isolated and applied for chromium hexavalent remediation.

Pseudomonas sp. grown under anaerobic conditions was the first chromium-reducing bacteria isolated in the late 1970s from wastewater when Romanenko and KorenKov (1977) discovered its Cr(VI) reduction capabilities (Fernandez et al., 2018). Since that day, a diversity of microbes has been identified and isolated from a wide range of contaminated environments with the capability to transform Cr(VI) into lesser toxic Cr(III).

Numerous studies have reported the use of microorganisms that are responsible for reducing Cr(VI) to Cr(III) under different experimental conditions, these include initial Cr(VI) concentration, temperature, pH, degree of agitation, anaerobically and aerobic, cell-free Cr(VI) reductase, nutrient supplementation, electron transport addition, design changes to the reactor, cell immobilizers, to mention a few (Barrera-Diaz et al., 2012; Dhal et al., 2013). A number of studies that have isolated and utilised chromium-reducing bacteria from different sources under aerobic and anaerobic conditions are summarised in Table 2-1.

Table 2-1: Summary of some known Cr(VI)-reducing bacteria

Name of species	isolation sources	Isolation conditions	Growth medium/carbon source	Cr(VI) initial Conc. (mg/L)	References
Acenetobacter calcoaciticus	Chromite ore mines	Aerobic	Luria–Bertani broth	100 - 1100	Mishra et al. (2010)
Mixed culture	Sand drying beds Belt filter press Dried sludge	Aerobic/Anaerobic	Luria-Bertani broth/agar	5 - 400	Bansal et al. (2019)
Bacillus cereus	Coalmine soil	-	Luria-Bertani broth	0 - 500	Banerjee et al. (2019)
Pseudomonas species	Alloy manufacturing effluent	-	Nutrient agar/broth	0 - 100	Wani et al. (2019)
mixed bacterial consortium	Municipal waste sludge	Aerobic	Mineral salt medium	0 - 400	Kholisa et al. (2021)
Bacillus sp.	Tannery waste disposal site	Aerobic	Luria-Bertani (LB) agar	50 - 250	Wu et al. (2019)
Klebsiella pneumoniae /Mangrovibacter yixingensis	Chromium contaminated tannery effluent	Aerobic	Mueller Hinton agar	20 - 100	Sanjay et al. (2018)
Caldicellulosiruptor saccharolyticus	-	Anaerobic	DSM640 medium	0 - 160	Bai et al. (2018)

Cellulosimicrobium sp.	Tannery wastewater	Aerobic	Luria-Bertani (LB) agar	0 - 300	Bharagava & Mishra (2018)
Bacillus sp. FY1 /*Arthrobacter* sp. WZ2	Chromium-contaminated soil (electroplating industry)	Aerobic	Luria-Bertani (LB) medium	200 - 1000	Xiao et al. (2018)
Achromobacter xylosoxidans SHB 204	Industrial effluent/paint and steel sludge/ drainage water	anaerobic	Nutrient broth	100 - 500	Rao et al. (2017)
Pseudomonas Aeruginosa	Leather tannery effluent	Aerobic	Nutrient agar	0 - 200	Munawaroh et al. (2017)
Pseudomonas gessardii strain LZ-E	-	Aerobic	BH liquid medium + naphthalene	0 - 40	Huang et al. (2016)
Bacillus subtilis	Rare-earth ore mine	Aerobic	Mineral salt media + yeast extract	200	Zheng et al. (2015)
Pseudomonas mendocina	Lab culture collection	Aerobic/anaerobic	Nutrient Broth + galactronic acid or glucuronic acid	50	Dogan et al. (2014)
Pseudochrobactrum saccharolyticum LY10	Chromium contaminated site	Aerobic	Luria-Bertani media	55 - 360	Long et al. (2013)
Staphylococcus arlettae strain Cr11	Tannery effluent	Aerobic	Tryptone soyapeptone media	100 - 5000	Sagar et al. (2012)

Bacillus sp.	Chromium contaminated site	Aerobic	Nutrient broth + glucose	50 - 600	Kathiravan et al. (2011)
Arthrobacter sp.	Creosote polluted site	Aerobic	Luria-Bertani media + Glucose	5 - 500	Ziagova et al. (2015)
Bacillus cereus	chromite mine	Aerobic	Nutrient agar (NA) medium	100 - 1000	Das et al. (2015)

2.6 PERMEABLE REACTIVE BARRIERS

2.6.1 Overview of permeable reactive barriers

In underground environments, pollutants are mainly transported by groundwater flow. The flowing contaminants with groundwater tend to create plumes and spread out over extended distances in the aquifer. For this reason, permeable reactive barriers (PRBs) have been developed to prevent further dissemination of contaminants and to reduce the risk and impact of the pollutants onto sensitive receptors, such as drinking water wells (Obiri-nyarko et al., 2014).

PRBs are defined as a zone of reactive materials that have been placed in the subsurface and are designed to intercept and treat the contaminant plume while permitting the groundwater to flow through the reactive media (Figure 2-5). The success of this technology relies on the polluted groundwater to be transported through the reactive barrier by a natural aquifer gradient. As the contaminants flow through the barrier, they react with the reactive material as a result either are transformed into environmentally acceptable compounds or trapped inside the reactive zone (Suthersan et al., 2017). PRB is a passive, *in situ* technique with great potential to treat even shallow aquifers at a lower cost. It is considered as a green and innovative technology for groundwater remediation and, is an emerging alternative to traditional pump-and-treat systems.

The PRB decontamination occurs through physical, chemical and/or biological processes. These include precipitation, sorption, oxidation/reduction and biologically mediated reduction (Obiri-nyarko et al., 2014; Suthersan et al., 2017).

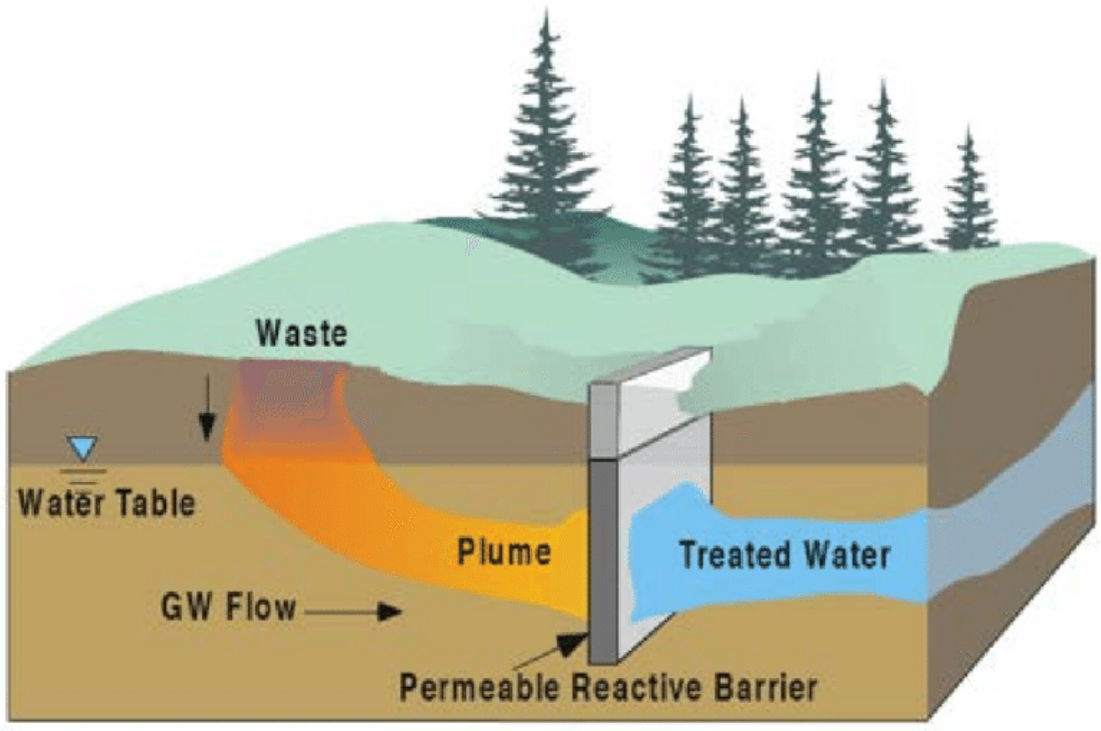


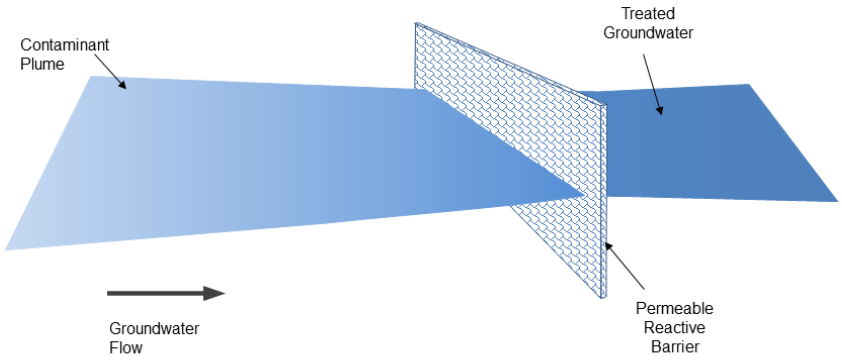
Figure 2-5: Typical permeable reactive barrier (Rad & Fazlali, 2020)

2.6.2 PRB configurations

In practice, there are two frequently used designs of PRB installations as shown in Figure 2-6: (a) the continuous permeable reactive barrier (CPRB) and (b) the funnel-and-gate system (FGS). The CPRB is the most commonly used design due to its minimal effect on the flow of groundwater (Al-Hashimi et al., 2021). A CPRB is a trench loaded with reactive material perpendicular to groundwater flow. Since this type of PRB does not have any structures, the contaminant plume passes through the reactive material due to the natural hydraulic gradient. So in order for the CPRB to be able to capture the contaminants plume in both horizontal and vertical directions, the -cross-sectional area of the reactive zone has to be slightly larger than that of the contaminated plume (Suthersan et al., 2017; Al-Hashimi et al., 2021). The FGS comprises impermeable barriers and a reactive zone. The funnel structures are made up of impermeable sheet piles or slurry walls to prevent contaminants'

plume flow and redirect it towards the reactive zone (gate). In the event that the contaminants' plume is very large, multiple reactive zones in series can be installed in the FGS (Al-Hashimi et al., 2021).

a



b

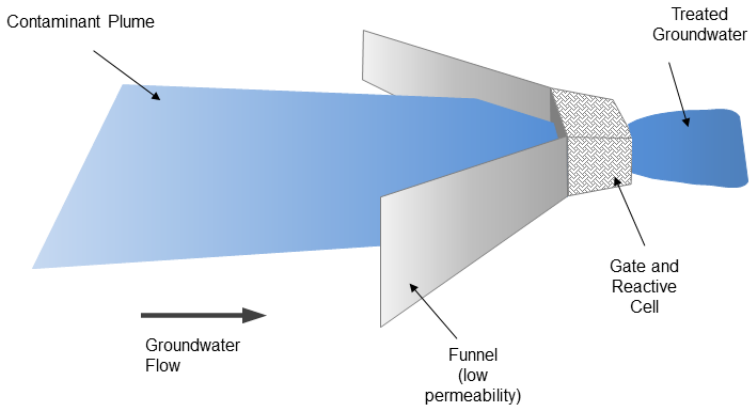


Figure 2-6: PRB designs: (a) continuous permeable reactive barrier and (b) funnel-and-gate system

2.6.3 Reactive media used in PRBs

Thus far, different permeable reactive barrier materials have been assessed for the treatment of groundwater contaminants, such as As, Ba, Be, Cd, Cr, Cu, Hg, Fe, Mn, Mo, Ni, Pb, Sb, Se, U, V, NO_3, PO_4, and SO_4 as shown in Table 2-2. The most commonly used reactive material is Zero-Valent Iron (ZVI). Apart from ZVI, there is a wide range of reactive

materials that have been studied including sawdust, oxygen-releasing compounds (ORC), zeolites, and activated carbon (AC) amongst others. The major drawback of such reactive materials is that they are either restricted to a certain group of contaminants or are available in small quantities and expensive (Upadhyay & Sinha, 2018; Köber et al., 2002). PRBs have been installed on a full scale for remediation of Cr(VI) and a variety of dissolved constituents such as phosphate and nitrate (Ludwig et al., 2002).

2.6.4 Bacterial Permeable Reactive Barriers (BPRB)

Biobarriers are specifically loaded with materials that enhance or stimulate microorganisms under aerobic or anaerobic environments to degrade pollutants in the treatment zone. Such a system has been investigated and employed in the removal of methyl-tert-butyl-ether (MTBE), tetrachloroethylene (TCE) from contaminated groundwater (S. J. Liu et al., 2006; Liu et al., 2017). In their respective studies, S. J. Liu et al. (2006) used a two-layered BPRB system for MTBE (Figure 2-7(**a**)), while Liu et al. (2017) designed a four-layered anaerobic/aerobic BPRB for TCE degradation) (Figure 2-7(**b**)). In the double-layered BRPB system, the first layer contains oxygen-releasing material that provides oxygen for microorganisms; in the second layer, aerobic degradation of MTBE occurs by the microorganisms to remediate the groundwater. However, in the four-layered BPRB system, the first layer comprises of oxygen-capturing materials that capture dissolved oxygen in groundwater to promote an anaerobic environment for the dechlorination of TCE by microbes. In the second layer, granular activated carbon is used as a microorganism support to allow anaerobic biodegradation of TCE. The third and fourth layers are similar to that of the two-layered BPRB system where oxygen-releasing materials are used.

Although there have been numerous studies on bioreduction of Cr(VI) using microorganisms, however, application of these microorganisms in PRB systems has been

seldom. The slow advancement toward the full-scale application of BPRB for Cr(VI) remediation has been due to the unavailability of microbes that can grow under nutrient-stressed environments, as well as the lack of information on the mobility of the reduced chromium species in the barrier.

Table 2-2: Summary of PRB materials used for different contamination sources (adapted from Upadhyay and Sinha (2018))

Contaminants	Mechanism	Reactive barrier	Limitation	References
Heavy metals, metalloids, chlorinated hydrocarbons, nutrients, radionuclides, pesticides	Reductive precipitation, Surface complexation, Adsorption, Co-precipitation	ZVI	Oxidises easily and enhances the pH which causes corrosion thus, reduces the permeability of reactive material.	Obiri-Naryko et al. 2014; Yang et al. 2010b
Heavy metals, lead, U(VI)	Surface adsorption, sorption, precipitation,	Apatite	-	Naryko et al. 2014
Phenols, PCE, TCE, BTEX	Sorption	Activated Carbon	Rapid breakthrough and low sorption capacity due to surface coating	Di-nardo et al. 2010
AMD, Cr, Cu, Fe, Ni, Pb, Zn	Adsorption, precipitation	TRM	-	laponite et al. 2006; Munro et al. 2004
Radionuclides, $NH4^{+}$, heavy metals, BTEX & PCE	Precipitation, sorption, ion exchange,	Zeolite	-	Perric et al., 2004

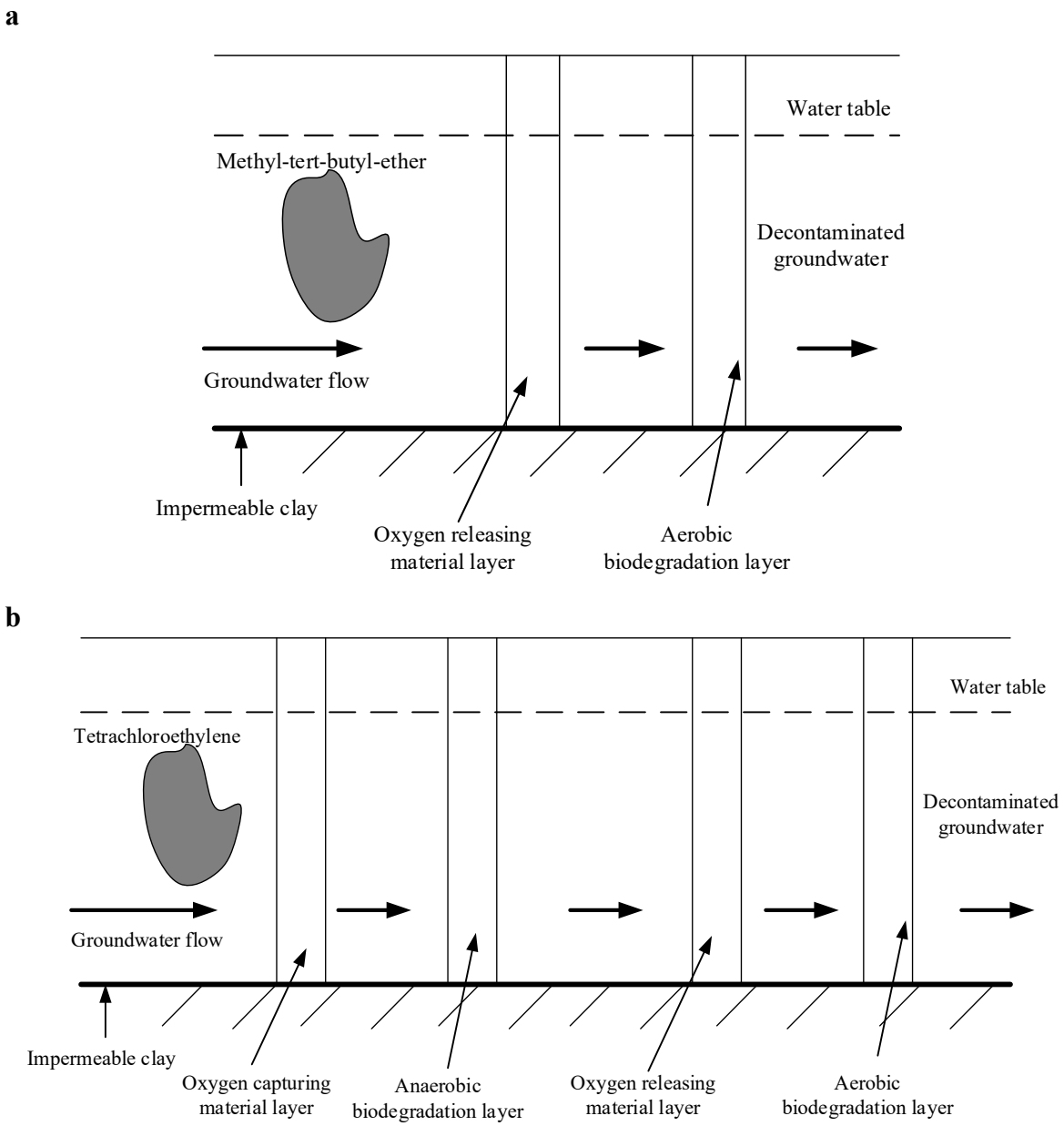


Figure 2-7: Different schematic biological permeable reactive barriers (a) A two-layered barrier for MTBE (b) four-layered barrier for TCE chromium reduction bacteria in PRB

The first attempt of applying CRB as a biobarrier was conducted by Jeyasingh et al. (2011). Their study was carried out on a pilot scale reactor to assess the feasibility of Cr(VI) bioremediation on contaminated aquifers using biobarrier. Experimental results showed that

the microbial biobarrier was able to remediate Cr(VI) plumes even at elevated Cr(VI) concentrations, provided that suitable conditions such as the thickness of biobarrier, initial biomass concentration, moderate flow velocity, and the number of injection wells are maintained. However, this study was limited to the assumption of homogeneousness and uniform spreading of bacteria.

Water Utilisation and Environmental Engineering group at the University of Pretoria isolated microbes that are capable of reducing and immobilizing Cr(VI) from sewage-dried sludge. These microorganisms were introduced in the columns packed with aquifer media in an attempt to reduce Cr(VI) from water flowing through the system. As the water flowed through the column system, the isolated microbes were able to reduce Cr(VI) and also survived under nutrient-stressed conditions (Molokwane et al., 2008; Molokwane & Nkhalambayausi-chirwa, 2009). Furthermore, the dried sludge microbes were also introduced in an open-top tank mesocosm reactor filled with aquifer material (Figure 2-8) to remediate 50 mg/L of Cr(VI) solution (Molokwane, 2010). The microbial reactive barrier was able to significantly reduce Cr(VI) by an average of approximately 90% in the final effluent. Molokwane et al. (2008) and Molokwane and Nkhalambayausi-chirwa (2009) concluded that the dried sludge microbes are autotrophic and use hypocarbonate as their carbon source. The findings could be useful in the formulation of BPRBs for the remediation of groundwater against the spread of Cr(VI) from hot spots in the area. However, despite the promising results, the mobility of the reduced chromium species in the soil and activity of the organisms in the barrier have not been studied.

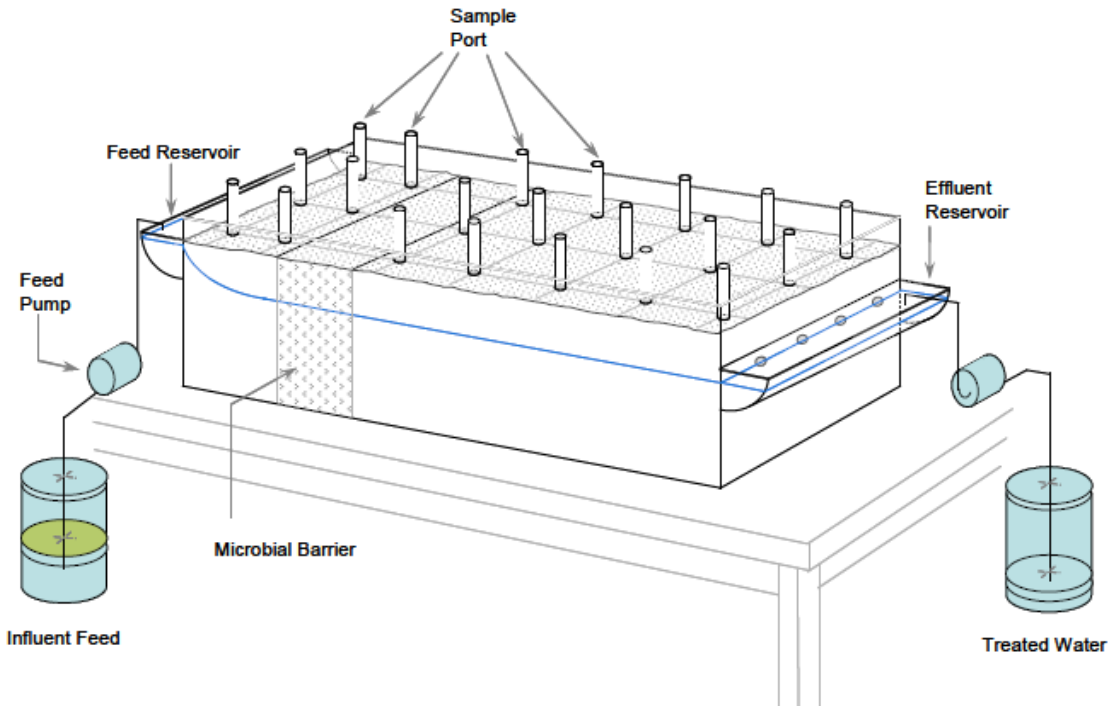


Figure 2-8: Experimental setup for biological permeable reactive barrier for Cr(VI) reduction (adapted from Molokwane (2010))

2.7 SUMMARY

The literature review of this study has established that improper management of chromium-containing waste from processing industries such as chromite ore processing, leather tanning, electroplating, steel production, wood preservation, wood pulp processing and textile are the main reason behind the groundwater contamination by Cr(VI). The intensity of the research in both extent and depth shows the deepening concern over extant Cr(VI) pollution problems around the world. The remediation of hexavalent chromium contaminated sites using traditional methods such as pump and treat or excavation followed by chemical treatment has been ineffective and generates large quantities of toxic sludge, which requires further treatment. Furthermore, it is disruptive to the ecosystems.

PRBs have been evaluated for the remediation of contaminants such as chlorinated organic compounds, heavy metals, radionuclides, and chromate. Biological permeable reactive barriers are a promising technique with many advantages, however, there have been very few studies conducted for the Cr(VI) removal. This has been due to the difficulties associated with removing the reduced metal precipitate from the aquifer. Also, the lack of information on the fate and the mobility of the reduced chromium species in the barrier is not known.

Chapter 3 METHODOLOGY

3.1 SOURCE OF MICROORGANISMS

The natural bacteria consortia were obtained from sludge collected at the Brits Wastewater Treatment Works (North West Province, South Africa). An abandoned sodium dichromate processing facility was reported to discharge high levels of Cr(VI) periodically into the sewage treatment works. The chrome processing facility was commissioned as early as 1996. Three sludge samples were collected, namely primary sludge, secondary sludge in the mixed liquor reactor and sludge cake after undergoing a belt filter dewatering process in the treatment plant. Although the nearby chrome foundry periodically discharges high levels of Cr(VI) to the treatment plant, at the time of collecting the samples for this study did not correspond with the discharging times.

Three sludge samples were collected at different locations within the wastewater treatment plant and were named sludge A, B, and C. Sludge A was a primary sludge, Sludge B was a settled Activated Sludge collected from secondary clarifiers and Sludge C: is dewatered sludge from Sand Drying Beds – the dried product of the combined sludge A and B.

3.2 GROWTH MEDIUMS

3.2.1 Commercial

Luria-Bertani (LB) broth, Luria-Bertani (LB) agar, and Plate count (PC) agar (Sigma-Aldrich Germany) were prepared by dissolving 25 g, 45 g, and 23 g in 1000 mL of distilled water respectively. The media were sterilized at 121°C at 115 kg/cm^2 for 15 minutes using

an autoclave then cooled at room temperature. The LB and PC agars after being cooled to 45 °C were dispensed into Petri dishes to form agar plates for colony development. The growth mediums were prepared according to the manufacturer's instructions.

3.2.2 Mineral salt medium

Mineral salt medium (MSM) consisted of 2.12 g K_2HPO_4, 2.12 g KH_2PO_4, 2 g NaCl, 1 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 4 g KNO_3 and 5 g glucose as a carbon source in 1,000 mL of distilled water (Jeyasingh & Philip, 2005).

3.3 CHEMICAL REAGENTS

3.3.1 Chemicals

Sodium chloride solution (0.85% NaCl) was prepared by dissolving 8.5 g of sodium chloride salt in 1000 mL distilled water and sterilized by autoclaving at 121°C for 15 minutes then cooled to room temperate and stored at 4°C.

Sulphuric acid solution (1N H_2SO_4) was prepared by adding 27.7 mL concentrated sulphuric acid (98%) into distilled water to make a 1000 mL solution.

3.3.2 Cr(VI) stock solution

A 1000 mg/L concentration Cr(VI) stock solution was prepared by dissolving 3.73 g of 99% pure K_2CrO_4 (analytical grade) in 1000 mL of deionized water. The 1000 mg/L Cr(VI) stock solution was used as a source of Cr(VI). The standard solutions of Cr(VI) were prepared from the Cr(VI) stock solutions in a 10 ml volumetric flask by diluting a certain volume of Cr(VI) stock solution with distilled water to give desirable final concentrations of (0, 1, 5,

10, 20, 50, 75 and 100) mg/L. From these data points (absorbance against concentration) a linear graph or calibration curve with the regression of 99.46% was obtained as shown in Figure B-0-1(see Appendix (B)).

3.3.3 DPC solution

Diphenyl carbazide (Merck, South Africa) solution was prepared for Cr(VI) reduction analysis by dissolving 0.5 g of 1,5 diphenyl carbazide in 100 mL of HPCL grade acetone and was stored in a brown bottle covered with a foil.

3.4 Cr(VI) REDUCTION EXPERIMENTS

3.4.1 Cr(VI) tolerance

Three sludge from different environments were identified as possible sources of Cr(VI) reducing cultures within the Brits wastewater treatment plant: (1) dried primary sludge (Sludge (A)), (2) secondary sludge in the mixed liquor reactor (Sludge (B)) and, (3) dried sludge after undergoing belt filter dewatering process (Sludge (C)). The consortia bacterial cultures were screened for Cr(VI) reduction on the basis of their reduction performance. 1 g of sludge sample was added to a 250 mL conical flask containing 100 mL of LB broth and incubated for 24 h at 37 °C by agitation at 120 rpm using a Labcon SPL-MP 15 Lateral Shaker (Labcon Laboratory Services, South Africa). The conical flask was closed with cotton to allow oxygen flow while preventing contaminants from entering the flask. Then, after 24 h of incubation 1 mL of this was transferred into a fresh 100 mL LB broth supplemented with 100 mg/L of Cr(VI). The fresh LB broth was incubated for 24 h under

the same conditions. This method was replicated by gradually increasing the Cr(VI) concentration in the growth medium up to 500 mg/L.

3.4.2 Aerobic batch experiments

The reduction of Cr(VI) by freshly grown cells of the bacterial consortia was determined in MSM. The experiments were conducted in a 250 mL Erlenmeyer flask containing 100 mL MSM supplemented with 50 – 400 mg/L Cr(VI) concentration as shown in Figure 3-1. The cells were harvested after 24 h incubation and washed thrice by centrifugation with 0.85% NaCl sterile solution and finally resuspended in the MSM. Flasks were inoculated with cells concentrated to a 5:1 ratio before adding Cr(VI), and the flasks were covered with cotton to allow oxygen while preventing microorganisms from entering. The flasks were incubated at 37 °C and under constant shaking of 120 rpm. All experiments were conducted in duplicate. 1 mL samples were taken at time intervals, determined by the observed rate of Cr(VI) removal. The samples were centrifuged at 6500 rpm for 10 min in a Hermle 2323 centrifuge (Hermle Laboratories, Wehigen, Germany) to remove suspended cells before analysis.

Figure 3-1: Erlenmeyer flask for Cr(VI) reduction experiment (a) 100 mg/L Cr(VI) before incubation (b) after 24 hours of incubation

3.4.3 Kinetic Parameter Estimation for Cr(VI) Reduction by Bacteria Consortia

The kinetics of Cr(VI) reduction by sludge bacteria consortia were evaluated using first and second-order rate laws.

3.4.3.1 *First-Order Kinetics*

The first-order kinetics assumes that when the Cr(VI) concentration decreases over time, the reaction rate also decreases linearly with Cr(VI) concentration. The rate of reaction (r) can be expressed using Equation (3-1):

$$r = -\frac{d[Cr(VI)]}{dt} = k_1[Cr(VI)] \quad 3\text{-}1$$

Eq. (1) is solved by integrating both sides between the limits $[Cr(VI)_0]$ at t = 0 and [Cr(VI)] at any time t gives the following expression by Equation (3-2):

$$ln\left(\frac{[Cr(VI)]}{Cr(VI)_0}\right) = k_1 t \quad 3\text{-}2$$

Where, k_1 is the first order rate constant (h^{-1}), Cr(VI) is Cr(VI) concentration (mg/L) and $Cr(VI)_0$ is the initial concentration (mg/L).

3.4.3.2 *Second-Order Kinetics*

The second-order kinetics assumes that when the Cr(VI) concentration changes over time, the rate of reaction also changes proportional to the square of the Cr(VI) concentration. The rate of reaction can be expressed by Equation (3-3):

$$r = -\frac{d[Cr(VI)]}{dt} = k_2[Cr(VI)]^2 \quad 3\text{-}3$$

Integrating Equation (3-3) between the limits [Cr(VI)] = $[Cr(VI)_0]$ at time t = 0 and [Cr(VI]) = [Cr(VI)] at any time t, yields the following Equation (3-4).

$$\frac{1}{Cr(VI)} = \frac{1}{Cr(VI)_0} + k_2 t \quad 3\text{-}4$$

Where k_2 is the second-order rate constant ($L.mg^{-1}.h^{-1}$).

3.5 BIOLOGICAL PARAMETER ESTIMATION FOR Cr(VI) REDUCTION BY BACTERIA CONSORTIA

3.5.1 Data Simulation

In Aquasim, the execution of data simulation is through the DASSL algorithm which uses backward differentiation formula (BDF) methods to solve a system of differential-algebraic equation (DAE) or Ordinary differential equation ODE (Li & Petzold, 2000). The techniques are variable-step and variable-order Gear integration. The system of equations is written in implicit ODE or DAE form. The main advantage of this technique is the use of numerically integrating system of ordinary and partial differential equations in time and simultaneously solving the algebraic equations. Spatial discretization of partial differential equations is done using conservative finite difference schemes (Reichert, 1998). Based on the differential conservation law, the equation below was derived and implemented in Aquasim. The implementation of the DASSL algorithm permits the use of full or branded Jacobian matrix (Equations 3-5 to 3-8) in solving the nonlinear system of algebraic equations (Reichart, 1998).

$$\frac{\partial \hat{p}}{\partial t} = \frac{\partial \hat{J}}{\partial x} + \hat{r} \qquad 3\text{-}5$$

Equation 3-5 is discretised as 3-6.

$$\frac{d}{dt}\hat{p}(x_i,t) = \frac{\hat{J}_{num}(x_{i+0.5},t) - \hat{J}_{num}(x_{i-0.5},t)}{x_{i+0.5} - x_{i-0.5}} + \hat{r}(x_i,t) \qquad 3\text{-}6$$

$$\underline{J} = \frac{\partial F}{\partial y} \qquad 3\text{-}7$$

$$\underline{J} = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial y_1} \\ \frac{\partial f_2}{\partial x_2} & \frac{\partial f_2}{\partial y_2} \end{bmatrix} \qquad 3\text{-}8$$

3.5.2 Parameter estimation

In Aquasim, model parameters that are represented by constant variables can be estimated by minimizing the sum of the squares of the weighted deviations between the measurements and calculating the results of the model using equation 3-9. The minimization is done through the simplex algorithm or secant algorithm (Reichert, 1998).

$$x^2(\lambda) = \sum_{i=1}^{n} \left[\frac{y_{meas,i} - y(\lambda)}{\alpha_{meas,i}} \right]^2 \qquad 3\text{-}9$$

where: $y_{meas,i}$ is the i-th measurement, $\alpha_{meas,i}$ is the standard deviation, $y(\lambda)$ is the calculated value of the model variable corresponding to the i-th measurement and evaluated at the time and location of this measurement, $\lambda = (\lambda_1, \ldots, \lambda_m)$ are the model parameters, and n is the number of data points and x^2 is the sum of the deviation for all the fit targets.

3.5.3 Sensitivity analysis

Sensitivity analysis in Aquasim combines the tasks of identifiability analysis and uncertainty analysis. The identifiability analysis is performed to test model parameters if they can be uniquely determined with the assistance of the available data and to evaluate the uncertainty of the parameter estimates. This can be done by estimating the standard errors and correlation coefficients of parameters during the parameter estimation procedure (Reichert, 1998). Equations 3-10 to 3-13 are the sensitivity functions that are distinguished by Aquasim:

$$\delta_{y,\lambda}^{a,a} = \frac{\partial y}{\partial \lambda} \qquad 3\text{-}10$$

$$\delta_{y,\lambda}^{r,a} = \frac{1}{y}\frac{\partial y}{\partial \lambda} \qquad 3\text{-}11$$

$$\delta_{y,\lambda}^{a,r} = \lambda\frac{\partial y}{\partial \lambda} \qquad 3\text{-}12$$

$$\delta_{y,\lambda}^{r,r} = \frac{\lambda}{y}\frac{\partial y}{\partial \lambda} \qquad 3\text{-}13$$

where: y is an arbitrary variable calculated by Aquasim and λ is a model parameter represented by a constant variable or by a measured variable.

However, in uncertainty analysis, the uncertainty of model parameters is propagated to the uncertainty of model results (Reichert, 1998). Aquasim uses the simplest error propagation

method, the linearized propagation of standard deviations of uncorrelated parameters. The error propagation formula using the linearized model and neglecting the parameter correlation and the error contribution of each parameter is given by equation 3-14 and 3-15 respectively:

$$\sigma_y = \sqrt{\sum_{i=1}^{m} \left(\frac{\partial p}{\partial \lambda_i}\right)^2 \sigma_{\lambda i}^2} \qquad 3\text{-}14$$

$$\delta_{y,\lambda}^{err} = \frac{\partial y}{\partial \lambda}\, \sigma_\lambda \qquad 3\text{-}15$$

where: λ_i are the uncertain model parameters, $\sigma_{\lambda i}$ are their standard deviations, $y(\lambda_1,..., \lambda_m)$ is the solution of the model equations for a given variable at a given location and time, and σ_y is the approximate standard deviation of the model result.

The sensitivity functions (3-10) to (3-13), the standard deviations of calculated variables from equation (3-14), and the contributions of parameter uncertainties to the total uncertainty from equation (3-15) are calculated using the finite difference approximation given by:

$$\frac{\partial y}{\partial \lambda_i} \approx \frac{y(\lambda_i + \Delta\lambda_i) - y(\lambda_i)}{\Delta\lambda_i} \qquad 3\text{-}16$$

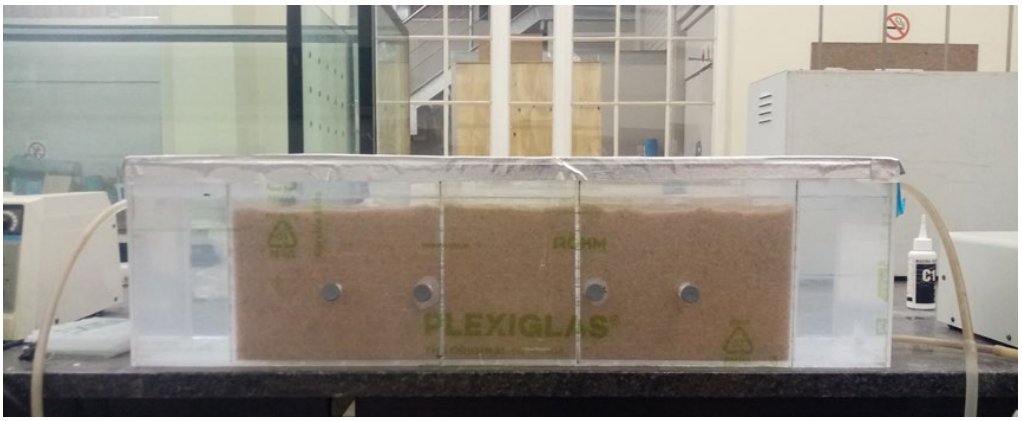
where: $\Delta\lambda_i$ is chosen to be 1% of the standard deviation $\sigma_{\lambda i}$ of the parameter λ_i.

3.6 CONTINUOUS REACTOR STUDY

3.6.1 Experimental system setup

The Cr(VI) remediation experiments were conducted in two horizontal flow tanks with the dimensions 820 x 170 x 200 mm (L x B x H) were constructed using 5 mm thick transparent Perspex sheets (Evonik Rohm GmbH, Essen, Germany) as shown in Figure 3-2. Each reactor consisted 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). Sand compartments were filled with thoroughly washed pure river sand with granular sizes ranging from 0.6 mm 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 70% to 30%. The compartment dividers were perforated with 90 holes of 1 mm size to ensure evenly distrusted flow. The reactors were operated as plug-flow systems with four sampling ports along the length. The first sampling point was placed 115 mm from the influent reservoir, the second and third were 10 mm before and after the biobarrier and the fourth was placed 495 mm from the influent reservoir. The flow was delivered using a Watson-Marlow 120U peristaltic pump.

(a)



(b)

(c)

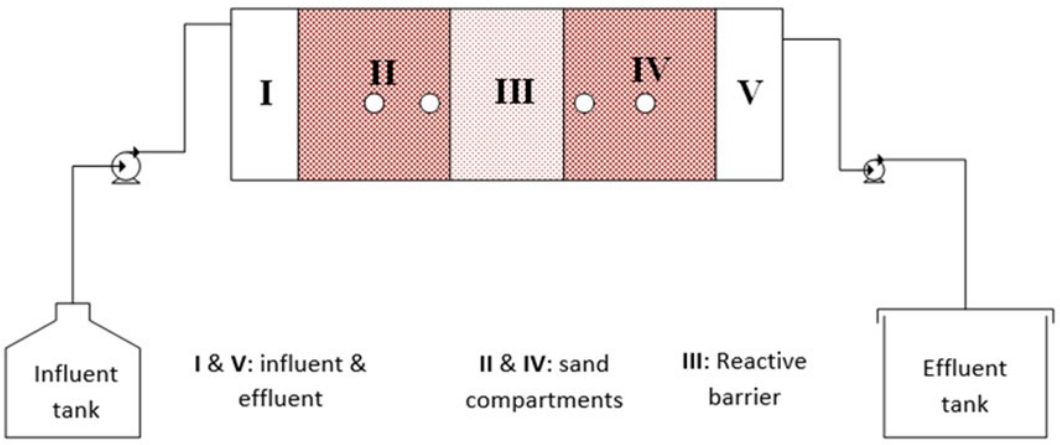


Figure 3-2: Bench scale set-up of a permeable reactive barrier system (a) control (b) BPRB (c) schematic

3.6.2 Reactor start-up

Two reactors were operated under a constant flow rate of 200 mL/h each. Before the starting up of experiments, the two reactors were saturated with distilled water for 10 days. The other reactor was filled with sand quartz only, to serve as a control. The influent solution of 40 mg/L Cr(VI) (initial pH = 6.8) was pumped into the reactors using a peristaltic pump and a liquid detention time of about 8 h in the biobarrier. After 30 days of continuous operation, Cr(VI) concentration was increased to 60 mg/L under the same conditions. 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 those already found in the sludge. The system is being developed for application in the groundwater environment, therefore, introducing foreign organic carbon sources is not desirable.

3.7 ANALYTICAL METHODS

3.7.1 Cr(VI) measurement

1 mL samples were collected overtime and centrifuged using 2 mL Eppendorf centrifuge tubes at 6000 rpm for 15 min in a Minispin® Microcentrifuge (Eppendorf, Hamburg, Germany) to separate the suspended cells from the solution. The cell-free supernatant was then extracted using a pipette without re-suspending the cells and was used for Cr(VI) analysis.

Then 0.2 mL of the extracted supernatant was added to a 10 mL volumetric flask followed by the addition of 1 mL (1N) H_2SO_4 solution for digestion of the sample. The flask was filled

with distilled water to the 10 mL mark. 0.2 mL of 1,5-DPC solution was then added to yield a purple colour. UV/Vis Spectrophotometer (WPA Lightwave II) from Labotech, South Africa was used for measuring the absorbance of the mixture at 540 nm wavelength, across a 10 mm light path. The intensity of the purple colour was proportional to the Cr(VI) concentration in the sample (APHA/AWWA/WEF, 2012).

3.7.2 Total Cr measurement

Varian AA - 1275 Series Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, CA (USA)) equipped with a 3 mA chromium hollow cathode lamp was used to measure the total Cr at a wavelength of 359.9 nm. The AAS was calibrated before total Cr analysis using 1-5 mg/L Cr(VI) concentration prepared from the Cr(VI) stock solution.

3.7.3 Cr(III) measurement

Cr(III) was determined as the difference between total Cr and Cr(VI) concentration.

3.7.4 pH and Temperature

pH and temperature were measured using a PL-700AL bench top multi-parameters meter.

Chapter 4 EVALUATION OF Cr(VI) REDUCTION USING INDIGENOUS BACTERIUM CONSORTIA ISOLATED FROM A MUNICIPAL WASTEWATER SLUDGE IN BATCH SYSTEM

4.1 INTRODUCTION

Chromium (Cr) and its compounds have been extensively used in many industrial processes, such as metal finishing, metal electroplating, steelworks manufacturing, wood preservation, leather tanning, textile dyeing, and synthesis of pigments (Dhal et al., 2013; Fernandez et al., 2018). As a result of this wide anthropogenic use of Cr, large quantities of Cr containing wastes have been produced and the lack of effective disposal methods of Cr effluents has led to the contamination of surface and groundwater environments, soils and aquatic sediments (Molokwane et al., 2008). Another major concern is that these high Cr(VI) effluents end up in municipal sewer lines and build up in the sludge because only a small quantity is discharged with the wastewater final effluent (Khakbaz et al., 2020). The application of municipal sludge in agricultural soils poses health risk threats. Cr is mainly present in the environment in two oxidation states trivalent [Cr(III)] and hexavalent [Cr(VI)] species (Troiano et al., 2013). Cr(VI) has been recognized as more hazardous due to its higher solubility, mobility, rapid permeability, and strong oxidizing ability to exert harmful effects on biological systems (Khambhaty et al., 2009; Kumar & Dwivedi, 2019). Consequently, USEPA has set an allowable limit for Cr(VI) in domestic water at 0.05 mg/L and 0.01 mg/L for aquatic life (Murugavelh & Mohanty, 2012). In comparison to Cr(VI), Cr(III) is an important microelement for sustaining human metabolism and homeostasis. It is less toxic

and readily forms highly insoluble hydroxide/oxides in the environment at pH values higher than 5.5 (Villacís-García et al., 2015; Gong et al., 2017; Fernandez et al., 2018). Thus, the reduction of Cr(VI) to relatively non-hazardous Cr(III) is an effective strategy to mitigate the risks to human health and the environment.

There are various conventional technologies available for minimizing the environmental impact of Cr(VI) and these include chemical reduction, ion exchange, electrochemical treatment, membrane separation (Ji et al., 2015; Li et al., 2017; Ma et al., 2018). However, most of these technologies are often ineffective and very expensive, especially for low concentrations of metals (Cheng et al., 2011). Additionally, the use of chemical reagents produces an enormous amount of hazardous sludge that requires further treatment (Mtimunye & Chirwa, 2014). Therefore, it is essential to develop an innovative, -cost-effective, and environmentally friendly alternative process to remediate the Cr(VI) contamination.

Bioreduction of toxic Cr(VI) to less toxic Cr(III) using microbial organisms is considered as a valuable, promising, and cost-effective approach for Cr(VI) remediation. The first case of microbial reduction of Cr(VI) was reported in the late 1970s by Romanenko & Koren'Kov (1977) where isolated *Pseudomonas strain* was tested for Cr(VI) reduction. Since then, numerous scholars have isolated new Cr(VI) reducing microbial strains under different conditions, such as *Bacillus* (Zheng et al., 2015; Zhu et al., 2019; Tan et al., 2020), *Pseudomonas* (Sathishkumar et al., 2017; Wani et al., 2019), *Microbacterium* (Kumar & Saini, 2019), *Desulfovibrio* (Elahi et al., 2019), *Enterobacter* (Mbonambi & Chirwa, 2019; Sun et al., 2020), *Halomonas* (Murugavelh & Mohanty, 2018), and *Escherichia* (Mohamed et al., 2020). Various environments such as industrial landfills, waste disposal sites, coal

mines, tannery effluents and contaminated sediments from rivers have been identified as main target areas to isolate these potential strains for in situ bioremediation.

Y. G. Liu et al. (2006) studied *Bacillus sp*. isolated from Cr landfill site for the reduction of Cr(VI) at an initial Cr(VI) concentration of 80 mg/L and observed a maximum of 81.5% reduction of Cr(VI) in 72 h. Banerjee et al. (2019) isolated the *Bacillus cereus strain* from an open-cast coalmine which completely reduced 200 mg/L Cr(VI) concentration within 16 h under heterotrophic conditions. Wani et al. (2019) isolated *Pseudomonas* species from Cr(VI) contaminated alloy manufacturing effluent and evaluated its Cr(VI) reduction performance. They observed a maximum Cr(VI) reduction of 86% at 100 mg/L under neutral pH conditions and 120 h incubation time. Li et al. (2019) studied the treatment of high-concentration chromium-containing wastewater using sulphate-reducing bacteria acclimated with ethanol under various conditions. Their results showed that the strain was capable of reducing Cr(VI) concentration up to 500 mg/L under the optimum pH value of 7, the temperature of 35 ºC, incubation time of 24 h, and the volume amount of chromium-containing wastewater to bacteria was 5:1. The distinctions in reduction capacities of bacterial strains are avowed to be directly dependent on the physicochemical parameters and heavy metal concentration in various environmental conditions. Consequently, exploration for native bacterial systems for in-situ bioremediation of that specific polluted location is always valuable.

This work aims to isolate and investigate the Cr(VI) reduction by a consortium bacterium from a sludge coming from a municipal wastewater treatment in Brits, South Africa which receives high periodic loads of hexavalent chromium from a chrome foundry nearby. Our previous study by Molokwane et al. (2008) showed that sludge bacteria from the Brits plant

had high Cr(VI) reduction capacity. The current study assesses how the Cr(VI) initial concentration, initial pH solution, and co-existing heavy metals affect the removal of Cr(VI) by consortia bacteria from the wastewater treatment plant. Furthermore, bacterium Cr(VI) reduction kinetics were also studied. This is an effort to expand the development of the bioremediation technique for Cr(VI) treatment of polluted sites in South Africa. South Africa holds the largest chrome ore reserves in the world and it is one of the largest producers of ferrochrome (Van Der Lingen & Paton, 2018).

4.2 BACTERIA SCREENING FOR Cr(VI) REDUCTION

The three sludge samples collected from different locations within the wastewater treatment plant were used as a source of indigenous microbial consortia and were screened and examined for their ability to reduce Cr(VI). Cr(VI) reducing ability was tested for each microbial consortia at Cr(VI) concentrations ranging from 100 mg/L to 500 mg/L. These experiments were carried out in LB broth supplemented with Cr(VI). All three microbial consortia showed good Cr(VI) reducing capability, as shown in Figure 4-1. It can be seen that complete Cr(VI) reduction of 100 mg/L Cr(VI) initial concentration was achieved by all the microbial consortia. However, as the initial Cr(VI) concentration was increased, Cr(VI) reduction decreased accordingly, and all the microbial consortia only managed a reduction of less than 7 % at the highest initial Cr(VI) concentration of 500 mg/L. The loss of Cr(VI) reduction capacity by the microbial consortia was due to Cr(VI) inhibition. Sludge C microbial consortia exhibited more Cr(VI) reducing power than sludge A and B microbial consortia at higher concentrations. This was due to better acclimation and longer exposure to Cr(VI), and sludge C which is a combination of sludge A and B. Therefore, microbial consortia from sludge C was used for further studies.

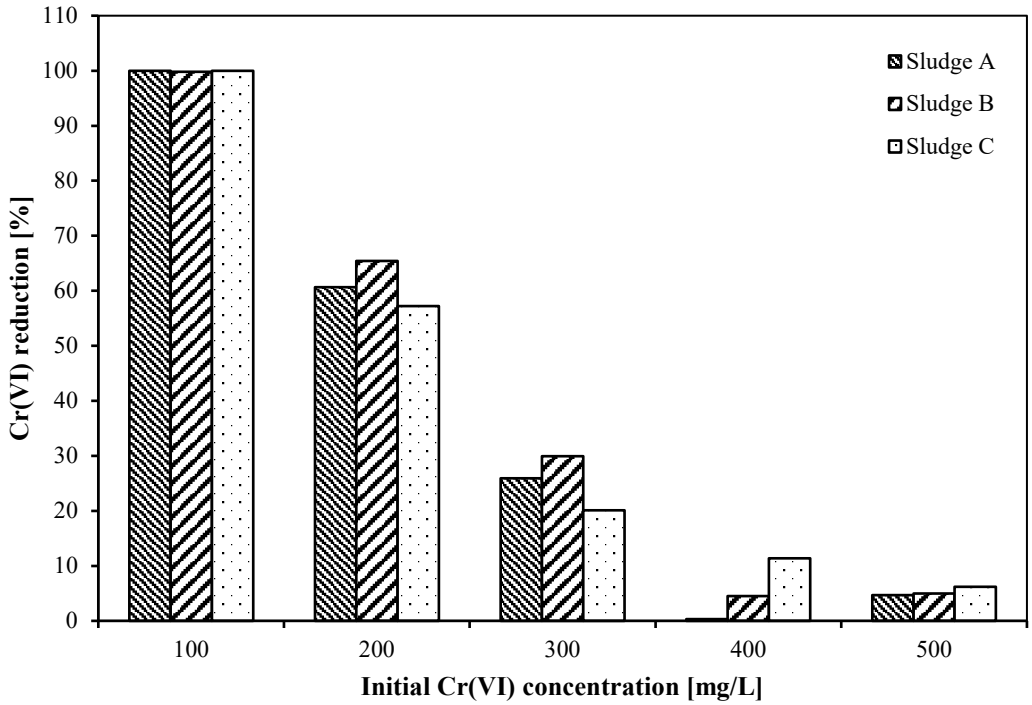


Figure 4-1: Percentage Cr(VI) reduction in consortia cultures from different sources (primary sludge, activated sludge, and dry sludge) under varying initial Cr(VI) concentrations incubated for 24 hours.

4.3 ABIOTIC CONTROLS

To examine the abiotic reduction of Cr(VI) by heat-killed and azide-inhibited cells, experiments were conducted using Cr(VI) concentration of 100 mg/L. The cells were killed by autoclaving at 121°C for 120 minutes. In these experiments, the extent of Cr(VI) removal was evaluated from the changes in Cr(VI) concentration in the aqueous phase. Figure 4-2 illustrates the extent of Cr(VI) reduction in a batch system containing cells-free, heat-killed and azide-inhibited cells. After 24 h of operation time, it was observed that only 4 %, 10 %, and 17 % of Cr(VI) was removed without cells, heat-killed and azide inhibited cells respectively. The low Cr(VI) reduction with heat-killed cells was due to the inactivation of the cells by heat. The 10 % Cr(VI) removed by heat-killed cells was due to the existence of

live cells that survived heat destruction or sorption. Cells inhibited with azide, also showed low Cr(VI) reduction capability due to cells inactivation under oxygen stressed environment. These results indicate that Cr(VI) reduction by live cells was not due to abiotic factors.

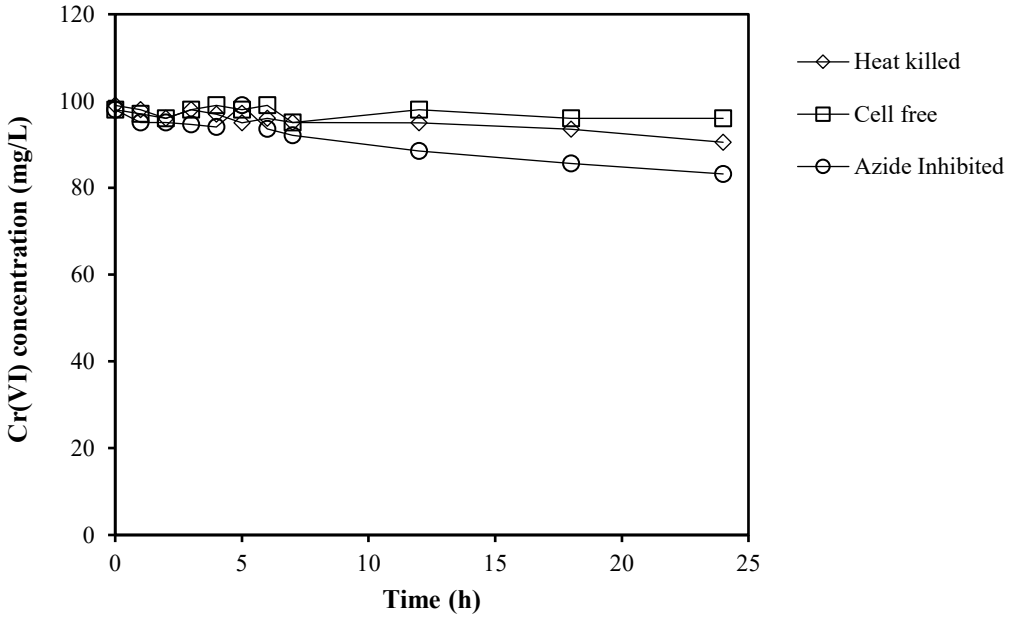


Figure 4-2: **Evaluation of abiotic reduction of Cr(VI) by heat-killed and azide inhibited reducing cells under aerobic conditions**

4.4 EFFECT OF Cr(VI) CONCENTRATION

The effect of initial Cr(VI) concentration on Cr(VI) reduction was studied over a range of 50 – 400 mg/L at a constant pH and temperature of 7.2 and 37 °C under aerobic conditions. As shown in Figure 4-3, the bacteria consortia could completely reduce Cr(VI) concentration of 50 mg/L within 5 h of incubation. As Cr(VI) initial concentration increased it took longer for the bacteria to completely reduce Cr(VI) as 100 mg/L, 150 mg/L and 200 mg/L were reduced in 18 h, 72 h, and 96 h respectively. However, above 200 mg/L Cr(VI) initial concentration complete Cr(VI) reduction was not observed as it can be seen that 300 mg/L and 400 mg/L concentrations were reduced by 92 % and 68 % after 120 h when the

experiment was terminated. The slow reduction capabilities at high concentrations can be ascribed to the Cr(VI) reduction bacteria reaching the Cr(VI) toxicity level. Molokwane et al. (2008) and Wang & Shen (1997) showed that the loss of Cr(VI) reduction capacities by bacteria are due to the loss of cell viability at high Cr(VI) concentrations. These results show that Cr(VI) toxicity does affect Cr(VI) reduction by microorganisms significantly.

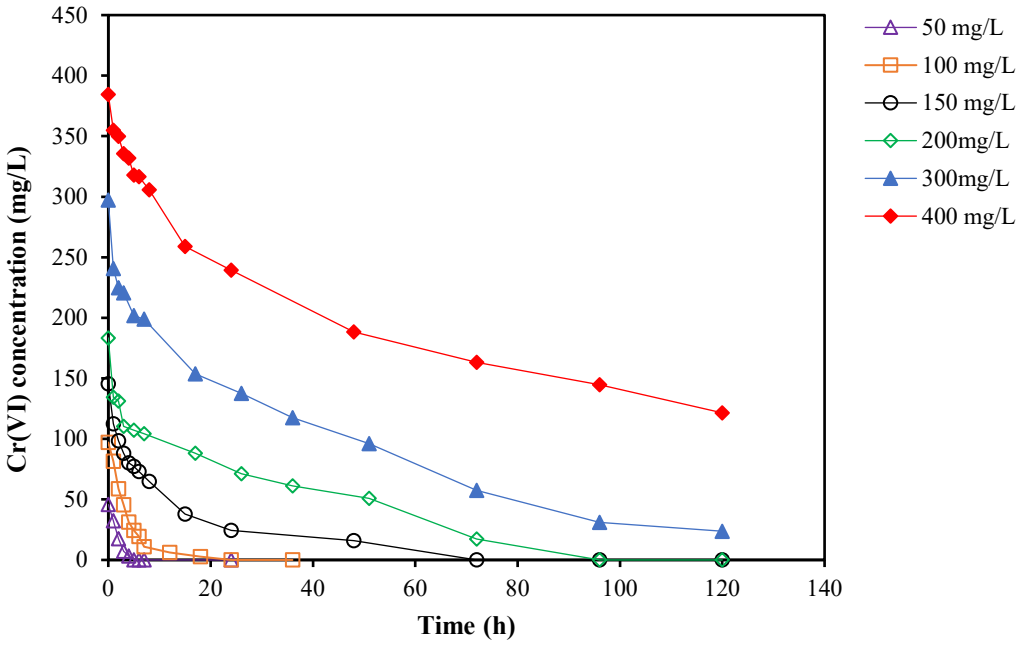


Figure 4-3: Effect of initial Cr(VI) concentration on Cr(VI) reduction using indigenous consortia bacteria

4.5 BACTERIA PERFORMANCE AT DIFFERENT Cr(VI) CONCENTRATION

The specific Cr(VI) reduction rates determined after 5 h and the overall specific reduction at varying initial Cr(VI) concentrations are given in Figure 4-4(a) and (b). The specific Cr(VI) reduction rate is defined as a measure of Cr(VI) reduction per unit mass of biomass per hour. It can be seen that the specific Cr(VI) reduction rate after 5 h decreased with increasing

initial Cr(VI) concentration reaching a minimum of 0.00043 mg Cr(VI)/mg biomass h at 150 mg/L, and remained constant at Cr(VI) concentration higher than 150 mg/L. However, the overall specific Cr(VI) reduction rate increased with increasing initial Cr(VI) concentration until a peak was reached at 300 mg/L and a further increase in Cr(VI) concentration to 400 mg/L resulted in a decrease in the overall specific rate, suggesting a possible Cr(VI) inhibition. These results show that the Cr(VI) reduction process is catalysed by microbial consortia saturation kinetics. Similar kinetic patterns have been reported for *Bacillus* strain (Elangovan et al., 2006); *Hypocrea tawa* strain (Morales-Barrera & Cristiani-Urbina, 2008); *Shewanella oneidensis* MR-1 (Middleton et al., 2003); however, in these studies the overall specific Cr(VI) reduction rate was determined in terms of the protein concentration and particulate organic carbon. Zakaria et al. (2007) and Jeyasingh & Philip (2005) also indicated that even though they did not observed complete Cr(VI) reduction, initial specific reduction rate increased with Cr(VI) concentration.

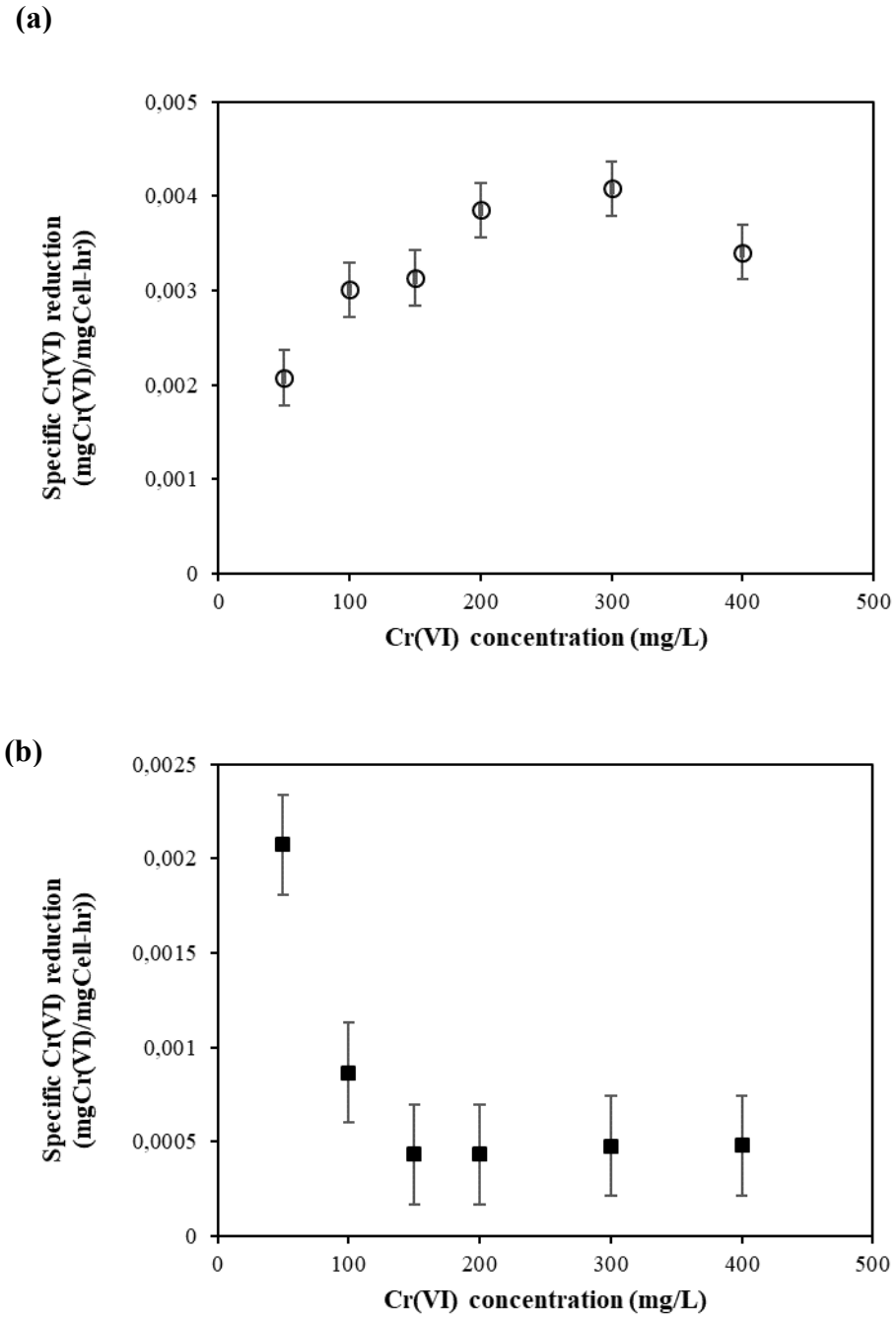


Figure 4-4: **Specific Cr(VI) reduction at varying Cr(VI) concentration (a) after 5 h (b) after the duration of the experiment**

4.6 EFFECT OF pH ON Cr(VI) REDUCTION

The pH of the solution is an important parameter, it can affect the activity of the bacteria, the degree of enzyme ionization and the accessibility of heavy metal ions (Tan et al., 2020; Karthika et al., 2016; Mangaiyarkarasi et al., 2011). Hence, the microbial removal of heavy metals efficiency is affected. The Cr(VI) residual concentration by bacteria consortia results at different pH levels are presented in Figure 4-5. Cr(VI) reduction by consortia bacterium was studied over a range of 2 – 11 initial pH levels in MSM medium amended with 50 mg/L Cr(VI) and incubated at 37 °C under aerobic conditions. As the initial pH increased, Cr(VI) residuals showed a decrease from the pH of 2 to 7, followed by an increasing Cr(VI) residuals from a pH of 7 to 10. The consortia showed an enhanced removal efficiency at neutral pH and near complete was observed within 5 h. Acidic and alkaline conditions severely inhibited Cr(VI) reduction by the indigenous bacteria consortia from wastewater sludge. These results highlight that Cr(VI) removal by indigenous bacteria consortia was higher in neutral to acidic conditions as compared to alkaline conditions. The widespread pH adaptability and efficient Cr(VI) removal ability under neutral–acidic conditions suggested that indigenous bacteria consortia could play a significant role in the bioremediation of acidic Cr polluted sites.

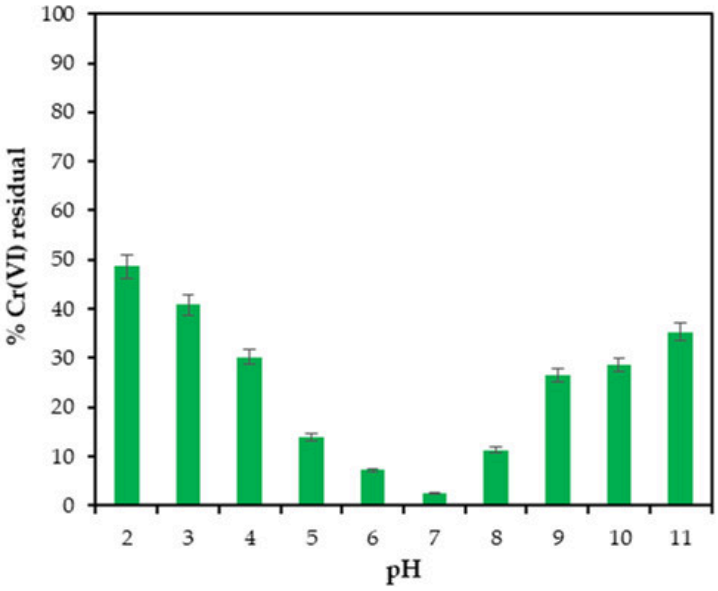


Figure 4-5: Effect of pH medium used for Cr(VI) reduction by indigenous bacteria consortia from wastewater sludge

4.7 EFFECTS OF COEXISTING HEAVY METALS ON Cr(VI) REDUCTION

Contaminated groundwater, soils and industrial wastewater usually contain other heavy metals and the presence of these co-existing heavy metal ions may have an effect on the Cr(VI) reduction by microorganisms. Thus, the effect of co-existing heavy metals on Cr(VI) reduction by mixed bacteria consortium was studied in this work (Figure 4-6). The influence of heavy metals on Cr(VI) reduction was studied using five individual metals (Ni^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Pb^{2+}) at 5 and 50 mg/L concentrations and Cr(VI) concentration was fixed at 50 mg/L. The microbial consortia completely reduced Cr(VI) within 5 h in the absence of other heavy metals. The presence of 5 mg/L of Ni^{2+}, Mn^{2+} and Pb^{2+} had no effect on Cr(VI) reduction, however, an enhanced reduction rate was observed with Cu^{2+} and Zn^{2+} as Cr(VI) reduction was complete within 2 h and 3 h respectively. However, enhanced Cr(VI) reduction was achieved in the presence of Cu^{2+} and Zn^{2+} with completely Cr(VI) reduction

being observed within the first hour of incubation, while with Zn^{2+} was achieved in 3 h at 50 mg/L metal concentration. Several researchers have reported the induced Cr(VI) reduction by microbial organisms in the presence of Cu^{2+}, including *Bacillus sp. CRB-B1 strain*, *Bacillus* strain TCL, *Acinetobacter haemolyticus* (Zakaria et al., 2007; Banerjee et al., 2019; Tan et al., 2020). The cause of the stimulating effect of Cu^{2+} and other metals on Cr(VI) reduction by microbial organisms is not yet clear. According to Tan et al. (2020) and Huang et al. (2021), the increase in Cr(VI) reduction caused by Cu^{2+} is due to the fact that it is one of the essential components of some antioxidizing agents such as superoxide dismutase and catalase. Moreover, it acts as an electron transporter for the oxidative respiratory system (Xu et al., 2015). At a higher heavy metal concentration of 50 mg/L, significant inhibition of Cr(VI) reduction was observed in the presence of Ni^{2+} and Pb^{2+} with 81 % and 43 % Cr(VI) reduction being achieved, whereas Mn^{2+} had no effect. Similar results were reported by Bhattacharya and Gupta (2013). The presence of Ni^{2+} and Pb^{2+} significantly inhibited chromate reduction by *Acinetobacter sp. B9*. The inhibition of Cr(VI) removal by some heavy metals at elevated concentrations may be due to the suppression of microbial activity by metal toxicity and the destruction of protein structures by heavy metals (Cheng et al., 2011).

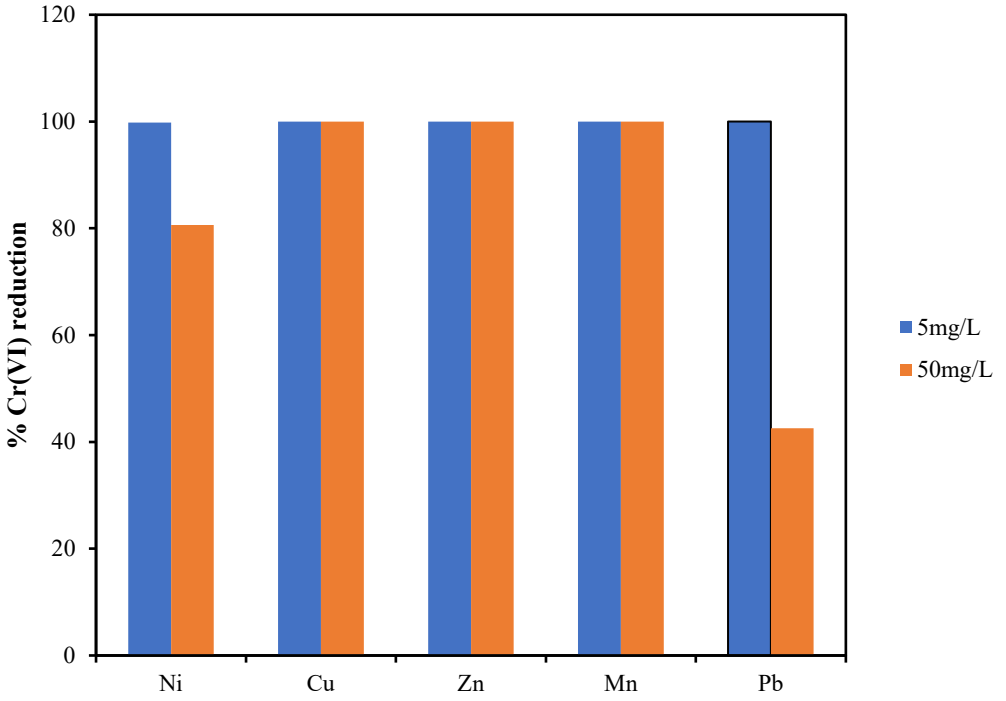


Figure 4-6: The effect of 5 and 50 mg/L of various heavy metals on reduction of 50 mg/L Cr(VI) by wastewater sludge bacteria consortia

4.1 KINETICS OF Cr(VI) REDUCTION BY BACTERIA CONSORTIA

To quantitatively determine the interaction between the reduction rate and time for Cr(VI) removal by bacterium consortia coming from a wastewater treatment plant, the kinetics for Cr(VI) reduction were conducted. The kinetics of the Cr(VI) bioreduction at varying initial Cr(VI) concentrations were studied by a first and second-order exponential decay (Bhattacharya & Gupta, 2013; Li et al., 2017; Xu et al., 2015; Huang et al., 2019; Zhu et al., 2019).

k_1 was determined as the slope from plotting $\ln([Cr(VI)]/[Cr(VI)_0])$ versus time. The estimated k_1 values and their coefficient of determination R^2 values resulting from linear regression are given in Table 4-1. As shown in Figure 4-7(a) and Table 4-1, the fitting R^2 of the initial concentration of 50 mg/L, 100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L were

0.96, 0.93, 0.82, 0.94, and 0.86 respectively, indicating that the first-order exponential decay described well the reduction process of Cr(VI) over time.The k_1 was found to decrease (0.615 h^{-1} to 0.011 h^{-1}) with increasing Cr(VI) concentration from 50 mg/L to 400 mg/L. Similar results to this study have been reported by Das et al. (2014) and Tan et al. (2020), however, their Cr(VI) reduction rate values were less by two orders of magnitude. This was due to the differences in experimental conditions such as Cr(VI) concentrations, reduction medium, and bacterial strains. Cr(VI) reducing bacterial strains have different reduction capabilities due to the use of dissimilar mechanisms.

The second-order rate constant k_2 was determined as the slope from plotting $1/[Cr(VI) - 1/[Cr(VI)_0]$ versus time. The second-order rate constants k_2 and their coefficient of determination R^2 values resulting from linear regression are given in Table 4-1. The fitting R^2 of different initial concentration 100 mg/L, 150 mg/L, 200 mg/L, 300 mg/L and 400 mg/L were 0.85, 0.99, 0.79, 0.91, and 0.97 respectively, indicating that the second-order exponential decay described well the reduction process of Cr(VI) over time as illustrated in Figure 4-7(b). However, the R^2 for 50 mg/L Cr(VI) concentration using the second-order was less than that of the first-order exponential model.

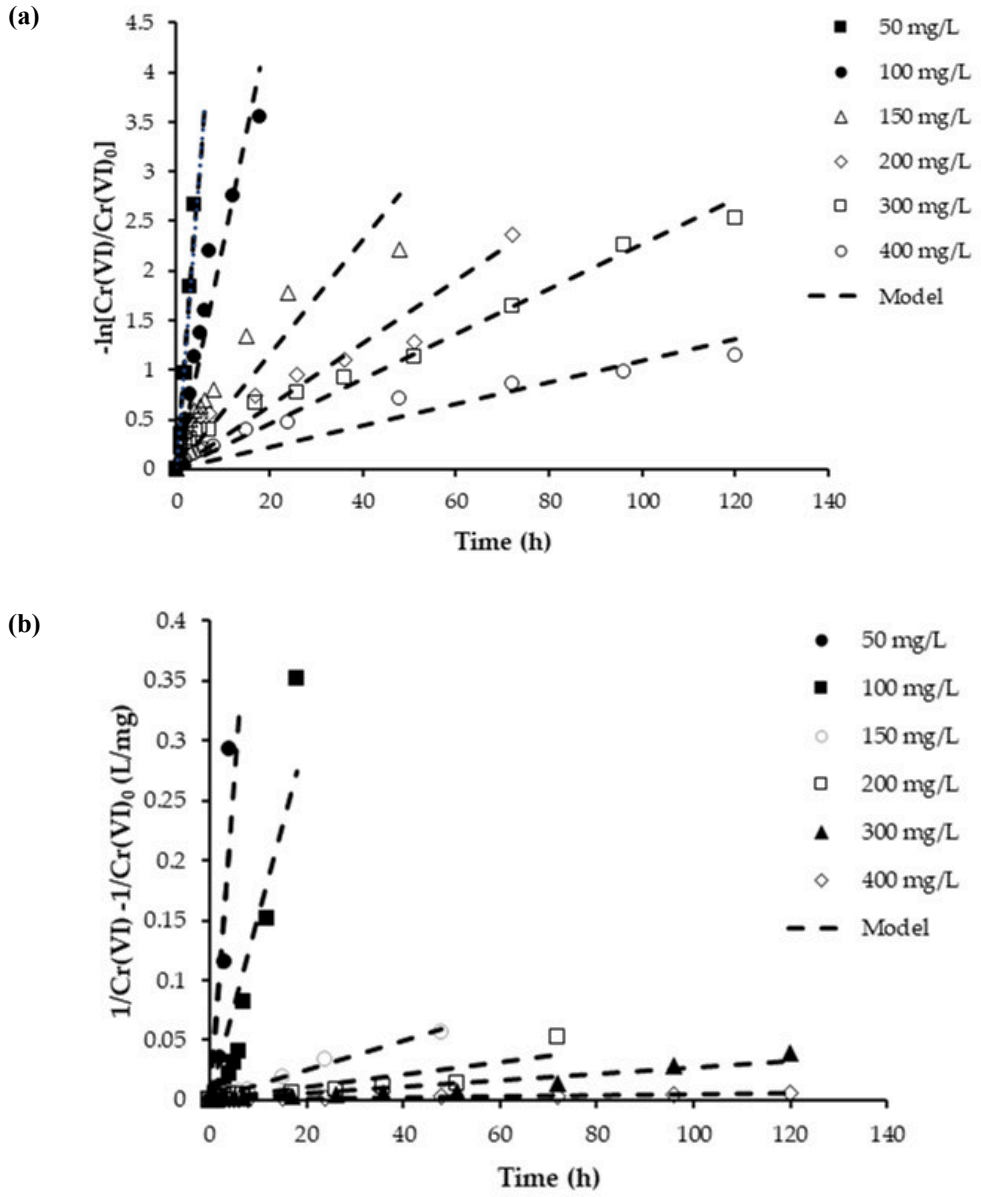


Figure 4-7: The kinetics of Cr(VI) reduction by bacterial consortia at different Cr(VI) initial concentration (a) pseudo-first-order kinetics (b) second-order kinetics

The k_2 values followed a similar trend as k_1 and were found to be decreasing from 0.0532 – $5x10^{-5}$ $L.mg^{-1}$. h^{-1} with increasing Cr(VI) concentration from 50 mg/L to 400 mg/L. Even though the overall Cr(VI) reduction rate declined with increasing Cr(VI) initial concentration, the total amount of Cr(VI) removed at higher initial concentrations was greater after 120 h.

Table 4-1: First and second order kinetic of Cr(VI) reduction by bacterial consortia and their correlation coefficient

Cr(VI) concentration	pseudo-first order		pseudo-second order	
	k_1	R^2	k_2	R^2
50	0.615	0.96	0.0532	0.74
100	0.225	0.93	0.0152	0.85
150	0.056	0.69	0.0012	0.99
200	0.032	0.82	0.0005	0.79
300	0.023	0.94	0.0003	0.91
400	0.011	0.86	0.00005	0.97

4.2 MICROBIAL CHARACTERISATION

The sludge C bacteria consortium was chosen for characterization due to its high performance. The bacterial isolates were identified based on 16S rDNA gene sequencing analyses and were carried out by the Microbiology Department, at the University of Pretoria to identify bacterial communities present after the sludge had been exposed to 100 mg/L Cr(VI). BLASTN analysis of the bacterial isolates X1, X2, X3, X4, X5, X6 and X7 are presented in Table 4-2 and shows four predominant species under aerobic conditions. The sequence for X1 was 99% similar to that of *Bacillus cereus* 213 16S and *Bacillus thuringiensis* strains. X2 and X3 isolates produced similar results and showed close association with *B. cereus* ATCC 10987, *Bacillus sp*. ZZ2 16s, *B. thuringiensis* str. *Al Hakam* having a 99% identity. While X4, X5 and X6 were in close association with *B.*

mycoides strain BGSC 6A13 16S, *B. thuringiensis serovar finitimus* strain BGSC 4B2 16S strains. The sequence for X7 was 99% similar to that of *Microbacterium sp.* S15-M4 and *Microbacterium foliorum*. A phylogenetic tree was constructed for the species from purified cultures grown under aerobic conditions based on a basic BLAST search of rRNA sequences in the NCBI database (Figure 4-8).

Table 4-2: Sludge Cr(VI)-Reducing Bacteria strain characterisation using 16S rRNA

Blast results	Pure Isolates							ID index
	X1	X2	X3	X4	X5	X6	X7	
B. cereus ATCC 10987		√	√					0.99
B. thuringiensis serovar finitimus strain BGSC 4B2 16S				√	√	√		0.99
B. thuringiensis str. Al Hakam		√	√					0.99
Bacillus cereus strain 213 16S	√							0.99
Bacillus mycoides strain BGSC 6A13 16S				√	√	√		0.99
Bacillus sp. ZZ2 16S		√	√					0.99
Bacillus thuringiensis 16S	√							0.99
Microbacterium foliorum							√	0.99
Microbacterium sp. S15-M4							√	0.99

Bacteria that are able to reduce and tolerate Cr(VI) have been reported by many researchers and mostly these microorganisms are from the chromium-contaminated sites. Soni et al. (2013) isolated four bacteria strains from soil irrigated with tannery wastewater which were

Bacillus sp., Microbacterium sp., Bacillus thuringiensis, and *Bacillus subtilis* and all were able to reduce Cr(VI) at varying concentrations. Upadhyay et al. (2017) reported that *Bacillus sp.* MNU16 isolated from coal contaminated mine was able to tolerate and reduce Cr(VI). Banerjee et al. (2019) also showed that *Bacillus cereus* MBGIPS 9 from coal mine lake has a high tolerance to Cr(VI) toxicity and reduction capacity.

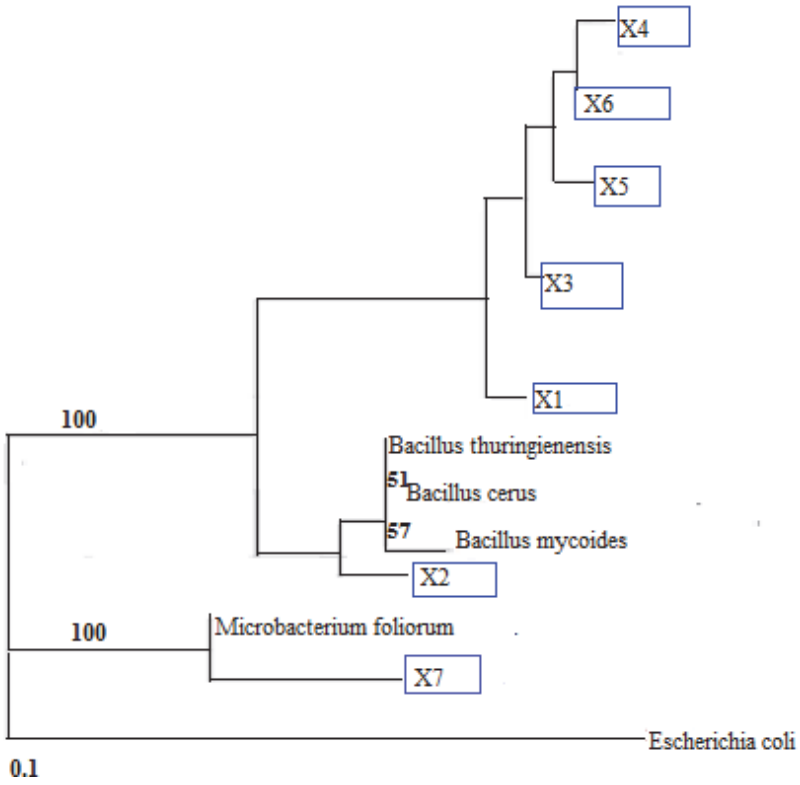


Figure 4-8: Phylogenetic tree constructed by neighbour-joining algorithm based on the partial 16S rRNA gene sequences and 1000 bootstrap replicates, showing the microbial diversity of Cr(VI) reducing consortium from Sludge C under aerobic conditions.

4.3 SUMMARY

In this study, aerobic microbial mixed culture isolated from a municipal wastewater treatment sludge was capable of reducing Cr(VI) at concentrations, up to 400 mg/L. It was

shown that Cr(VI) reduction was an enzyme-mediated process instead of adsorption. Complete Cr(VI) reduction of 50 mg/L concentration was observed in 6 h under aerobic and neutral pH conditions. The Cr(VI) reduction rate decreases with increasing initial Cr(VI) concentration due to Cr(VI) toxicity on bacterial cells. It was observed that Cr(VI) reduction increases with increasing the initial pH of the solution until the optimal pH of 7, a further increase in pH results in decreased Cr(VI) removal. Co-existing heavy metals did not have an effect on Cr(VI) reduction at both low and high heavy metal concentrations with exception of Cu^{2+} and Zn^{2+} which enhanced Cr(VI) reduction, while Pb^{2+} and Ni^{2+} showed inhibitory effects at high concentrations. The fitting of time course data to a first-order rate resulted in a rate constant in the range of 0.615 h^{-1} to 0.011 h^{-1} which decreased with increasing Cr(VI) concentration from 50 mg/L to 400 mg/L. Similarly, the second order rate constant was in the range of 0.0532 – $5x10^{-5}$ $L^{-1}.mg^{-1}.h$ and decreased with increasing initial concentration. The reduction ability of mixed bacterial consortium to treat Cr(VI) may be explored further for practical application and developing a sustainable bioremediation process for Cr(VI) contaminated areas. This is an effort to expand the development of bioremediation technique for Cr(VI) treatment of polluted sites in South Africa.

Chapter 5 PERFORMANCE AND MICROBIAL CULTURE SHIFT OF A BENCH-SCALE BIOLOGICAL PERMEABLE REACTIVE BARRIER FOR IN-SITU REMEDIATION OF Cr(VI)-CONTAMINATED GROUNDWATER

5.1 INTRODUCTION

Hexavalent chromium [Cr(VI)] pollution of soil and groundwater has become an environmental and public health problem across the globe (Jobby et al., 2018). It largely originates from many industrial applications, such as metal finishing, metal electroplating, steelworks manufacturing, wood preservation, leather tanning, textile dyeing, and synthesis of pigments (Zhao et al., 2018; Gong et al., 2018; Molokwane et al., 2008; Kholisa et al., 2021). The lack of proper storage and effective disposal methods of Cr(VI) effluents has also intensified the Cr(VI) contamination (Li et al., 2019; Qian et al., 2014). Cr(VI) is known to be soluble and mobile and it easily infiltrates into groundwater, through which it can end up into surface waterbodies (streams, rivers, lakes) and adversely affect ecosystems. Therefore, USEPA has set Cr(VI) concentration nominal limit in drinking water to 0.05 mg/L, and 0.01 mg/L for aquatic life (Murugavelh & Mohanty, 2018; Gong et al., 2017). In contrast, trivalent chromium Cr(III) is much less mobile and non-toxic and is an essential nutrient at low concentrations (Yin et al., 2017). Thus, remediation of Cr(VI) contaminated soil and groundwater is a necessity to avert the migration of Cr(VI) to further pollute a larger area and endanger the ecosystem and human health.

Conventional methods for removing, immobilising Cr(VI) and transforming Cr(VI) to Cr(III) are applied and these include membrane filtration, ion exchange, chemical precipitation, adsorption, chemical oxidation and reduction (Huang et al., 2021; Tan et al., 2020). The high cost and energy requirements, excessive chemical consumption and generation of large amounts of toxic sludge which require further treatment, are some of the disadvantages (Frade et al., 2018). Although processes such as ion exchange and adsorption possess low costs, when the adsorbent materials are deposited in landfills, desorption usually occurs resulting in soil and groundwater contamination again (Mbonambi et al., 2019; Yao et al., 2020). Therefore, it is essential to develop an innovative, cost-effective, and environmentally friendly alternative process to remediate Cr(VI) contamination.

The bioreduction of toxic Cr(VI) to less toxic Cr(III) using microbial organisms isolated from Cr(VI) contaminated sites is considered as a valuable, promising, and cost-effective approach for Cr(VI) remediation (Tan et al., 2020; Kholisa et al., 2021; Zeng et al., 2019). The advantage of such systems is that they can be operated with insignificant chemical by-products and require less energy inputs. The process may employ native, non-invasive strains of bacteria, thereby offsetting the environmental concerns over the possible introduction of alien species with possible unforeseeable detrimental effects to the native environment. Although many bacterial strains have been shown to mediate the reduction of Cr(VI) to Cr(III), few studies have examined the potential of in situ treatment of Cr(VI) using microorganisms.

Huang et al. (2021) studied the performances of Cr(VI) removal by *Sporosarcina saromensis* W5 attached to activated carbon or zero-valent iron as a bio-permeable reactive barrier. Enhanced Cr(VI) reduction performance was observed in both experiments. Murugavelh and

Mohanty (2018) evaluated the performance of *Halomonas* sp. to reduce Cr(VI) in a fixed film bioreactor. The reactor was operated under continuous flow and near complete Cr(VI) reduction was observed for 10 and 20 mg L^{-1} initial Cr(VI) concentration. Jeyasingh et al. (2011) evaluated a pilot scale feasibility of bioremediation of Cr(VI) contaminated aquifers using biobarrier and reactive zone technologies, using Cr(VI) reducing bacteria. Complete Cr(VI) reduction was obtained when the plume contained 50 mg/L Cr(VI) concentration and the biobarrier was 10 cm thick with an initial biomass concentration of 0.44mg/g of soil. Molokwane and Nkhalambayausi-Chirwa (2009) investigated microbial Cr(VI) reduction in groundwater aquifer media using a microcosm reactor. Near complete Cr(VI) removal was observed in the reactor while operating under a low hydraulic loading and Cr(VI) influent concentration of 40 mg/L.

In this study, the performance and microbial culture shift were assessed using a bench-scale biological permeable reactive barrier. The process simulates a microbial inoculated barrier system using sludge from sand-drying beds at a wastewater treatment plant (WWTP) receiving periodic loadings of Cr(VI) in the influent.

5.2 REACTOR PERFORMANCE

Cr(VI) removal from groundwater by biotic and abiotic BPRB was carried out by using horizontal rectangular reactors and the results are presented in Figure 5-1. Both reactors operated at hydraulic loading of 200 mL/h and were fed with distilled water for 14 days to saturate the reactors, remove air space between the pores and acclimatize the bacteria. The control reactor compartments were packed with sand quartz only to study the abiotic effect on the Cr(VI) removal and was fed with a Cr(VI) concentration of 40 mg/L as shown in

Figure 5-1(a). It can be seen that effluent Cr(VI) concentration in the control reactor gradually increased until day 6. After day 6, the reactor reached a steady state as the influent Cr(VI) concentration was the same as effluent. Pure quartz sand is not particularly relevant when referring to sorption regardless of the solution chemistry (Tadeo-jalife et al., 2021). Tang et al. (2021) pointed out that quartz sand is easily saturated and has low adsorption capacity.

In the BPRP reactor, compartments 2 and 4 were packed with quartz sand while compartment 3 was packed with 70% and 30% sand-sludge mixture. After the saturation phase which lasted 14 days, the BPRB reactor was fed with distilled water containing Cr(VI) concentration of 40 mg/L and the results are shown in Figure 5-1(b). The 20 cm barrier had a hydraulic retention time of 8 h. After feeding the reactor with Cr(VI) for 30 days, no Cr(VI) was detected in the effluent for this period. This indicated that the Cr(VI) removal was 100% in the BPRB. Cr(VI) concentration was then increased by 20 mg/L and continued to operate at 60 mg/L Cr(VI) concentration for another 30 days. Cr(VI) concentration was not detected in the effluent, showing 100% efficiency. On day 47, 4.11 mg/L of Cr(VI) concentration was first detected, and the following day (48) Cr(VI) was 100% removed. From day 52, a Cr(VI) concentration of 2.2 mg/L was noticed and a sharp increase in Cr(VI) concentration from 2.7 mg/L to 20.9 mg/L in the effluent from day 54 to day 57. Cr(VI) concentration in the effluent continued to increase up to 23.1 mg/L in day 61 which is equivalent to 38% Cr(VI) removal. This increase in Cr(VI) concentration in the effluent was attributed to the depletion of the carbon source from the sludge. Microorganisms utilize a variety of organic carbon sources, either as an energy source or as an electron donor to facilitate Cr(VI) bioreduction (Han et al., 2021). Han et al. (2021) further explained that organic carbon

sources play an important role in enhancing Cr(VI) bioreduction by the stimulation of microorganisms for providing more electron donors.

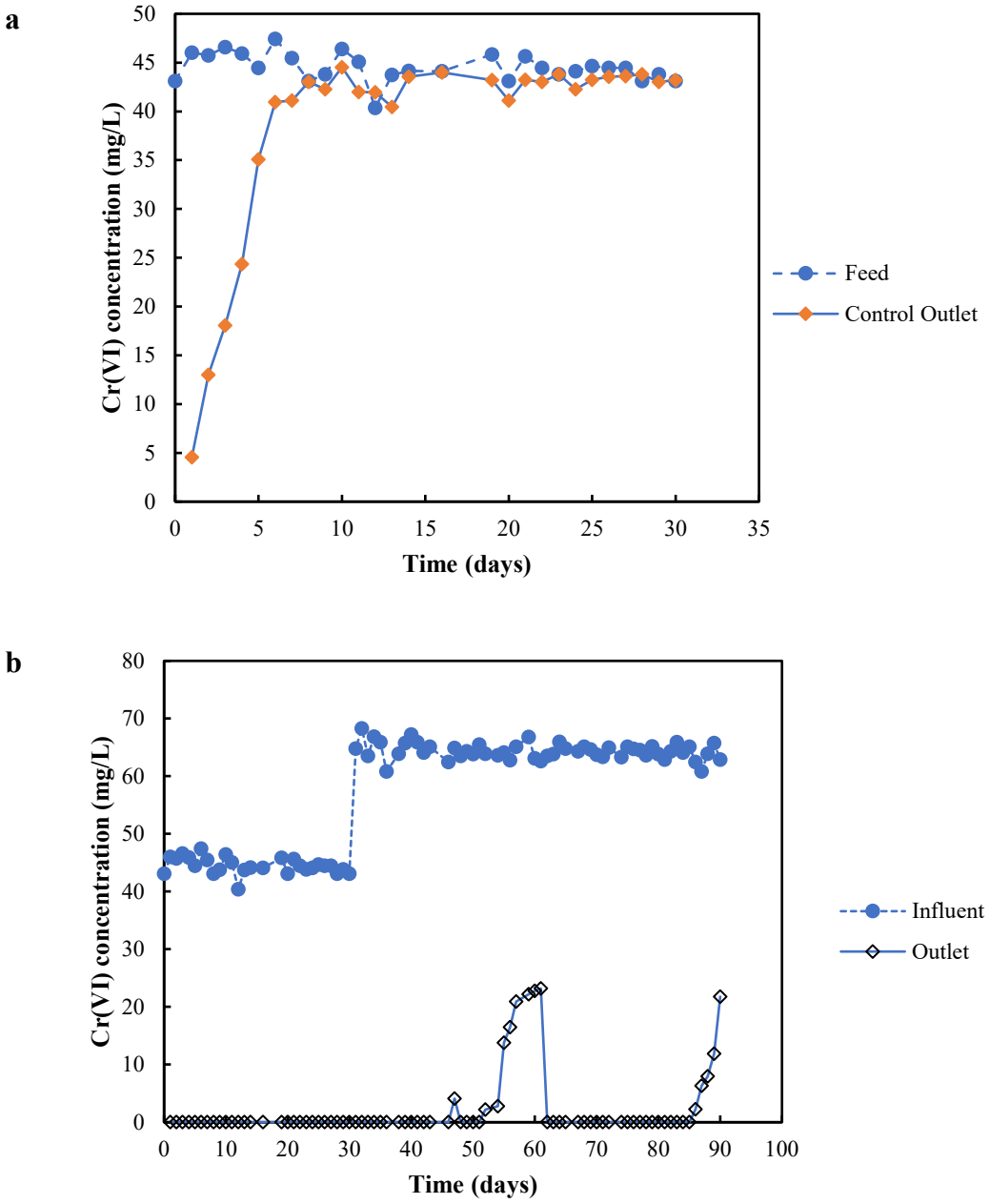


Figure 5-1: Cr(VI) removal of (a) abiotic and (b) biotic in the permeable reactive barrier system

Due to depletion of carbon source and low Cr(VI) reduction, a 5 g/L glucose was added to the reactor feed to provide microorganisms with carbon and energy. After, adding the glucose on day 61, the microorganisms completely reduce Cr(VI) as observed on day 62. Cr(VI) was not detected in the effluent until day 86, when Cr(VI) increased to 21.77 mg/L within 3 days. This increase in Cr(VI) concentration was attributed to low pH in the effluent. The overall performance of both reactors is summarised in Table 5-1. These results show that a BPRB technology studied here could perform well at concentrations as high as 60 mg/L. During the course of this study clogging of the reactor due to the biomass increase was not observed. The influent flow rate remained the same throughout the experiment and the fluid height in the reactor approximately remained stationary. However, Boni and Sbaffoni (2009) cautioned about the long-term behaviour and the durability of such a system, therefore, greater attention should be paid in terms of its hydraulic properties.

Table 5-1: Overall performance of BRPB and control reactors under various conditions

Reactor	Inlet concentration	Conditions	Effluent concentration	Removal efficiency	days of operation
Control	46.03 ± 1.41	Control	43.21	6.11	30
BRPB	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

5.3 ENVIRONMENTAL PARAMETERS

There are many parameters that can affect the performance of Cr(VI) reduction by microorganisms, temperature and pH being among the ones (Wani et al., 2019; Tan et al., 2020). For this reason, influent and effluent pH and temperatures were monitored throughout the entire duration of the study.

5.3.1 Time course of pH

The influent pH into the reactors ranged between 6.5 and 7.5 while the effluent pH of the BPRB reactor ranged from 5.2 to 7.5 and the control reactor ranged from 6.39 to 6.91 as shown in Figure 5-2: Time course of pH during Cr(VI) removal operation. The influent pH values were consistent with an average input value of 6.96 throughout the whole experiment. Similarly, the effluent pH values for both the control and BPRB reactors were stable for the first 30 days. Indicating no significant variation in the influent and effluents pH values. However, due to operational problems, the control reactor was discontinued after 31 days. The BPRB reactor continued to operate for another 30 days and during this time effluent pH was stable at 6.91. After 61 days of operation, the carbon source from the sludge was depleted and glucose was used as the sole carbon source. When glucose was used as the carbon source, a drastic decline in effluent pH from 6.91 to below 5.5 was observed within two days. The decrease in pH values was ascribed to the fermentation of glucose forming several types of organic acids by different *Bacillus* species and other bacterial species which result in a subsequent drop in medium pH (Upadhyay et al., 2017; Cherif-silini et al., 2013; Shukla et al., 2012; Sanghi & Srivastava, 2010). Also, oily scum and bubbles were observed on the top surface of the reactor, indicating that there was a production of weakly acidic

substances such as volatile fatty acids and gas (Rahman & Thomas, 2021; X. Zhang et al., 2020; Upadhyay et al., 2017; Sun et al., 2020). The influent pH range of 6.5 –7.5 through the PRB reactor, indicated that Cr(VI) mainly existed as CrO_4^{2-} and $HCrO_4^-$.

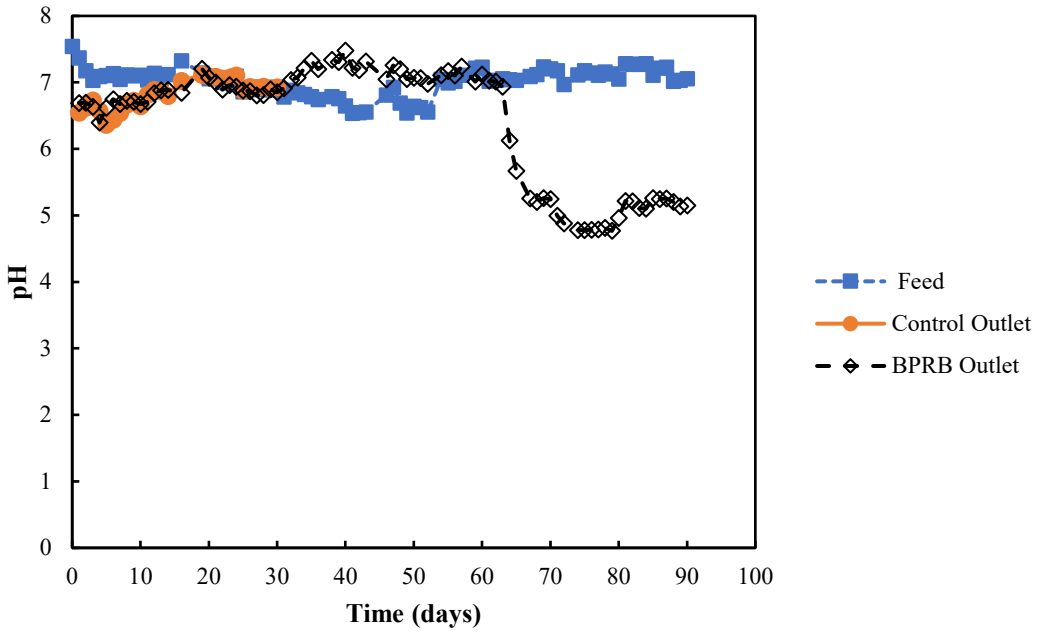


Figure 5-2: Time course of pH during Cr(VI) removal operation

5.3.2 Time course of Temperature

The time series plot for influent and effluent temperatures for both reactors is shown in Figure 5-3. The influent and effluent temperatures did not show significant variation during the course of this study. The influent temperature varied between 16.3 °C and 24.6 °C, while the effluent control varied between 21.4 °C and 27.4 °C for the 30 days of operation and the BPRB reactor effluent ranged between 21.3 °C to 29.7 °C. The near-surface groundwater temperature is typically between 0 °C and 30 °C depending on the location (Schweighofer et al., 2021). Temperatures above and/or below this range tend to affect negatively the

organisms living in the groundwater. Slight temperature changes can have a drastic effect on microbial activity and chemical reactions. However, the temperature fluctuations in this study did not have any significant impact on the microbial Cr(VI) reduction as 100% reduction was achieved with the exception of when the carbon source was depleted.

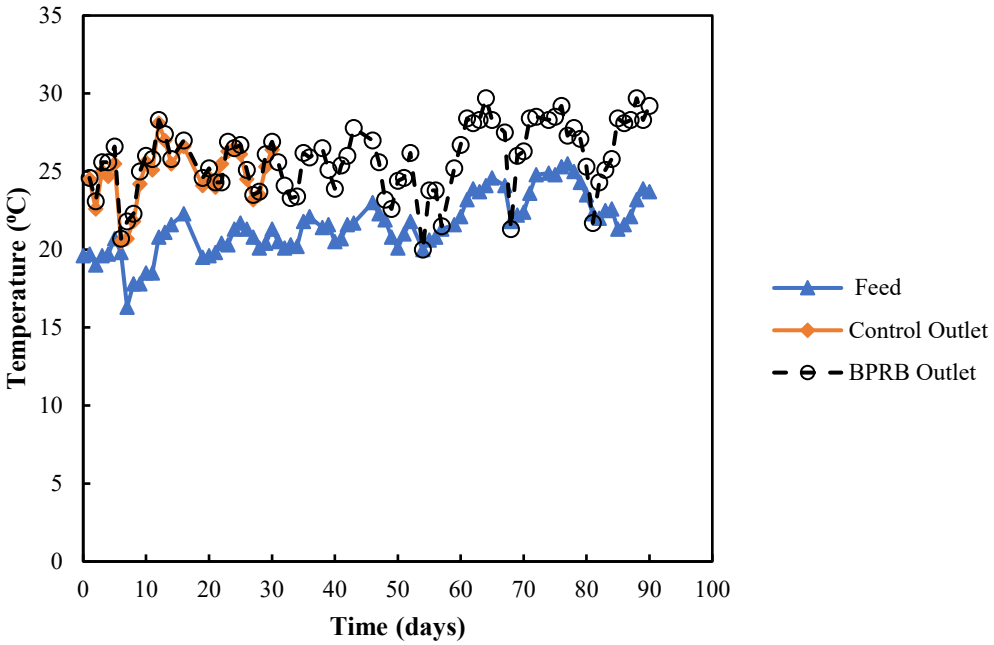


Figure 5-3: Time course of temperature variation during Cr(VI) removal operation

5.4 Cr(III) PRECIPITATION

Numerous studies have shown that Cr(VI) is reduced to Cr(III) by microbial cultures in batch systems (Kholisa et al., 2021; Ma et al., 2018; Zhu et al., 2019; Kumar & Dwivedi, 2019) and continuous systems (Zhang et al., 2020; Kathiravan et al., 2011; Murugavelh & Mohanty, 2018). In many batch systems, the removed Cr(VI) was accounted for as Cr(III) (Kholisa et al., 2021). However, in continuous systems, it has not been always the case. Cr(III) precipitates as ($Cr(OH)_3$) under basic or even slightly acidic conditions (Liu et al.,

2014). In the current study, total Cr measurements in the BPRB reactor effluent were similar to that of Cr(VI) indicating that Cr(III) was trapped within pore spaces in the reactor as Cr(OH)3(s). This was further characterised by the presence of dark-green colour after PRB, showing an accumulation of Cr(III). Molokwane and Nkhalambayausi-chirwa (2009) experienced a decrease in flow rate demonstrating that there was a reduction in spore spaces in the reactor which hindered the free flow of water due to continuous Cr(III) precipitation. However, in this study clogging of pore spaces in the reactor or hindered flow was not observed. The tested run times were shorter than the typical operational times in the field.

5.5 SPATIAL Cr(VI) CONCENTRATION PROFILE

Cr(VI) removal across the BRPB reactor was evaluated over 90 days of operation using data collected from sampling ports placed across the reactor. Figure 5-4(a), Figure 5-4(b) and Figure 5-4(c) show no Cr(VI) removal in sampling points before the barrier (port 1 and port 2) while high Cr(VI) removal is observed in sampling ports after the barrier (port 3 and port 4) at the initial Cr(VI) feed concentration of 45 and 65 mg/L, respectively. The insignificant Cr(VI) removal observed in sampling port 1 and port 2 was because the compartment before the barrier was only filled with sand, hence no Cr(VI) reduction occurred. It can be seen in Figure 5-4(a) that in port 3, no Cr(VI) concentration was detected in the first 6 days. After day 6, an increase in Cr(VI) concentration was observed and continued to increase up to 15 mg/L on day 10. Thereafter, 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 Cr(VI) concentration in port 2 is approximately 45 mg/L while port 3 and port 4 are nearly 0 mg/L. After 30 days of operation, the feed Cr(VI) concentration was

increased to 65 mg/L as shown in Figure 5-4(b). The system reached a steady state again on day 36, as the port 1 and port 2 Cr(VI) concentrations were equally to feed concentration. No Cr(VI) was detected in port 3 and port 4 until day 55 of operation. The Cr(VI) concentration in these ports continued to increase reaching 26 mg/L in port 3 and 16 mg/L in port 4. This increase in Cr(VI) concentration in port 3 and port 4 was attributed to the depletion of carbon sources from the sludge. Microorganisms utilize a variety of organic substances, either as an energy source or as electron donors to facilitate Cr(VI) bioreduction (Han et al., 2021). Due to depletion of carbon source and low Cr(VI) reduction, a 5 g/L glucose was added to the reactor feed to provide microorganisms with carbon and energy. After, continuously adding the glucose from day 60, the microorganisms completely reduced Cr(VI) as observed on day 61 as shown in Figure 5-4(c). Cr(VI) was not detected until day 83. Cr(VI) concentration continued to increase in both ports reaching 29 mg/L in port 3 and 23 mg/L port 4 on day 90. The deterioration of Cr(VI) reduction was due to decreasing pH (5.2) in the system. The decrease in pH values was ascribed to the fermentation of glucose forming several types of organic acids by different bacterial species which resulted in a subsequent drop in medium pH (Upadhyay et al., 2017; Cherif-silini et al., 2013; Shukla et al., 2012; Sanghi & Srivastava, 2010). These findings demonstrate the significance of metal-cell interactions within the bioreactive permeable barrier matrix in reducing Cr(VI).

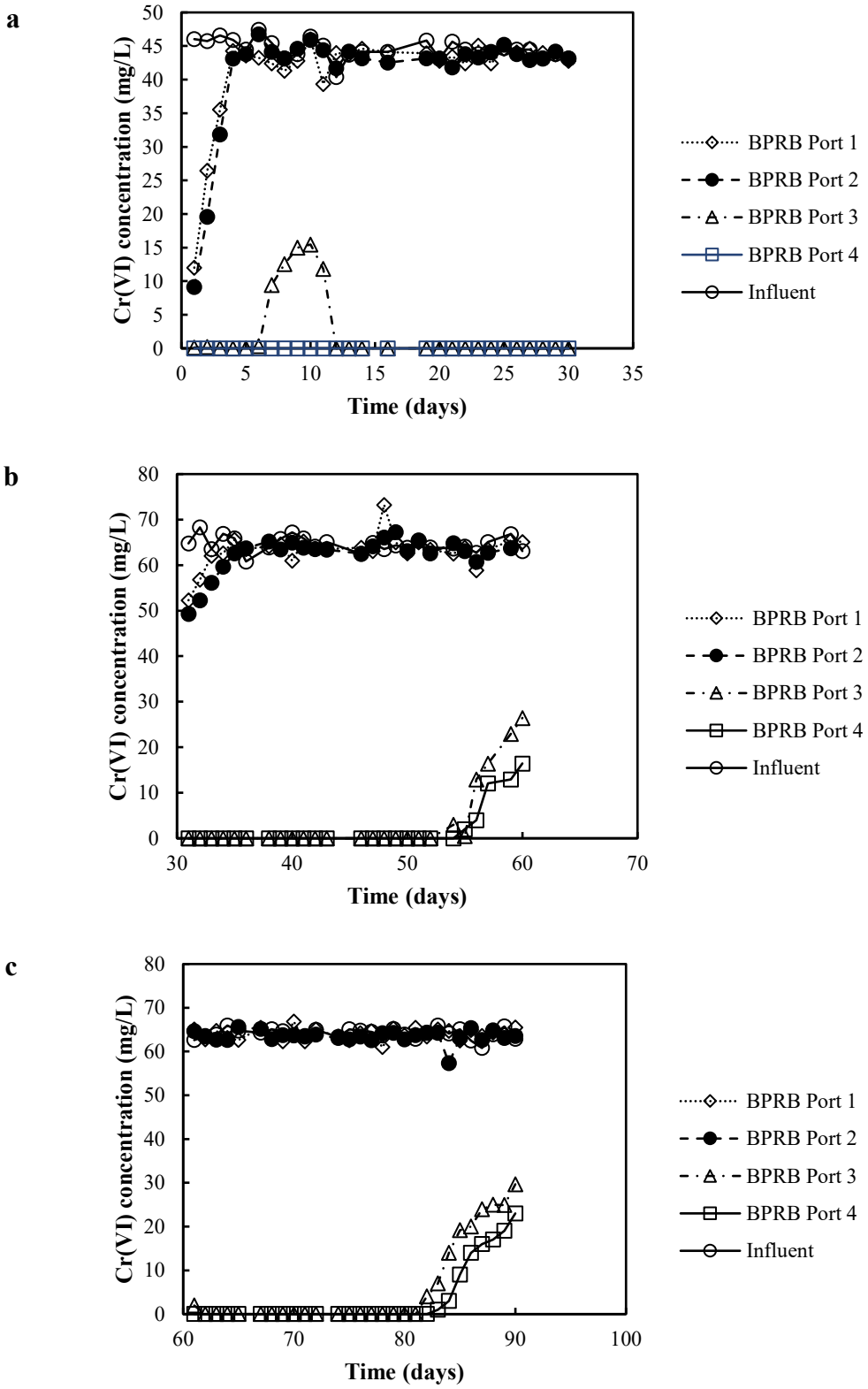


Figure 5-4: Cr(VI) concentration across the reactor at (a) 45 mg/L, (b) 65 mg/L, and (c) 65 mg/L and external carbon source

5.6 MICROBIAL CULTURE DYNAMICS

Changes to microbial culture composition after 13 weeks (90 days) of exposure to Cr(VI) were monitored by the 16S rRNA fingerprinting method. The results are presented in Figure 5-5 and Table 5-2, and the predominant species under nutrient and oxygen stress conditions were the *Pseudomonas* groups – *Pseudomonas fluorescens*, *Pseudomonas shahriarae*, *Pseudomonas hibiscicola*, *Pseudomonas gessardi*, *Pseudomonas geniculata* and *Comamonas testosterone* and *Stenotrophomonas maltophilia* at 100% identity index. These bacterial species are different from the initially identified species before the operation. The significant changes in the microbial community after the reactor operation may be due to operating the reactor under different conditions. It is well known that changes in carbon source in the reactor may effectively change the microbial species in the system (Wall & Krumholz, 2006). Also, the pH changes in the system was significant, which may have resulted in a microbial shift in the reactor (Pal et al., 2005).

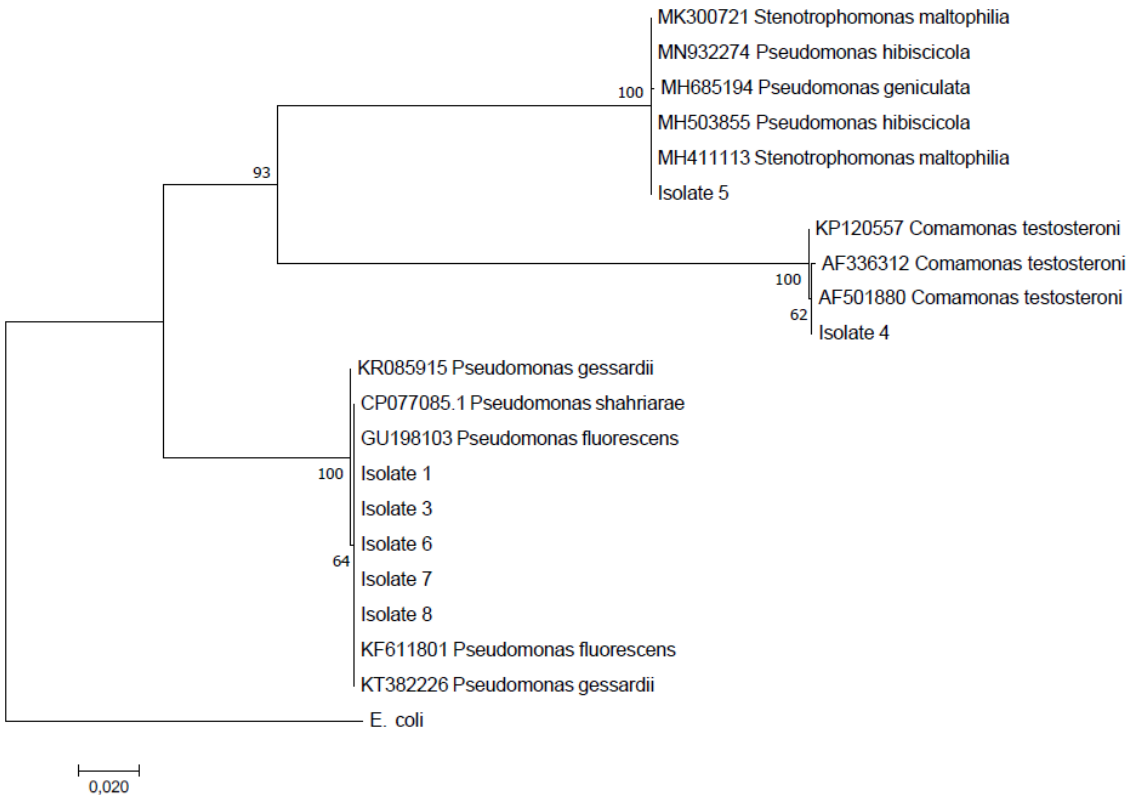


Figure 5-5: Phylogenetic tree showing the microbial shift and diversity after 90 days of operation

Table 5-2: Microbial characterisation 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 testosterone*	100
Y4	*Pseudomonas hibiscicola*	100
Y5	*Stenotrophomonas maltophilia*	100
Y6	*Pseudomonas gessardii*	100
Y7	*Pseudomonas geniculata*	100

5.7 PRB COST

Although this study did not focus on or perform any cost analysis for the biobarrier system, it important to have an understanding how expensive similar system were. It is understood that after PRB systems are installed, maintenance costs are very low for at least five to ten years. It is expected that there should be no other operating cost, except for the costs of monitoring performance and utility (Bortone et al., 2013). The use and selection of reactive medium is based on various conditions such as the type of target contaminants (e.g. organic or inorganic), their concentration, mechanisms needed to remove them (biodegradation, adsorption, etc.), and hydrological conditions of the aquifer, environmental effects, availability and cost of materials (Rad & Fazlali, 2020; Naidu et al., 2014).

According to the PRB cost analysis conducted by Bortone et al. (2013), it found that the cost of the reactive material exceeded 70% of the total the PRB cost. While Rad & Fazlali (2020) showed that of the total PRB cost, 37% was related to construction costs and 67% was the cost of reactive material used in PRB. These clearly shows that reactive medium used in the PRB systems contributes a large portion of the total cost. Therefore, using a low-cost reactive material such as wastewater sludge could make this technique cheaper.

5.8 SUMMARY

The effectiveness of bioremediation of Cr(VI) contaminated water using biological permeable reactive barrier technology was evaluated through bench-scale studies. Successful Cr(VI) reduction was achieved over the 90 days operational period of the BPRB system. Therefore, it can be concluded that the indigenous bacteria obtained in 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 co-exist with Cr(VI) in the environment and to evaluate the effect of operating the BPRB under various HRTs while occasionally backwashing or dislodging the accumulated precipitate from the system.

Chapter 6 MODELLING BIOLOGICAL Cr(VI) REDUCTION IN A BATCH SYSTEM

6.1 INTRODUCTION

The microbial reduction of Cr(VI) to Cr(III) has been shown to be a valuable, promising, and cost-effective approach for Cr(VI) remediation (Tan et al., 2020; Kholisa et al., 2021; Zeng et al., 2019). The biologically mediated Cr(VI) reduction is a complex process in the environment. To simplify matters in this study, the Cr(VI) reduction rate in the system is time-dependent, hence batch experiments were first conducted on the isolated mixed culture from the Cr(VI) environment. The effect of Cr(VI) on the reduction rate was evaluated at various initial Cr(VI) concentrations ranging from 50 to 400 mg/L, and the results were discussed in Chapter 4. A clear interrelation between the Cr(VI) reduction rate and the bacterial activity was observed as Cr(VI) reduction rate decreased with increasing initial Cr(VI) concentration. Furthermore, the bacterial cells were inhibited at high initial Cr(VI) concentrations due to the toxicity of Cr(VI). Similar observations were observed in several studies by Molokwane et al. (2008); Nkhalambayausi-Chirwa & Wang (2004); and Shen & Wang (1994), in which both bacteria growth and Cr(VI) reducing activity in pure and mixed cultures were inhibited by high Cr(VI) concentration. The experimental data obtained in this study was used to estimate Cr(VI) biokinetics using an enzyme-based model. The model was primarily developed to validate the toxic effect of Cr(VI) by integrating enzyme kinetics and Cr(VI) reduction capacity. The reduction capacity describes the maximum amount of Cr(VI) that a batch culture can reduce, and the loss of Cr(VI) reduction capacity in the bacterial cultures may be associated with the toxic effects of Cr(VI).

6.2 MODEL DEVELOPMENT FOR ENZYMATIC Cr(VI) REDUCTION

The biological reduction of Cr(VI) is facilitated through the membrane-electron transport mechanism in CRB (Shen & Wang, 1994). Viamajala et al. (2003) proposed that Cr(VI) reduction is mediated by the enzymes that are not substrate-specific for Cr(VI) and that "chromate reductases" may be unanticipated contributors to Cr(VI) reduction while achieving other physiological functions. There are numerous Cr(VI) reducing species of bacteria that exist in mixed cultures and their net Cr(VI) reducing activity may be described by one composite enzyme, E_t.

From the first principles, the single enzymic kinetic expression is based on the following reaction:

$$\text{Cr(VI)} + E \underset{k_b}{\overset{k_a}{\rightleftharpoons}} E^*\text{Cr(VI)} \xrightarrow{k_d} E + \text{Cr(III)} \qquad 6\text{-}1$$

where: E = the Cr(VI) reductase enzyme for mixed culture which is proportional to biomass concentration X (ML^{-3}); E^*Cr(VI) = the transitional enzyme-Cr(VI) complex; k_a = rate constant for complex formulation, k_b = rate constant for reverse complex formulation, k_d = rate constant for Cr(IV) formation.

Let C be the Cr(VI) concentration and E^*Cr(VI) be E^*

Therefore, the enzymatic rate of formation of E* from reaction 6-1 is expressed as follows:

$$\frac{dE^*}{dt} = k_a C(E - E^*) - k_b E^* - k_d E^* \qquad 6\text{-}2$$

Under steady-state conditions, *E** can either be formed or destroyed spontaneously such that that $\frac{dE^*}{dt}$ is approaching zero, hence $\frac{dE^*}{dt} \approx 0$. Therefore, Equation 6-2 become.

$$k_a C(E - E^*) - k_b E^* - k_d E^* = 0 \qquad 6\text{-}3$$

Solving Equation 6-3 for E* the resulting expression was obtained:

$$E^* = \frac{k_a CE}{k_a C + k_b + k_d} = \frac{CE}{C + \frac{k_b + k_d}{k_a}} \qquad 6\text{-}4$$

From Equation 6-2, the Cr(VI) reduction rate can be expressed as follows:

$$-\frac{dC}{dt} = \frac{k_d CE}{C + \frac{k_b + k_d}{k_a}} \qquad 6\text{-}5$$

Equation 6-5 is similar to Monod kinetic equation 6-6

$$-\frac{dC}{dt} = \frac{k_m C}{C + K_c} X \qquad 6\text{-}6$$

where: *C* = Cr(VI) concentration at time, *t* (mg/L); k_d is equivalent to the maximum specific Cr(VI) reduction rate, k_m (mg Cr(VI)/ mg cells/h); $\frac{k_b + k_d}{k_a}$ is equivalent to the half-saturation

constant, K_c (mg/L); E = the Cr(VI) reductase enzyme for mixed culture which is proportional to biomass concentration, X (mg cells/L).

The extent of Cr(VI) reduction in batch systems is proportional to the number of cells in the reactor and the capacity of Cr(VI) reduction (R_c) of each cell. For a batch system where, pre-concentrated washed resting cells are used, cell growth kinetics become irrelevant as the concentration of cells is too high to allow the production of new cells. Thus the amount of Cr(VI) reduced is proportional to the number of cells inactivated by Cr(VI) (Shen & Wang, 1994). Therefore, the biomass concentration at any time can be estimated using Equation 6-7:

$$X = X_0 - \left(\frac{C_0 - C}{R_c}\right) \qquad 6\text{-}7$$

where: C_0 = initial Cr(VI) concentration (mg/L); R_c is the Cr(VI) reduction capacity of cells (mg Cr(VI)/ mg cells); X_0 is the initial biomass concentration (mg cells/L).

Substituting Equation 6-7 into Equation 6-6 yields a modified Monod kinetic equation:

$$-\frac{dC}{dt} = \frac{k_m C}{C + K_c}\left(X_0 - \frac{C_0 - C}{R_c}\right) \qquad 6\text{-}8$$

In a study by Molokwane (2010), a modified Monod model (Equation 6-9) to account for non-competitive inhibition rate kinetics due to an increase in initial Cr(VI) concentration was proposed.

$$-\frac{dC}{dt} = \left(\frac{k_m}{1 + {C_0}/{K_i}}\right)\left(\frac{C}{K_c + C}\right)\left(X_0 - \frac{C_0 - C}{R_c}\right) \qquad 6\text{-}9$$

K_i is the kinetic inhibition constant in mg/L.

6.3 MODELING Cr(VI) REDUCTION BY BACTERIA CONSORTIUM

6.3.1 Cr(VI) bioreduction simulation

The batch experimental data obtained was simulated using the model in Equation 6-8. The unknown model kinetic parameters, k_m, K_c, and R_c were estimated by fitting the model to the experimental data using Aquasim. For each parameter, initial values were guessed, and the simulation was carried out. Upper and lower constraints were set for each parameter to omit nonsensical or unsound parameter values. Re-estimation of model parameters was done repeatedly until the best fit values were obtained. Parameter optimization was done using the objective function in Equation 3-9.

The model kinetic parameters were initially estimated using the 100 mg/L Cr(VI) initial concentration data. The obtained model parameters rom 100 mg/L Cr(VI) were used to simulate the entire range of Cr(VI) concentrations and the results were plotted against the experimental data as shown in Figure 6-1. The model captured well the trend of data under all experimental conditions. This confirms that the kinetic parameter values obtained at 100 mg Cr(VI)/L simulated Cr(VI) reduction data very well for a broader range of Cr(VI) concentrations. Although, there was a slight difficulty in fitting higher concentration above 150 mg/L mainly due to excessive loss of viable cells as shown in Table 6-1. This was expected due to Cr(VI) toxicity towards the biomass, and it is consistent with the results

obtained in Chapter 4. The model fitted reasonably well with the experimental data at a wide range of Cr(VI) initial concentrations with best fits obtain at 50 mg/L and 100 mg/L initial Cr(VI) concentrations as shown by a lower Chi^2.

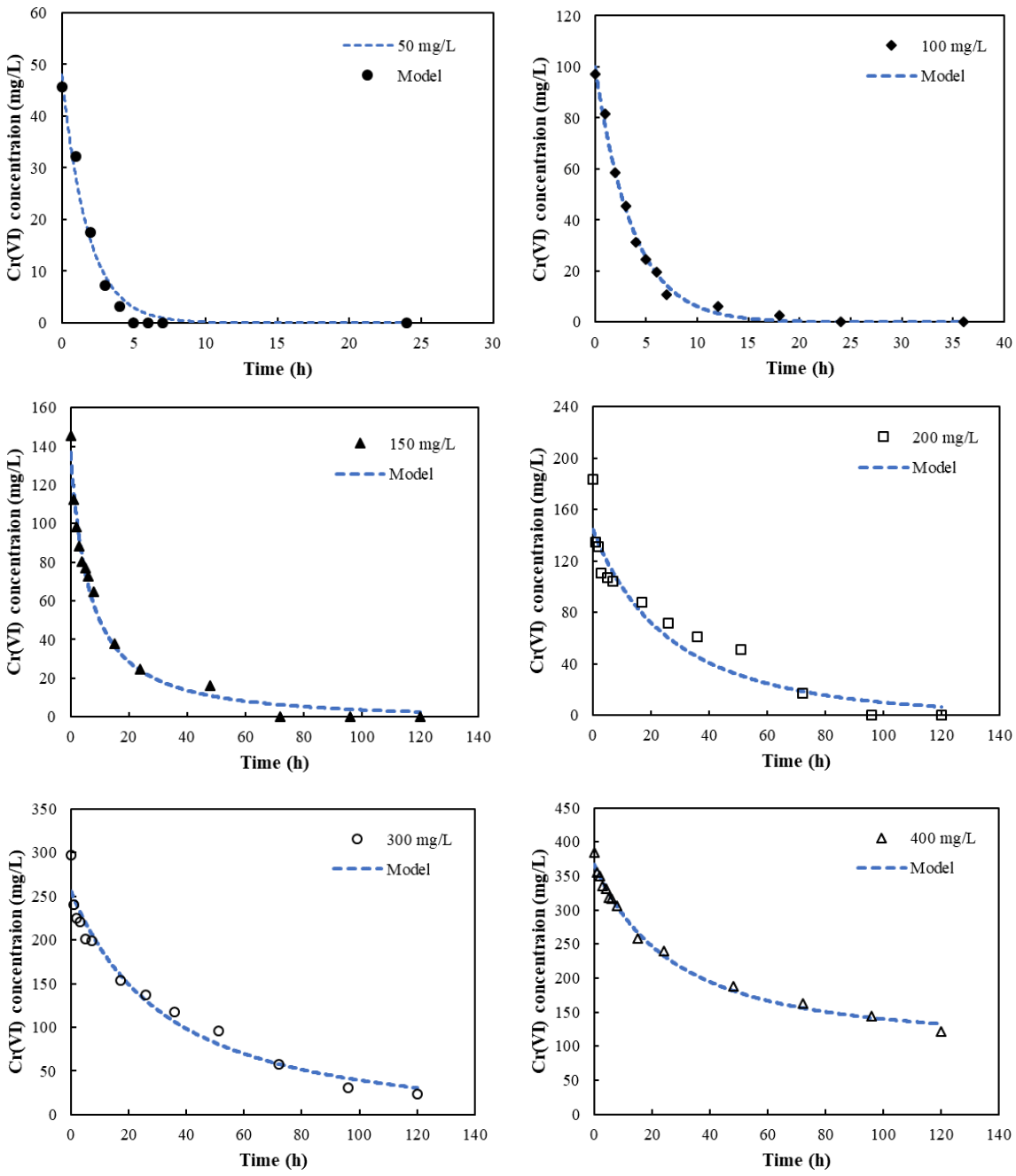


Figure 6-1: Batch consortium model simulation at various initial Cr(VI) concentration of (a) 50 mg/L, (b) 100 mg/L, (c) 150 mg/L, (d) 200 mg/L, (e) 300 mg/L and, (f) 400 mg/L

Table 6-1: Optimum kinetic parameters in batch consortium culture

Initial Concentration (mg/L)	K_c (mg/L)	k_m (mg/L.h^{-1})	R_c (mg/mg)	Chi^2
50	932.85	0.1041	0.9285	47.08
100	900.21	0.1009	0.9543	67.11
150	908.1	0.1007	0.9631	323.66
200	919.94	0.1007	0.9543	2933.49
300	904.62	0.1061	0.9401	3205.06
400	905.44	0.1225	0.8956	744.26

6.3.2 Sensitivity Analysis

The sensitivity (identifiability and uncertainty) analysis was performed to evaluate and compare the effect model parameters (Equation 6-8). Figure 6-2 shows the dependency of sensitivity response curve of k_m, K_c and R_c. The sensitivity decreases with increasing k_m until reaches minimum approximately 3 h then increases again to zero while K_c was the opposite. This high response in the first 3 h indicates high cell Cr(VI) reduction activity.

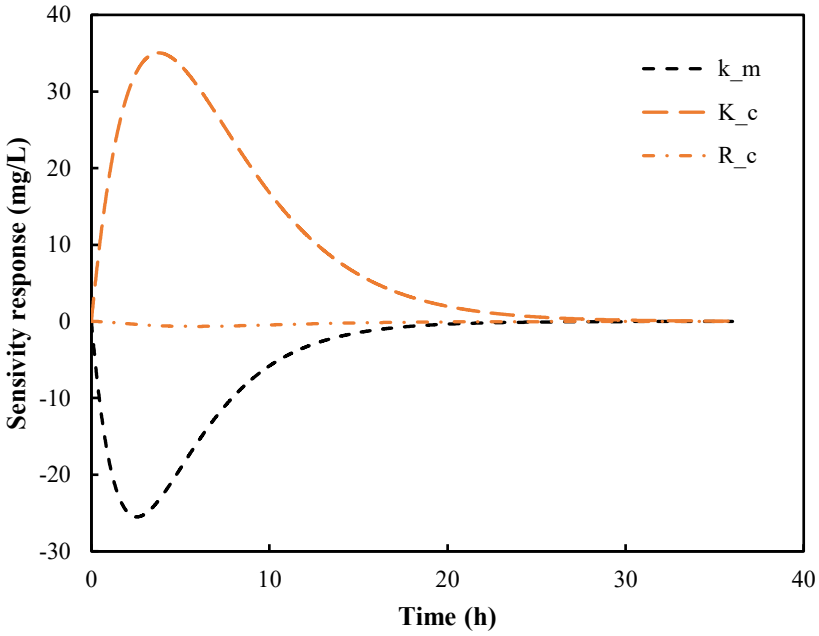


Figure 6-2: Batch consortium sensitivity at 100 mg/L with respect to k_m, K_c and R_c.

6.4 SUMMARY

In this chapter, the kinetic parameters affecting Cr(VI) reduction rate in a batch system employing consortium culture of bacteria were evaluated using a modified non-competitive inhibition model based on the Michaelis-Menten model. This enzymatic model was selected as it is capable to describe the complexity of microbial kinetics for Cr(VI) reduction in previous studies. The estimated (k_m, K_c and R_c) values using 100 mg/L initial Cr(VI) concentration data were able to predict Cr(VI) reduction for a broader range of initial Cr(VI) concentrations. The sensitivity of the model on each parameter was also assessed and the results showed kinetic parameters k_m and K_c to be significant. This indicate that the two kinetic parameters would be important in the scale up process of the reactor. This model provides a quantitative understanding of the kinetics for Cr(VI) reduction by microorganisms

and could be valuable for estimating reactor designs and enhanced for advance reactive transport modelling.

Chapter 7 MODELLING BIOLOGICAL Cr(VI) REDUCTION IN A PERMEABLE BIOREACTIVE BARRIER SYSTEM

7.1 INTRODUCTION

Nearly, all surface water in the environment do come into contact with groundwater one way or another. This interaction does not only influence water quantity, but also the fate, transport and transformation of solute and pollutants (Zheng et al., 2020). Given the toxicity of Cr(VI), the interaction between surface and groundwater is an important factor that cannot be ignored in the study of the transport and transformation of Cr(VI). Due to health and environmental threats posed by Cr(VI), needs to be reduced to its stable form Cr(III) for its remediation. From the previous studies, it has been shown that the Cr(VI) in the environment can be reduced by the presence of ferrous ions, and sulphides, in addition, it can be used as a terminal electron acceptor by native microorganisms during organic matter degradation (Wang & Choi, 2013). This indicates that both biotic and abiotic processes have an effect on the fate and migration of Cr(VI) in the environment (Ghiasi et al., 2020). Thus, it is vital to develop a mathematical model to improve our understanding of how various biogeochemical and physical processes affect Cr(VI) fate and transport and to predict this phenomenon. The general mathematical framework is based on the effects of complex sets of microbiological and geochemical reactions on Cr(VI) transport and bioavailability.

7.2 MATHEMATICAL MODELS

Generally, transport and removal of Cr(VI) in aquifers is predominantly due to three mechanisms, (i) dispersion governed by the interstitial velocity v (LT^{-1}), (ii) mass transport into media particles governed by mass transport rate coefficient k_L (LT^{-1}), (iii) adsorption rate governed by mass transport and surface reaction, (iv) Cr(VI) reduction governed by the reaction rate kinetics, and (v) cell replacement rate with the cells acting as the catalyst in the Cr(VI) reduction process. These fundamental processes in the reactor during transient state operation can be represented by Equations 7-1 to 7-5 below:

Advection

The advection transport is defined as the movement of dissolved Cr(VI) species from one point to another governed by bulk movement of fluid is described as follows:

$$-r_{adv} = -\frac{d(CV)}{dt} = Av(C_{in} - C) \qquad 7\text{-}1$$

where: r_{adv} is the mass rate movement of Cr(VI) (MT^{-1}); C is the effluent Cr(VI) concentration at any time, t (ML^{-3}); V is the volume of the reactor (L^3); t = time (T); C_{in} is the influent Cr(VI) concentration (ML^{-3}); A is the cross-sectional area of the reactor (L^2); v is the flow velocity (LT^{-1}); and Av is equivalent with influent or compartment flow rate (Q) (L^3T^{-1}).

Molecular Dispersion

The mass transport of all dissolved species across the boundary layer (L_w) into the biofilm is

due to the random thermal motion of molecules at temperatures above absolute zero. Mass transfer within the attached cell layer follows Fick's law of diffusion. The mass transfer is a function of external mass transfer resistance (k_L) across the biofilm surface area and bulk Cr(VI) concentration is described by Equation 7-2:

$$-\frac{d(CV)}{dt} = k_L A_f \frac{dC}{dx} = \frac{k_L}{L_w} A_f (C_b - C_s) = -J_c A_f \qquad 7\text{-}2$$

where: k_L is the dispersion coefficient of Cr(VI) in water (L^2T^{-1}); L_w is the thickness of biofilm (L); A_f is the biofilm surface area (L^2); C_b is the bulk liquid Cr(VI) concentration at time, t ((ML^{-3}); C_s is the liquid-biofilm interface Cr(VI) concentration (ML^{-3}).

In most mass transfer limited reactions $C_b >>> C_s$, thus C_s is negligible. Equation 7-2 is modified to Equation 7-3.

$$-J_c A_f = \left(\frac{k_L C_b}{L_w}\right) A_f \qquad 7\text{-}3$$

Adsorption

The rate of removal of Cr(VI) in the reactor is determined by the rate at which the Cr(VI) is transported and adsorbed in the reactor's biofilm, as well as the reaction taking place on the surface area. The Cr(VI) removal rate by adsorption is described by Equation 7-4:

$$-q_c = -\frac{dC}{dt} = k_{ad}(C_{eq} - C) \qquad 7\text{-}4$$

where: q_c is the rate of Cr(VI) removal by adsorption ($ML^{-3}T^{-1}$); k_{ad} is the Cr(VI) adsorption rate coefficient (T^{-1}); C_{eq} is the Cr(VI) equilibrium concentration at the surface (ML^{-3}).

Microbial reduction

The bioreactor in this study was operated predominately under oxygen-stressed conditions. Molokwane and co-authors (2008) developed an expression to describe microbial Cr(VI) reduction rate under anaerobic conditions in Equation 7-5. It has been shown that resting cells are able to reduce Cr(VI) without cell growth in batch studies (Molokwane et al., 2008; Li et al., 2019). Wang and Shen (1997) and Nkhalambayausi-Chirwa and Wang, (2004) stated that under resting cell conditions the amount of Cr(VI) reduced is proportional to the cells inactivated by Cr(VI).

$$-r_c = -\frac{dC}{dt} = \frac{k_m C}{(C + K_c)\left(K^{\left(1-\frac{C_r}{C_{in}}\right)}\right)}\left(X_0 - \frac{C_{in} - C}{R_c}\right) \qquad 7\text{-}5$$

where: r_c is the Cr(VI) reduction rate ($ML^{-1}T^{-1}$); K is the dimensionless Cr(VI) inhibition constant (MM^{-1}); C_r is the Cr(VI) toxicity threshold concentration.

7.3 REACTOR MASS BALANCE

The overall reactor mass balance which incorporates all the non-linear ODEs (Equation 7-1 to Equation 7-5) for modelling the fate and transport of Cr(VI) in a packed-bed reactor within the transient state, is presented as follows:

$$-\frac{d(CV)}{dt} = -r_{adv} - J_c A_f - (q_c + r_c)\Delta V \qquad 7\text{-}6$$

Substitution Equation 7-1 - Equation 7-5 into Equation 7-6 to obtain a transient state expression.

$$-\frac{dC}{dt} = \frac{Q}{V}(C_{in} - C) + \left(\frac{k_L C_b}{L_w}\right)\frac{A_f}{V} + \frac{k_m C}{(C + K_c)\left(K^{\left(1-\frac{C_r}{C_{in}}\right)}\right)}\left(X_0 - \frac{C_{in} - C}{R_c}\right) + k_{ad}(C_{eq} - C) \qquad 7\text{-}7$$

Data simulation and optimisation were performed using Aquasim 2.0 to obtain kinetic parameter values. This was done by setting upper and lower limits for each parameter to exclude invalid parameter values. In the event that the estimated parameter value was close to the limit, then the limit was relaxed until it did not force the model. This process was repeated until the estimated parameter values lie away from the lower and upper limit values. The continuous flow reactors in this study were modelled as plug flow reactors with the Cr(VI) removal using Equation 7-7 under the following assumption:

- The flow in the reactor is plug and one-dimensional
- The porous media is homogenous.
- pH and Temperature are constant at steady-state operation.

- The reactor approaches steady-state operation.
- All substances dissolved in water flow.
- Due to biotransformation, Cr(III) formed is either precipitated and retained or adsorbed onto the soil matrix almost immediately.
- Some microbes are mobile, and some are immobile.

7.4 MODEL SIMULATION

The transport of Cr(VI) without the biotransformation in the reactor has been shown previously in Figure 5-1(a). The mass balance model (Equation 7-7) was applied to the operation of the cell-free reactor and the results showed a characteristic exponential curve indicating saturation of physical processes in the system within the first 5 days. The cell-free reactor data was used to estimate the physical parameters in the system. Nkhalambayausi-Chirwa and Wang (2001) and Igboamalu and Chirwa (2015) in their respective studies observed no significant Cr(VI) adsorption and neither transformation nor accumulation during the operation of the cell-free reactor. This suggests that in the long term, Cr(VI) removal is mainly due to bioreduction by bacteria, advection and mass transport. Therefore, adsorption parameters in Equation 7-7 were not included in the simulation.

To simulate the BRP reactor performance accurately, the viable biomass in the reactor needs to be predicted precisely. Direct measurement of viable cell concentration inside the reactor proved to be impractical. As a result, biomass concentration was based on the initial biomass concentration obtained in the batch studies. The initial guess parameters for simulation of the Cr(VI) removal rate across reactors were based on previous studies (Nkhalambayausi-Chirwa & Wang, 2005; Molokwane & Chirwa, 2013; Mtimunye & Chirwa, 2014).

Numerical solutions without practical initial guess parameters or constraints may result in converging to false optimum values for various parameters. To counter the false optimum values, upper and lower constraints were set for each parameter to omit invalid parameter values. Using the optimised parameter values and other operating parameters, the model (Equation 7-7) was used to calculate the time series Cr(VI) concentration within the reactor. The estimated Cr(VI) concentration data was then compared to measured experimental data. The derivation between the experimental and estimated Cr(VI) concentrations was used to determine the accuracy of the model using Equation **Error! Reference source not found.**.

Optimum values of kinetic parameters for Cr(VI) reduction in the biological barrier were found as follows: k_m = 1.068 mg/L/d, K_c = 11.14 mg/L, and R_c = 0.75 mg/mg for Cr(VI) reduction in the biological barrier and these are summarised in Table 7-1. Figure 7-1 shows the results of influent and effluent simulation within the biological barrier reactor. The kinetic parameters k_m (1.068 mg/L/d) and K_c (11.14 mg/L) obtained from the continuous flow bioreactor for Cr(VI) removal show that the biological activity was lower than observed previously in the batch system k_m (0.1043 mg/L/h) and K_c (786.4 mg/L). Cr(VI) reduction rates were expected to be higher in the continuous flow reactor than in the batch process due to the shielding effect of mass transport resistance against toxic effects on cells (Vickstrom et al., 2017; Azizian & Semprini, 2016). Semprini and McCarty (1991) further explained that bacteria culture acclimatises to toxic compounds and results in higher bioreduction of the toxic organic compounds due to longer contact times in continuous-flow reactor systems. However, in this study, the lower Cr(VI) reduction rates in the continuous flow may be due to a decrease in pH in the reactor. Therefore, it would be interesting to study how would a controlled pH inside the reactor affect the Cr(VI) bioreduction rates and the overall performance of the reactor. The model predicted effluent Cr(VI) concentration with more

than 99.6% confidence. Overall, the transient-state model successfully simulated the trends in influent and effluent Cr(VI) concentrations under different Cr(VI) loadings. Even though the effluent Cr(VI) concentration trend in the reactor was successfully traced by the model, adjustments would be needed to take into account the loss of working volume and decreasing flow rate due to the growth of biomass in the reactor.

Table 7-1: Physical parameters and optimum values of kinetic parameters for Cr(VI) reduction in the biological barrier

Parameter	Description	Units	Optimum
k_m	Specific Cr(VI) reduction reaction rate	mg/L/d	1.068
K_c	Half velocity concentration	mg/L	11.14
R_c	Cell Cr(VI) reduction capacity	mg/mg	0.75
θ	Porosity	_	0.42
rho_s	Solid particle density	kg/m^3	2300
Qin	Inflow rate	m^3/d	0.0048
k_L	Dispersion coefficient	m^2/d	$6.7x10^{-6}$
A	Cross-sectional area	m^2	0.0165

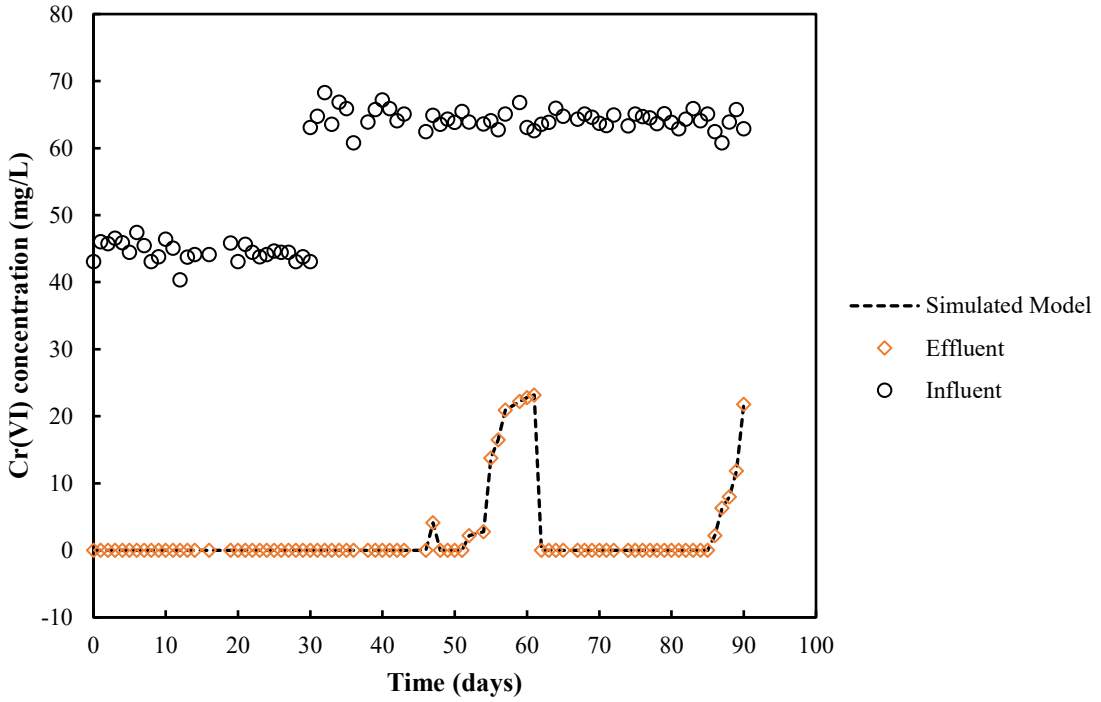


Figure 7-1: Simulation and optimization of influent and effluent Cr(VI) in the biological barrier

7.5 SUMMARY

This chapter evaluated the transient-state model defined by a complex system of non-linear equations to optimise kinetic parameters in a biological system and to predict effluent Cr(VI) concentration under different operation conditions using the optimum parameter values. The continuous-flow packed bed reactor was modelled as a plug flow system based on a one-dimensional advective-diffusive transport. The obtained kinetic parameters in this study showed lower removal kinetics than those obtained previously in batch systems. This was ascribed to low pH values in the reactor which reduced the bacteria activity. The developed model predicted the Cr(VI) effluent well under various Cr(VI) influent concentration loadings (40–60 mg/L) with 99.6% confidence. Despite the fact, that the effluent Cr(VI) concentration trend in the reactor was successfully traced by the model, adjustments would

be needed to take into account the loss of working volume and decreasing flow rate due to the growth of biomass in the reactor. The model modification may result in a proper application in engineered biological systems for the treatment of groundwater with higher Cr(VI) concentrations and multiple toxic contaminants.

Chapter 8 CONCLUSION AND RECOMMENDATIONS

8.1 CONCLUSIONS

Environmental pollution is a global problem that affects both developed and developing countries by contaminating soil and water, threatening biodiversity, ecosystems, and human health. South Africa holds the largest chrome ore reserves in the world, and it is one of the largest producers of ferrochrome. The production process of steel and chromate generates enormous quantities of ferrochrome wastes which are discharged in dumps. These waste has been shown to contain significantly higher levels of Cr(VI) than the maximum acceptable risk concentration that is allowed for waste disposal in South Africa, which becomes a serious concern for soil and groundwater pollution. Thus, research into the remediation of Cr(VI) pollution has attracted widespread attention.

The current study was aimed at exploring and evaluating the prospect of Cr(VI) contamination control in groundwater aquifers at contaminated sites using natural microbial processes. Experiments were conducted in batch and continuous flow bioreactor systems for Cr(VI) reduction using wastewater sludge microorganisms.

Batch experiments under varying initial Cr(VI) concentrations of 50 - 400 mg/L in mineral salt media with harvested and concentrated cells were capable of reducing Cr(VI) at concentrations, up to 400 mg/L. Complete Cr(VI) reduction of 50 mg/L concentration was observed in 6 h under aerobic and neutral pH conditions. The Cr(VI) reduction rate decreases with increasing initial Cr(VI) concentration, due to Cr(VI) toxicity on bacterial cells. It was observed that Cr(VI) reduction increases with increasing the initial pH of the solution until

the optimal pH of 7, a further increase in pH results in decreased Cr(VI) removal. Co-existing heavy metals did not have an effect on Cr(VI) reduction at both low and high heavy metal concentrations with exception of Cu^{2+} and Zn^{2+} which enhanced Cr(VI) reduction, while Pb^{2+} and Ni^{2+} showed inhibitory effects at high concentrations.

The effectiveness of bioremediation of Cr(VI) contaminated water using biological permeable reactive barrier technology was evaluated through bench-scale studies. Successful Cr(VI) reduction was achieved with 95.9% removal over the 90 days operational period of the BPRB system. When glucose was used as the carbon source, a drastic decline in effluent pH from 6.91 to below 5.5 was observed in the effluent. The decrease in pH values was ascribed to the oxidation of glucose forming several types of organic acids by different *Bacillus* species and other bacterial species which result in a subsequent drop in medium pH. However, it did not influence the overall reactor performance. The results suggest that the biological permeable reactive barrier technology using indigenous bacterial strains has potential application for Cr(VI) remediation in contaminated environments.

The reaction kinetic parameters k_m, K_c, R_c, and k_L were estimated successfully using both the batch and continuous experiment data, and this was simulated using Aquasim 2.0. The batch modelling results showed that the performance of the mixed bacteria culture was well represented by the non-competitive model with cell inactivation for various Cr(VI) initial concentrations (50 mg/L - 400 mg/L) under aerobic conditions. Though there was slight difficulty in fitting Cr(VI) concentration above 200 mg/L due to excessive loss of biomass which is not captured by the model. The sensitivity of the estimated parameters showed that k_m, and K_c were the most sensitive parameters to the model prediction than R_c.

The mass transport kinetics and Cr(VI) removal in the permeable reactive barrier was represented by a diffusion-reduction model formulated using a set of ODEs which were solved by Aquasim for numerical solutions. The model simulated the operation of a mixed reactor with dispersion and a plug flow regime. The developed model predicted the Cr(VI) effluent well under various Cr(VI) influent concentration loadings (40–60 mg/L) with 99.6% confidence.

8.2 RECOMMENDATIONS

The studies presented here provide insight into fundamental processes involved in the areas of Cr(VI) mass transport in aquifers, and the application of wastewater sludge as a permeable reactive barrier for Cr(VI) remediation. This biological permeable reactive barrier technology is presently in its infancy, and future studies are anticipated to continually fill in the existing gaps of knowledge. Some specific recommendations for future work being considered are as follows.

The permeable reactive barrier reactor was operated for 90 days and therefore longer study period is required to understand how reactor performance will be affected.

Further studies are required to understand the interaction of bacteria with other heavy metals that co-exist with Cr(VI) in the environment and also to evaluate the effect of operating the BPRB under various HRTs while occasionally backwashing or dislodging the accumulated precipitate from the system.

To evaluate the effect of controlling pH inside the reactor on the overall performance of permeable reactive barrier.

Finally, experiments should be conducted with real contaminated groundwater to study the effect of different chemical compositions and conditions of contaminated water on the Cr(VI) removal efficiency by bacteria and the hydraulic behaviour of the used mixtures.

REFERENCES

Ackerley, D.F., Matin, A., Keyhan, M., Park, C.H., Blake, R. & Gonzalez, C.F. 2004. Chromate-Reducing Properties of Soluble Flavoproteins from Pseudomonas putida and Escherichia coli. *Applied and Environmental Microbiology*, 70(2): 873–882.

Al-Hashimi, O., Hashim, K., Loffill, E., Cebasek, T.M., Nakouti, I., Faisal, A.A.H. & Al-Ansari, N. 2021. A Comprehensive Review for Groundwater Contamination and Remediation: Occurrence, Migration and Adsorption Modelling. *Molecules*, 26: 1–28.

Alghanmi, S.I., Al Sulami, A.F., El-Zayat, T.A., Alhogbi, B.G. & Abdel Salam, M. 2015. Acid leaching of heavy metals from contaminated soil collected from Jeddah, Saudi Arabia: kinetic and thermodynamics studies. *International Soil and Water Conservation Research*, 3(3): 196–208. http://dx.doi.org/10.1016/j.iswcr.2015.08.002.

Alloway, B.J. 2013. *Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability*. 3rd ed. Dordrecht: Springer. http://www.springerlink.com/index/10.1007/978-94-007-4470-7_10.

Asadi, A., K Huat, B.B., Nahazanan, H. & Keykhah, H.A. 2013. Theory of Electroosmosis in Soil. *Int. J. Electrochem. Sci*, 8: 1016–1025. www.electrochemsci.org.

Ashley, K., Howe, A.M., Demange, M. & Nygren, O. 2003. Sampling and analysis considerations for the determination of hexavalent chromium in workplace air. *Journal of Environmental Monitoring*, 5(5): 707–716.

Ashraf, A., Bibi, I., Niazi, N.K., Ok, Y.S., Murtaza, G., Shahid, M., Kunhikrishnan, A., Li, D. & Mahmood, T. 2017. Chromium(VI) sorption efficiency of acid-activated banana

peel over organo-montmorillonite in aqueous solutions. *International Journal of Phytoremediation*, 19(7): 605–613. http://dx.doi.org/10.1080/15226514.2016.1256372.

Azizian, M.F. & Semprini, L. 2016. Simultaneous anaerobic transformation of tetrachloroethene and carbon tetrachloride in a continuous flow column. *Journal of Contaminant Hydrology*, 190(December): 58–68. http://dx.doi.org/10.1016/j.jconhyd.2016.04.002.

Baharuddin, N.H., Sulaiman, N.M.N. & Aroua, M.K. 2014. Unmodified starch as water-soluble binding polymer for Chromium ions removal via polymer enhanced ultrafiltration system. *Journal of Environmental Health Science and Engineering*, 12(1): 1–10.

Bandehali, S., Parvizian, F., Moghadassi, A.R., Hosseini, S.M. & Shen, J.N. 2020. Fabrication of thin film-PEI nanofiltration membrane with promoted separation performances: Cr, Pb and Cu ions removal from water. *Journal of Polymer Research*, 27(4).

Banerjee, S., Misra, A., Chaudhury, S. & Dam, B. 2019. A Bacillus strain TCL isolated from Jharia coalmine with remarkable stress responses, chromium reduction capability and bioremediation potential. *Journal of Hazardous Materials*, 367(July 2018): 215–223. https://doi.org/10.1016/j.jhazmat.2018.12.038.

Bansal, N., Coetzee, J.J. & Chirwa, E.M.N. 2017. In Situ Bioremediation of Chromium (VI) In a Simulated Ferrochrome Slag In Situ Bioremediation of Chromium (VI) In a Simulated Ferrochrome Slag. *CHEMICAL ENGINEERING TRANSACTIONS*, 61(October): 55–60.

Bansal, N., Coetzee, J.J. & Chirwa, E.M.N. 2019. In situ bioremediation of hexavalent chromium in presence of iron by dried sludge bacteria exposed to high chromium concentration. *Ecotoxicology and Environmental Safety*, 172: 281–289. https://doi.org/10.1016/j.ecoenv.2019.01.094.

Baral, A. & Engelken, R.D. 2002. Chromium-based regulations and greening in metal finishing industries in the USA. *Environmental Science and Policy*, 5(2): 121–133.

Barrera-Díaz, C.E., Lugo-Lugo, V. & Bilyeu, B. 2012. A review of chemical, electrochemical and biological methods for aqueous Cr(VI) reduction. *Journal of Hazardous Materials*, 223–224: 1–12. http://dx.doi.org/10.1016/j.jhazmat.2012.04.054.

Beukes, J.P., Van Zyl, P.G. & Ras, M. 2012. Treatment of Cr(VI)-containing wastes in the South African ferrochrome industry - A review of currently applied methods. *Journal of the Southern African Institute of Mining and Metallurgy*, 112(5): 347–352.

Bhattacharya, A. & Gupta, A. 2013. Evaluation of Acinetobacter sp . B9 for Cr (VI) resistance and detoxification with potential application in bioremediation of heavy-metals-rich industrial wastewater. *Environmental Science and Pollution Research*, 20: 6628–6637.

Bhaumik, M., Choi, H.J., Seopela, M.P., McCrindle, R.I. & Maity, A. 2014. Highly effective removal of toxic Cr(VI) from wastewater using sulfuric acid-modified avocado seed. *Industrial and Engineering Chemistry Research*, 53(3): 1214–1224.

Bhowal, A., Bhattacharyya, G., Inturu, B. & Datta, S. 2012. Continuous removal of hexavalent chromium by emulsion liquid membrane in a modified spray column. *Separation and Purification Technology*, 99: 69–76.

http://dx.doi.org/10.1016/j.seppur.2012.08.026.

Bhuvaneshwari, S., Sivasubramanian, V. & Senthilrani, S. 2011. Biosorption of Chromium from Aqueous Waste Water using Chitosan and Desorption of Chromium from Biosorbent for Effective Reuse. *Research Journal of Chemistry and Environment*, 15(2): 185–191.

Bielicka, A., Bojanowska, I. & Wiśniewski, A. 2005. Two faces of chromium - Pollutant and bioelement. *Polish Journal of Environmental Studies*, 14(1): 5–10.

Bilgin, M. & Tulun, S. 2016. Removal of heavy metals (Cu, Cd and Zn) from contaminated soils using EDTA and FeCl3. *Global Nest Journal*, 18(1): 98–107.

Björkegren, S., Karimi, R.F., Martinelli, A., Jayakumar, N.S. & Hashim, M.A. 2015. A new emulsion liquid membrane based on a palm oil for the extraction of heavy metals. *Membranes*, 5(2): 168–179.

Boni, M.R. & Sbaffoni, S. 2009. The potential of compost-based biobarriers for Cr(VI) removal from contaminated groundwater: Column test. *Journal of Hazardous Materials*, 166: 1087–1095.

Bortone, I., Chianese, S., Nardo, A. Di, Natale, M. Di, Erto, A. & Musmarra, D. 2013. A comparison between pump & treat technique and permeable reactive barriers for the remediation of groundwater contaminated by chlorinated organic compounds. *Chemical Engineering Transactions*, 32: 31–36.

Cameselle, C. 2015. Enhancement of Electro-Osmotic Flow during the Electrokinetic Treatment of A Contaminated Soil. *Electrochimica Acta*, 181: 31–38. http://dx.doi.org/10.1016/j.electacta.2015.02.191.

Çeribasi, I.H. & Yetis, U. 2001. Biosorption of Ni(ii) and Pb(ii) by Phanerochaete chrysosporium from a binary metal system - Kinetics. *Water SA*, 27(1): 15–20.

Chai, L., Huang, S., Yang, Z., Peng, B., Huang, Y. & Chen, Y. 2009. Cr (VI) remediation by indigenous bacteria in soils contaminated by chromium-containing slag. *Journal of Hazardous Materials journal*, 167: 516–522.

Cheng, S.F., Huang, C.Y. & Tu, Y.T. 2011. Remediation of soils contaminated with chromium using citric and hydrochloric acids: The role of chromium fractionation in chromium leaching. *Environmental Technology*, 32(8): 879–889.

Cherif-silini, H., Silini, A., Ghoul, M., Yahiaoui, B. & Arif, F. 2013. Solubilization of phosphate by the Bacillus under salt stress and in the presence of osmoprotectant compounds. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Agriculture*, 37: 4562–4571.

Cheung, K.H. & Gu, J. 2007. Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: A review. *International Biodeterioration and Biodegradation*, 59: 8–15.

Chirwa, E.M.N., Venter, I. & Wienand, H. 2013. Biological Cr (VI) immobilisation in saturated aquifer zone using culture inoculated soil columns. *Water Science and Technology*, 67(10): 2321–2329.

Chojnacka, K. 2010. Biosorption and bioaccumulation - the prospects for practical applications. *Environment International*, 36(3): 299–307. http://dx.doi.org/10.1016/j.envint.2009.12.001.

Coetzee, J.J., Bansal, N. & Chirwa, E.M.N. 2018. Chromium in Environment, Its Toxic Effect from Chromite-Mining and Ferrochrome Industries, and Its Possible

Bioremediation. *Exposure and Health*, (0123456789): 1–12. https://doi.org/10.1007/s12403-018-0284-z.

Comte, S., Guibaud, G. & Baudu, M. 2008. Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. *Journal of Hazardous Materials*, 151(1): 185–193.

Cramer, L.A., Basson, J. & Nelson, L.R. 2004. The impact of platinum production from UG2 ore on ferrochrome production in South Africa. *Journal of The South African Institute of Mining and Metallurgy*, 104(9): 517–527.

Dąbrowski, A., Hubicki, Z., Podkościelny, P. & Robens, E. 2004. Selective removal of the heavy metal ions from waters and industrial wastewaters by ion-exchange method. *Chemosphere*, 56(2): 91–106.

Das, S., Mishra, J., Kumar, S., Pandey, S., Srinivas, D., Chakraborty, A., Sudarshan, M., Das, N. & Thatoi, H. 2014. Investigation on mechanism of Cr(VI) reduction and removal by Bacillus amyloliquefaciens, a novel chromate tolerant bacterium isolated from chromite mine soil. *Chemosphere*, 96: 112–121. http://dx.doi.org/10.1016/j.chemosphere.2013.08.080.

Dermont, G., Bergeron, M., Mercier, G. & Richer-Laflèche, M. 2008. Soil washing for metal removal: A review of physical/chemical technologies and field applications. *Journal of Hazardous Materials*, 152(1): 1–31.

Dey, S. & Paul, A.K. 2013. Hexavalent chromium reduction by aerobic heterotrophic bacteria indigenous to chromite mine overburden. *Brazilian Journal of Microbiology*, 44(1): 307–315.

Dhal, B., Thatoi, H., Das, N. & Pandey, B.D. 2010. Reduction of hexavalent chromium by

Bacillus sp. isolated from chromite mine soils and characterization of reduced product. *Journal of Chemical Technology and Biotechnology*, 85(11): 1471–1479.

Dhal, B., Thatoi, H.N., Das, N.N. & Pandey, B.D. 2013. Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining / metallurgical solid waste: A review. *Journal of Hazardous Materials*, 250–251: 272–291. http://dx.doi.org/10.1016/j.jhazmat.2013.01.048.

Diep, P., Mahadevan, R. & Yakunin, A.F. 2018. Heavy metal removal by bioaccumulation using genetically engineered microorganisms. *Frontiers in Bioengineering and Biotechnology*, 6.

Dognani, G., Hadi, P., Ma, H., Cabrera, F.C., Job, A.E., Agostini, D.L.S. & Hsiao, B.S. 2019. Effective chromium removal from water by polyaniline-coated electrospun adsorbent membrane. *Chemical Engineering Journal*, 372(April): 341–351. https://doi.org/10.1016/j.cej.2019.04.154.

Doke, S.M. & Yadav, G.D. 2014. Process efficacy and novelty of titania membrane prepared by polymeric sol-gel method in removal of chromium(VI) by surfactant enhanced microfiltration. *Chemical Engineering Journal*, 255: 483–491.

Elahi, A., Ajaz, M., Rehman, A., Vuilleumier, S., Khan, Z. & Hussain, S.Z. 2019. Isolation, characterization, and multiple heavy metal-resistant and hexavalent chromium-reducing Microbacterium testaceum B-HS2 from tannery effluent. *Journal of King Saud University - Science*, 31(4): 1437–1444. https://doi.org/10.1016/j.jksus.2019.02.007.

Elangovan, R., Abhipsa, S., Rohit, B., Ligy, P. & Chandraraj, K. 2006. Reduction of Cr(VI) by a Bacillus sp. *Biotechnology Letters*, 28(4): 247–252.

Fedje, K.K., Yillin, L. & Strömvall, A.M. 2013. Remediation of metal polluted hotspot areas through enhanced soil washing - Evaluation of leaching methods. *Journal of Environmental Management*, 128: 489–496. http://dx.doi.org/10.1016/j.jenvman.2013.05.056.

Fernandez, P.M., Vinarta, S.C., Bernal, A.R., Cruz, E.L. & Figueroa, L.I.C. 2018. Bioremediation strategies for chromium removal : Current research , scale-up approach and future perspectives Chemosphere Bioremediation strategies for chromium removal : Current research , scale-up approach and future perspectives. *Chemosphere*, 208: 139–148. https://doi.org/10.1016/j.chemosphere.2018.05.166.

Fonseca, B., Pazos, M., Tavares, T. & Sanromán, M.A. 2012. Removal of hexavalent chromium of contaminated soil by coupling electrokinetic remediation and permeable reactive biobarriers. *Environmental Science and Pollution Research*, 19(5): 1800–1808.

Frade, P.R., Notini, L., Santos, S. V., Moreira, R.F.P.M., Leão, M.M.D. & Amorim, C.C. 2018. Feasibility study of the use of basic oxygen furnace sludge in a permeable reactive barrier. *Journal of Hazardous Materials*, 351: 188–195. https://doi.org/10.1016/j.jhazmat.2018.02.035.

Frois, S.R., Grassi, M.T., Fernandes, T.C., Dos Santos Barreto, R.A. & Abate, G. 2011. Preconcentration of CR(III) and speciation analysis of chromium employing montmorillonite saturated with potassium ions | Pré-concentração de Cr(III) e análise de especiação de cromo empregando montmorilonita saturada com íons potássio. *Quimica Nova*, 34(3): 462–467.

Fu, F. & Wang, Q. 2011. Removal of heavy metal ions from wastewaters: A review. *Journal*

of Environmental Management, 92(3): 407–418. http://dx.doi.org/10.1016/j.jenvman.2010.11.011.

Ghiasi, B., Hossein, M. & Mahdavi, A. 2020. Co-transport of chromium(VI) and bentonite colloidal particles in water- saturated porous media: Effect of colloid concentration, sand gradation, and flow velocity. *Journal of Contaminant Hydrology*, 234(May).

Gidudu, B. & Nkhalambayausi Chirwa, E.M. 2020. Application of biosurfactants and pulsating electrode configurations as potential enhancers for electrokinetic remediation of petrochemical contaminated soil. *Sustainability (Switzerland)*, 12(14).

Gitipour, S., Ahmadi, S., Madadian, E. & Ardestani, M. 2011. Soil washing of chromium- and cadmium-contaminated sludge using acids and ethylenediaminetetra acetic acid chelating agent. *Environmental Technology (United Kingdom)*, 37(1): 145–151.

Gong, Y., Gai, L., Tang, J., Fu, J., Wang, Q. & Zeng, E.Y. 2017. Reduction of Cr(VI) in simulated groundwater by FeS-coated iron magnetic nanoparticles. *Science of the Total Environment*, 595: 743–751. http://dx.doi.org/10.1016/j.scitotenv.2017.03.282.

Gong, Y., Zhao, D. & Wang, Q. 2018. An overview of field-scale studies on remediation of soil contaminated with heavy metals and metalloids: Technical progress over the last decade. *Water Research*, 147: 440–460. https://doi.org/10.1016/j.watres.2018.10.024.

Han, H., Hou, J., Zhang, D., Zhong, Y., Chen, C. & Zhang, Q. 2021. External Carbon Source Facilitates Indirect Cr (VI) Bioreduction Process by Anaerobic Sludge Produced from Kitchen Waste. , (Vi): 1–15.

Han, S., Zang, Y., Gao, Y., Yue, Q., Zhang, P., Kong, W., Jin, B., Xu, X. & Gao, B. 2020. Co-monomer polymer anion exchange resin for removing Cr(VI) contaminants: Adsorption kinetics, mechanism and performance. *Science of the Total Environment*,

709: 136002. https://doi.org/10.1016/j.scitotenv.2019.136002.

Hashim, M.A., Mukhopadhyay, S., Sahu, J.N. & Sengupta, B. 2011. Remediation technologies for heavy metal contaminated groundwater. *Journal of Environmental Management*, 92(10): 2355–2388. http://dx.doi.org/10.1016/j.jenvman.2011.06.009.

Haynes, W.M. 2016. *CRC Handbook of Chemistry and Physics*. 97th ed. Boca Raton: CRC Press , Taylor & Francis Group.

Higgins, M.R. & Olson, T.M. 2009. Life-cycle case study comparison of permeable reactive barrier versus pump-and-treat remediation. *Environmental Science and Technology*, 43(24): 9432–9438.

Hlihor, R.M., Diaconu, M., Fertu, D., Chelaru, C., Sandu, I., Tavares, T. & Gavrilescu, M. 2013. Bioremediation of Cr(VI) Polluted Wastewaters by Sorption on Heat Inactivated Saccharomyces cerevisiae Biomass. *International Journal of Environmental Research*, 7(3): 581–594.

Hosseini, M.S. & Sarab, A.R.R. 2007. Cr(III)/Cr(VI) speciation in water samples by extractive separation using Amberlite CG-50 and final determination by FAAS. *International Journal of Environmental Analytical Chemistry*, 87(5): 375–385.

Huang, X.N., Min, D., Liu, D.F., Cheng, L., Qian, C., Li, W.W. & Yu, H.Q. 2019. Formation mechanism of organo-chromium (III)complexes from bioreduction of chromium (VI)by Aeromonas hydrophila. *Environment International*, 129: 86–94.

Huang, Y., Zeng, Q., Hu, L., Zhong, H. & He, Z. 2021. Bioreduction performances and mechanisms of Cr(VI) by Sporosarcina saromensis W5 , a novel Cr(VI)-reducing facultative anaerobic bacteria. *Journal of Hazardous Materials*, 413(February): 125411. https://doi.org/10.1016/j.jhazmat.2021.125411.

Idoine, N.E., Raycraft, E.R., Shaw, R.A., Hobbs, S.F., Deady, E.A., Everett, P., Evans, E.J. & Mills, A.. 2022. *World Mineral Production 2016-2020*. Keyworth, Nottingham.

Igboamalu, T.E. & Chirwa, E.M.N. 2015. Kinetic Studies of Cr(VI) Reduction in an Indigenous Mixed Culture of Bacteria in the Presence of As(III). *Proceedings of the Water Environment Federation*, 2014(13): 5308–5327.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. & Beeregowda, K.N. 2014. Toxicity , mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2): 60–72.

Jeyasingh, J. & Philip, L. 2005. Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions. *Journal of Hazardous Materials*, 118(1–3): 113–120.

Jeyasingh, J., Somasundaram, V., Philip, L. & Bhallamudi, S.M. 2011. Pilot scale studies on the remediation of chromium contaminated aquifer using bio-barrier and reactive zone technologies. *Chemical Engineering Journal*, 167(1): 206–214. http://dx.doi.org/10.1016/j.cej.2010.12.024.

Ji, Q., Yu, D., Zhang, G., Lan, H., Liu, H. & Qu, J. 2015. Microfluidic Flow through Polyaniline Supported by Lamellar-Structured Graphene for Mass-Transfer-Enhanced Electrocatalytic Reduction of Hexavalent Chromium. *Environmental Science and Technology*, 49(22): 13534–13541.

Jobby, R., Jha, P., Yadav, A.K. & Desai, N. 2018. Chemosphere Biosorption and biotransformation of hexavalent chromium [Cr(VI]: A comprehensive review. *Chemosphere*, 207: 255–266. https://doi.org/10.1016/j.chemosphere.2018.05.050.

Kabir, M.M., Naieum, A. & Fakhruddin, M. 2018. Isolation and characterization of

chromium(VI)-reducing bacteria from tannery effluents and solid wastes. *World Journal of Microbiology and Biotechnology*, 34(126): 1–17. http://dx.doi.org/10.1007/s11274-018-2510-z.

Kalola, V. & Desai, C. 2020. Biosorption of Cr(VI) by Halomonas sp. DK4, a halotolerant bacterium isolated from chrome electroplating sludge. *Environmental Science and Pollution Research*, 27(22): 27330–27344.

Karthika, N., Jananee, K. & Murugaiyan, V. 2016. Remediation of contaminated soil using soil washing-a review. *Journal of Engineering Research and Applications*, 6(1–2): 13–18. http://www.ijera.com/papers/Vol6_issue1/Part - 2/C601021318.pdf.

Kathiravan, M.N., Karthick, R. & Muthukumar, K. 2011. Ex situ bioremediation of Cr(VI) contaminated soil by Bacillus sp.: Batch and continuous studies. *Chemical Engineering Journal*, 169(1–3): 107–115. http://dx.doi.org/10.1016/j.cej.2011.02.060.

Khakbaz, A., De Nobili, M., Mainardis, M., Contin, M., Aneggi, E., Mattiussi, M., Cabras, I., Busut, M. & Goi, D. 2020. Monitoring of heavy metals, eox and las in sewage sludge for agricultural use: A case study. *Detritus*, 12: 160–168.

Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I. & Dumat, C. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration*, 182: 247–268.

Khambhaty, Y., Mody, K., Basha, S. & Jha, B. 2009. Biosorption of Cr(VI) onto marine Aspergillus niger: Experimental studies and pseudo-second order kinetics. *World Journal of Microbiology and Biotechnology*, 25(8): 1413–1421.

Khan, M.A., Zhao, H., Zou, W., Chen, Z., Cao, W., Fang, J., Xu, J., Zhang, L. & Zhang, J. 2018. *Recent Progresses in Electrocatalysts for Water Electrolysis*. Springer

Singapore. https://doi.org/10.1007/s41918-018-0014-z.

Khedr, S., Shouman, M., Fathy, N. & Attia, A. 2014. Effect of Physical and Chemical Activation on the Removal of Hexavalent Chromium Ions Using Palm Tree Branches. *ISRN Environmental Chemistry*: 1–10.

Kholisa, B. & Chirwa, E.M.N. 2021. Bioreduction of Cr (VI) Using Bacterial Consortia Isolated from a Municipal Wastewater Sludge Experiencing Cr (VI) Loading from an Abandoned Chrome Foundry. *Chemical Engineering Transactions*, 88: 115–120.

Kholisa, B., Matsena, M. & Chirwa, E.M.N. 2021. Evaluation of cr(Vi) reduction using indigenous bacterial consortium isolated from a municipal wastewater sludge: Batch and kinetic studies. *Catalysts*, 11(9).

Köber, R., Schäfer, D., Erbert, M. & Dahmke, A. 2002. Coupled in situ reactors using Fe0 and activated carbon for the remediation of complex contaminant mixtures in groundwater. *Groundwater Quality 2001*, 275(275): 435–518.

Kotaś, J. & Stasicka, Z. 2000. Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution*.

Kožuh, N., Štupar, J. & Gorenc, B. 2000. Reduction and oxidation processes of chromium in soils. *Environmental Science and Technology*, 34(1): 112–119.

Krishna, K.R. & Philip, L. 2005. Bioremediation of Cr (VI) in contaminated soils. *Journal of Hazardous Materials*, 121: 109–117.

Ksheminska, H., Fedorovych, D., Honchar, T., Ivash, M. & Gonchar, M. 2008. Yeast tolerance to chromium depends on extracellular chromate reduction and Cr(III) chelation. *Food Technology and Biotechnology*, 46(4): 419–426.

Kumar, M. & Saini, H.S. 2019. Reduction of hexavalent chromium (VI) by indigenous alkaliphilic and halotolerant Microbacterium sp. M5: comparative studies under growth and nongrowth conditions. *Journal of Applied Microbiology*, 127(4): 1057–1068.

Kumar, V. & Dwivedi, S.K. 2019. Hexavalent chromium reduction ability and bioremediation potential of Aspergillus flavus CR500 isolated from electroplating wastewater. *Chemosphere*, 237: 124567. https://doi.org/10.1016/j.chemosphere.2019.124567.

Lai, K.C.K. & Lo, I.M.C. 2008. Removal of chromium (VI) by acid-washed zero-valent iron under various groundwater geochemistry conditions. *Environmental Science and Technology*, 42(4): 1238–1244.

Landrot, G., Tappero, R., Webb, S.M. & Sparks, D.L. 2012. Arsenic and chromium speciation in an urban contaminated soil. *Chemosphere*, 88(10): 1196–1201. http://dx.doi.org/10.1016/j.chemosphere.2012.03.069.

Li, J., Ding, Y., Wang, K., Li, N., Qian, G., Xu, Y. & Zhang, J. 2020. Comparison of humic and fulvic acid on remediation of arsenic contaminated soil by electrokinetic technology. *Chemosphere*, 241: 125038. https://doi.org/10.1016/j.chemosphere.2019.125038.

Li, L.L., Feng, X.Q., Han, R.P., Zang, S.Q. & Yang, G. 2017. Cr(VI) removal via anion exchange on a silver-triazolate MOF. *Journal of Hazardous Materials*, 321: 622–628.

Li, S. & Petzold, L. 2000. Software and algorithms for sensitivity analysis of large-scale differential algebraic systems. *Journal of Computational and Applied Mathematics*, 125(1–2): 131–145.

Li, X., Fan, M., Liu, L., Chang, J. & Zhang, J. 2019. Treatment of high-concentration

chromium-containing wastewater by sulfate-reducing bacteria acclimated with ethanol. *Water Science and Technology*, 80(12): 2362–2372.

Li, Y., Gao, Y., Zhang, Q., Wang, R., Li, C., Mao, J., Guo, L., Wang, F., Zhang, Z. & Wang, L. 2021. Flexible and free-standing pristine polypyrrole membranes with a nanotube structure for repeatable Cr(VI) ion removal. *Separation and Purification Technology*, 258(July 2020).

Lin, S.H. & Kiang, C.D. 2003. Chromic acid recovery from waste acid solution by an ion exchange process: Equilibrium and column ion exchange modeling. *Chemical Engineering Journal*, 92(1–3): 193–199.

Van Der Lingen, E. & Paton, A. 2018. Market implications for technology acquisition modes in the South African ferrochrome context. *Journal of the Southern African Institute of Mining and Metallurgy*, 118(10): 1087–1094.

Liu, L., Li, W., Song, W. & Guo, M. 2018. Remediation techniques for heavy metal-contaminated soils: Principles and applicability. *Science of the Total Environment*, 633: 206–219. https://doi.org/10.1016/j.scitotenv.2018.03.161.

Liu, S., Chen, M., Cao, X., Li, G., Zhang, D., Li, M., Meng, N., Yin, J. & Yan, B. 2020. Chromium (VI) removal from water using cetylpyridinium chloride (CPC)-modified montmorillonite. *Separation and Purification Technology*, 241(December 2019): 116732. https://doi.org/10.1016/j.seppur.2020.116732.

Liu, S.J., Jiang, B., Huang, G.Q. & Li, X.G. 2006. Laboratory column study for remediation of MTBE-contaminated groundwater using a biological two-layer permeable barrier. *Water Research*, 40(18): 3401–3408.

Liu, S.J., Yang, Q.M., Yang, Y.K., Ding, H. & Qi, Y. 2017. In situ remediation of

tetrachloroethylene and its intermediates in groundwater using an anaerobic/aerobic permeable reactive barrier. *Environmental Science and Pollution Research*, 24(34): 26615–26622.

Liu, W., Ni, J. & Yin, X. 2014. Synergy of photocatalysis and adsorption for simultaneous removal of Cr(VI) and Cr(III) with TiO2 and titanate nanotubes. *Water Research*, 53(Iii): 12–25. http://dx.doi.org/10.1016/j.watres.2013.12.043.

Liu, Y.G., Xu, W.H., Zeng, G.M., Li, X. & Gao, H. 2006. Cr(VI) reduction by Bacillus sp. isolated from chromium landfill. *Process Biochemistry*, 41(9): 1981–1986.

Losi, M.E., Amrhein, C. & Frankenberger, W.T. 1994. FACTORS AFFECTING CHEMICAL AND BIOLOGICAL REDUCTION OF HEXAVALENT CHROMIUM IN SOIL. *Environmental Toxicology and Chemistry*, 13(11): 1727–1735.

Ludwig, R.D., McGregor, R.G., Blowes, D.W., Benner, S.G. & Mountjoy, K. 2002. A Permeable Reactive Barrier for Treatment of Heavy Metals. *Ground Water*, 40(1): 59–66.

Luo, R.H. & Keh, H.J. 2021. Electrophoresis and electric conduction in a salt-free suspension of charged particles. *Electrophoresis*, 42(21–22): 2134–2142.

Luo, W. & Li, T. 2012. The use of chromium minerals in the 4th-3rd century BC China? A preliminary study of a bronze Pan unearthed from Jiuliandun Graves, Hubei Province, central southern China. *Journal of Raman Spectroscopy*, 43(2): 303–306.

Lyu, H., Tang, J., Huang, Y., Gai, L., Zeng, E.Y., Liber, K. & Gong, Y. 2017. Removal of hexavalent chromium from aqueous solutions by a novel biochar supported nanoscale iron sulfide composite. *Chemical Engineering Journal*, 322: 516–524. http://dx.doi.org/10.1016/j.cej.2017.04.058.

Ma, S., Song, C.S., Chen, Y., Wang, F. & Chen, H.L. 2018. Hematite enhances the removal of Cr(VI) by Bacillus subtilis BSn5 from aquatic environment. *Chemosphere*, 208: 579–585. https://doi.org/10.1016/j.chemosphere.2018.06.037.

Mackay, D.M. & Cherry, J.A. 1989. Groundwater contamination: Pump-and-treat remediation. *Environmental Science and Technology*, 23(6): 630–636.

Makino, T., Kamewada, K., Hatta, T., Takahashi, Y. & Sakurai, Y. 1998. Determination of optimal chromium oxidation conditions and evaluation of soil oxidative activity in soils. *Journal of Geochemical Exploration*, 64: 435–441.

Mangaiyarkarasi, M.S.M., Rao, T.S. & Tata, B.V.R. 2011. Bioreduction of Cr (VI) by alkaliphilic Bacillus subtilis and interaction of the membrane groups. *Saudi Journal of Biological Sciences*, 18(2): 157–167. http://dx.doi.org/10.1016/j.sjbs.2010.12.003.

Mbonambi, N.C. & Chirwa, E.M.N. 2019. Biological remediation of chromium (VI) in aquifer media columns. *Chemical Engineering Transactions*, 76(Vi): 1333–1338.

Mbonambi, N.C., Mojela, S.M. & Chirwa, E.M.N. 2019. Microbial chromium (VI) immobilization in saturated aquifer columns using culture inoculated soil. *Chemical Engineering Transactions*, 74: 1459–1464.

Megharaj, M., Avudainayagam, S. & Naidu, R. 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. *Current Microbiology*, 47(1): 51–54.

Michalak, I., Chojnacka, K. & Witek-Krowiak, A. 2013. State of the art for the biosorption process - A review. *Applied Biochemistry and Biotechnology*, 170(6): 1389–1416.

Middleton, S.S., Latmani, R.B., Mackey, M.R., Ellisman, M.H., Tebo, B.M. & Criddle, C.S.

2003. Cometabolism of Cr(VI) by Shewanella oneidensis MR-1 produces cell-associated reduced chromium and inhibits growth. *Biotechnology and Bioengineering*, 83(6): 627–637.

Mishra, S. & Bharagava, R.N. 2016. Toxic and genotoxic effects of hexavalent chromium in environment and its bioremediation strategies. *Journal of Environmental Science and Health - Part C Environmental Carcinogenesis and Ecotoxicology Reviews*, 34(1): 1–32. http://dx.doi.org/10.1080/10590501.2015.1096883.

Mishra, V, Samantaray, D.P., Dash, S.K., Mishra, B.B. & Swain, R.K. 2010. Study on Hexavalent Chromium Reduction by Chromium Resistant Bacterial Isolates of Sukinda Mining Area. *Our Nature*: 63–71.

Mishra, V., Samantaray, D.P., Dash, S.K., Sethi, A.K. & Mishra, B.B. 2010. Aerobic hexavalent chromium reduction by Acenetobacter calcoaciticus. *Biosciences Biotechnology Research Asia*, 7(2): 963–967.

Mohamed, M.S.M., El-Arabi, N.I., El-Hussein, A., El-Maaty, S.A. & Abdelhadi, A.A. 2020. Reduction of chromium-VI by chromium-resistant Escherichia coli FACU: a prospective bacterium for bioremediation. *Folia Microbiologica*, 65(4): 687–696.

Mohan, D. & Pittman, C.U. 2006. Activated carbons and low cost adsorbents for remediation of tri- and hexavalent chromium from water. *Journal of Hazardous Materials*, 137(2): 762–811.

Molokwane, P.E. 2010. *SIMULATION OF IN SITU BIOREMEDIATION OF Cr(VI) IN GROUNDWATER AQUIFER ENVIRONMENTS USING A MICROBIAL CULTURE BARRIER*.

Molokwane, P.E. & Chirwa, E.M.N. 2013. Modelling biological Cr(VI) reduction in aquifer

microcosm column systems. *Water Science and Technology*, 67(12): 2733–2738.

Molokwane, P.E., Meli, K.C. & Nkhalambayausi-chirwa, E.M. 2008. Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa). *Water Research*, 42: 4538–4548.

Molokwane, P.E. & Nkhalambayausi-chirwa, E.M. 2009. Microbial culture dynamics and chromium (VI) removal in packed-column microcosm reactors. *Water Science and Technology*, 60(2): 381–388.

Morales-Barrera, L. & Cristiani-Urbina, E. 2008. Hexavalent chromium removal by a Trichoderma inhamatum fungal strain isolated from tannery effluent. *Water, Air, and Soil Pollution*, 187(1–4): 327–336.

Mount, D.R. & Hockett, J.R. 2000. Use of toxicity identification evaluation methods to characterize, identify, and confirm hexavalent chromium toxicity in an industrial effluent. *Water Research*, 34(4): 1379–1385.

Moutsatsou, A., Gregou, M., Matsas, D. & Protonotarios, V. 2006. Washing as a remediation technology applicable in soils heavily polluted by mining-metallurgical activities. *Chemosphere*, 63(10): 1632–1640.

Mtimunye, P.J. & Chirwa, E.M.N. 2014. Finite difference simulation of biological chromium (VI) reduction in aquifer media columns. *Water SA*, 40(2): 359–368.

Mulligan, C.N., Yong, R.N. & Gibbs, B.F. 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology*, 60: 193–207.

Murthy, Y.R., Tripathy, S.K. & Kumar, C.R. 2011. Chrome ore beneficiation challenges &

opportunities - A review. *Minerals Engineering*, 24(5): 375–380. http://dx.doi.org/10.1016/j.mineng.2010.12.001.

Murugavelh, S. & Mohanty, K. 2012. Bioreduction of hexavalent chromium by free cells and cell free extracts of Halomonas sp. *Chemical Engineering Journal*, 203: 415–422. http://dx.doi.org/10.1016/j.cej.2012.07.069.

Murugavelh, S. & Mohanty, K. 2018. Performance of Halomonas sp. to reduce hexavalent chromium in batch and continuous fixed film reactor. *Journal of Environmental Chemical Engineering*, 6(2): 2561–2567. https://doi.org/10.1016/j.jece.2018.03.037.

Naidu, R., Bekele, D.N. & Birke, V. 2014. Permeable reactive barriers: Cost-effective and sustainable remediation of groundwater. In *Permeable Reactive Barrier: Sustainable Groundwater Remediation*. 1–23.

Nam, A., Choi, U.S., Yun, S.T., Choi, J.W., Park, J.A. & Lee, S.H. 2018. Evaluation of amine-functionalized acrylic ion exchange fiber for chromium(VI) removal using flow-through experiments modeling and real wastewater. *Journal of Industrial and Engineering Chemistry*, 66: 187–195. https://doi.org/10.1016/j.jiec.2018.05.029.

Niroumand, H., Nazir, R. & Kassim, K.A. 2012. The performance of electrochemical remediation technologies in soil mechanics. *International Journal of Electrochemical Science*, 7(6): 5708–5715.

Nkhalambayausi-Chirwa, E.M. & Wang, Y.-T. 2005. Modeling Cr(VI) Reduction and Phenol Degradation in a Coculture Biofilm Reactor. *Journal of Environmental Engineering*, 131(11): 1495–1506.

Nkhalambayausi-Chirwa, E.M. & Wang, Y.T. 2004. Modeling hexavalent chromium removal in a Bacillus sp. fixed-film bioreactor. *Biotechnology and Bioengineering*,

87(7): 874–883.

Nkhalambayausi-Chirwa, E.M. & Wang, Y.T. 2001. Simultaneous chromium(VI) reduction and phenol degradation in a fixed-film coculture bioreactor: Reactor performance. *Water Research*, 35(8): 1921–1932.

Obiri-nyarko, F., Grajales-mesa, S.J. & Malina, G. 2014. An overview of permeable reactive barriers for in situ sustainable groundwater remediation. *Chemosphere*, 111: 243–259. http://dx.doi.org/10.1016/j.chemosphere.2014.03.112.

Pagnanelli, F., Cruz Viggi, C., Cibati, A., Uccelletti, D., Toro, L. & Palleschi, C. 2012. Biotreatment of Cr(VI) contaminated waters by sulphate reducing bacteria fed with ethanol. *Journal of Hazardous Materials*, 199–200: 186–192. http://dx.doi.org/10.1016/j.jhazmat.2011.10.082.

Pal, A., Dutta, S. & Paul, A.K. 2005. Reduction of Hexavalent Chromium by Cell-Free Extract of Bacillus sphaericus AND 303 Isolated from Serpentine Soil. , 51: 327–330.

Pattanapipitpaisal, P., Mabbett, A.N., Finlay, J.A., Beswick, A.J., Paterson-Beedle, M., Essa, A., Wright, J., Tolley, M.R., Badar, U., Ahmed, N., Hobman, J.L., Brown, N.L. & Macaskie, L.E. 2002. Reduction of cr(vi) and bioaccumulation of chromium by gram positive and gram negative microorganisms not previously exposed to cr-stress. *Environmental Technology*, 23(7): 731–745.

Poljsak, B., Pócsi, I., Raspor, P. & Pesti, M. 2010. Interference of chromium with biological systems in yeasts and fungi: A review. *Journal of Basic Microbiology*, 50(1): 21–36.

Pradhan, D., Behari, L., Sawyer, M., Rahman, P.K.S.M.S.M., Sukla, L.B., Sawyer, M. & Rahman, P.K.S.M.S.M. 2017. Recent bioreduction of hexavalent chromium in wastewater treatment : A review. *Journal of Industrial and Engineering Chemistry*, 55:

1–20. http://dx.doi.org/10.1016/j.jiec.2017.06.040.

Pugazhenthi, G., Sachan, S., Kishore, N. & Kumar, A. 2005. Separation of chromium (VI) using modified ultrafiltration charged carbon membrane and its mathematical modeling. *Journal of Membrane Science*, 254(1–2): 229–239.

Qian, A., Liao, P., Yuan, S. & Luo, M. 2014. Efficient reduction of Cr(VI) in groundwater by a hybrid electro-Pd process. *Water Research*, 48(1): 326–334. http://dx.doi.org/10.1016/j.watres.2013.09.043.

Rad, P.R. & Fazlali, A. 2020. Optimization of permeable reactive barrier dimensions and location in groundwater remediation contaminated by landfill pollution. *Journal of Water Process Engineering*, 35(October 2019): 101196. https://doi.org/10.1016/j.jwpe.2020.101196.

Rahman, Z. & Thomas, L. 2021. Chemical-Assisted Microbially Mediated Chromium (Cr) (VI) Reduction Under the Influence of Various Electron Donors, Redox Mediators, and Other Additives: An Outlook on Enhanced Cr(VI) Removal. *Frontiers in Microbiology*, 11(January): 1–19.

Rapti, S., Pournara, A., Sarma, D., Papadas, I.T., Armatas, G.S., Tsipis, A.C., Lazarides, T., Kanatzidis, M.G. & Manos, M.J. 2016. Correction: Selective capture of hexavalent chromium from an anion-exchange column of metal organic resin-alginic acid composite. *Chemical Science*, 7(3): 2438.

Reichert, P. 1998. Aquasim - A tool for simulation and data analysis of aquatic systems. , 30(2 pt 2): 21–30.

Ren, B., Zhang, Q., Zhang, X., Zhao, L. & Li, H. 2018. Biosorption of Cr(vi) from aqueous solution using dormant spores of Aspergillus niger. *RSC Advances*, 8(67): 38157–

38165.

Rock, M.L., James, B.R. & Helz, G.R. 2001. Hydrogen peroxide effects on chromium oxidation state and solubility in four diverse, chromium-enriched soils. *Environmental Science and Technology*, 35(20): 4054–4059.

Rossouw, P.S. 2009. *Environmental extractability of chromium (III) and nickel from soils of South Africa's Eastern Highveld*. University of Pretoria.

Saha, R., Nandi, R. & Saha, B. 2011. Sources and toxicity of hexavalent chromium. *Journal of Coordination Chemistry*, 64(10): 1782–1806.

Sahu, S.K., Meshram, P., Pandey, B.D., Kumar, V. & Mankhand, T.R. 2009. Removal of chromium(III) by cation exchange resin, Indion 790 for tannery waste treatment. *Hydrometallurgy*, 99(3–4): 170–174. http://dx.doi.org/10.1016/j.hydromet.2009.08.002.

Sakellariou, L. & Papassiopi, N. 2018. An Approach to Electrokinetic Removal of Cr(VI) from Soil and Kaolin Samples. *Bulletin of Environmental Contamination and Toxicology*, 101(6): 718–724. http://dx.doi.org/10.1007/s00128-018-2432-3.

Sanghi, R. & Srivastava, A. 2010. Long-term chromate reduction by immobilized fungus in continuous column. *Chemical Engineering Journal*, 162(1): 122–126. http://dx.doi.org/10.1016/j.cej.2010.05.011.

Sardohan, T., Kir, E., Gulec, A. & Cengeloglu, Y. 2010. Removal of Cr(III) and Cr(VI) through the plasma modified and unmodified ion-exchange membranes. *Separation and Purification Technology*, 74(1): 14–20. http://dx.doi.org/10.1016/j.seppur.2010.05.001.

Sathishkumar, K., Murugan, K., Benelli, G., Higuchi, A. & Rajasekar, A. 2017. Bioreduction of hexavalent chromium by Pseudomonas stutzeri L1 and Acinetobacter baumannii L2. *Annals of Microbiology*, 67(1): 91–98. http://dx.doi.org/10.1007/s13213-016-1240-4.

Saucedo-Rivalcoba, V., Martínez-Hernández, A.L., Martínez-Barrera, G., Velasco-Santos, C., Rivera-Armenta, J.L. & Castaño, V.M. 2011. Removal of hexavalent chromium from water by polyurethane-keratin hybrid membranes. *Water, Air, and Soil Pollution*, 218(1–4): 557–571.

Schulte, R.F. 2018. *2018 Minerals Yearbook*. https://www.usgs.gov.

Schweighofer, J.A. V, Wehrl, M., Baumgärtel, S. & Rohn, J. 2021. Detecting Groundwater Temperature Shifts of a Subsurface Urban Heat Island in SE Germany.

Seh-Bardan, B.J., Othman, R., Wahid, S.A., Husin, A. & Sadegh-Zadeh, F. 2012. Bioleaching of heavy metals from mine tailings by aspergillus fumigatus. *Bioremediation Journal*, 16(2): 57–65.

Semprini, L. & McCarty, P.L. 1991. Comparison Between Model Simulations and Field Results for In-Situ Biorestoration of Chlorinated Aliphatics: Part 1. Biostimulation of Methanotrophic Bacteria. *Groundwater*, 29(3): 365–374.

Sharma, K. 2002. MICROBIAL Cr(VI) REDUCTION: ROLE OF ELECTRON DONORS, ACCEPTORS, AND MECHANISMS, WITH SPECIAL EMPHASIS ON CLOSTRIDIUM spp. , (Vi).

Shen, H. & Wang, Y. 1994. Modeling Hexavalent Chromium Reduction in Escherichia coli 33456. *Biotechnology and Bioengineering*, 43: 293–300.

Shi, T., Wang, Z., Liu, Y., Jia, S. & Changming, D. 2009. Removal of hexavalent chromium

from aqueous solutions by D301, D314 and D354 anion-exchange resins. *Journal of Hazardous Materials*, 161(2–3): 900–906.

Shukla, K.P., Sharma, S., Singh, N.K. & Singh, V. 2012. Deciphering Rhizosphere Soil System for Strains Having Plant Growth Promoting and Bioremediation Traits. *Agricultural Research*, 1(3): 251–257.

Soffritti, I., D'Accolti, M., Lanzoni, L., Volta, A., Bisi, M., Mazzacane, S. & Caselli, E. 2019. The potential use of microorganisms as restorative agents: An update. *Sustainability (Switzerland)*, 11(14): 1–17.

Soni, S.K., Singh, R. & Awasthi, A. 2013. In vitro Cr(VI) reduction by cell-free extracts of chromate-reducing bacteria isolated from tannery effluent irrigated soil. *Environmental Science and Pollution Research*, 20: 1661–1674.

Sun, Y., Lan, J., Du, Y., Guo, L., Du, D., Chen, S., Ye, H. & Zhang, T.C. 2020. Chromium(VI) bioreduction and removal by Enterobacter sp. SL grown with waste molasses as carbon source: Impact of operational conditions. *Bioresource Technology*, 302(September 2019): 121974. https://doi.org/10.1016/j.biortech.2019.121974.

Suthersan, S.S., Horst, J., Schnobrich, M., Welty, N. & McDonough, J. 2017. *RemediationEngineering: Design Concepts*. Second. Boca Raton: CRC Press , Taylor & Francis Group.

Tadeo-jalife, N.R., Vasquez-medrano, R. & Quevedo, I.R. 2021. Assessing the Adsorption of Bipyridinium Herbicides on Model Soil Granular Media. , 2(January).

Tan, H., Wang, C., Zeng, G., Luo, Y., Li, H. & Xu, H. 2020. Bioreduction and biosorption of Cr(VI) by a novel Bacillus sp. CRB-B1 strain. *Journal of Hazardous Materials*, 386(August 2019): 121628. https://doi.org/10.1016/j.jhazmat.2019.121628.

Tang, W., Peng, Z., Li, L., Yue, T., Wang, J., Li, Z., Li, R., Chen, J., Colvin, V.L. & Yu, W.W. 2012. Porous stainless steel supported magnetite crystalline membranes for hexavalent chromium removal from aqueous solutions. *Journal of Membrane Science*, 392–393: 150–156. http://dx.doi.org/10.1016/j.memsci.2011.12.013.

Tang, Y., Liao, X., Zhang, X., Peng, G., Gao, J. & Chen, L. 2021. Science of the Total Environment Enhanced adsorption of hexavalent chromium and the microbial effect on quartz sand modi fi ed with Al-layered double hydroxides. *Science of the Total Environment*, 762: 143094. https://doi.org/10.1016/j.scitotenv.2020.143094.

Tekerlekopoulou, A.G., Tsiflikiotou, M., Akritidou, L., Viennas, A., Tsiamis, G., Pavlou, S., Bourtzis, K. & Vayenas, D. V. 2013. Modelling of biological Cr(VI) removal in draw-fill reactors using microorganisms in suspended and attached growth systems. *Water Research*, 47(2): 623–636. http://dx.doi.org/10.1016/j.watres.2012.10.034.

Timková, I., Sedláková-Kaduková, J. & Pristaš, P. 2018. Biosorption and bioaccumulation abilities of actinomycetes/streptomycetes isolated from metal contaminated sites. *Separations*, 5(4).

Troiano, J.M., Jordan, D.S., Hull, C.J. & Geiger, F.M. 2013. Interaction of Cr(III) and Cr(VI) with hematite studied by second harmonic generation. *Journal of Physical Chemistry C*, 117(10): 5164–5171.

Turpeinen, R., Kairesalo, T. & Häggblom, M.M. 2004. Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils. *FEMS Microbiology Ecology*, 47(1): 39–50.

Upadhyay, N., Vishwakarma, K., Singh, J., Mishra, M., Tripathi, D.K. & Sharma, S. 2017. Tolerance and Reduction of Chromium (VI) by Bacillus sp . MNU16 Isolated from

Contaminated Coal Mining Soil. *Frontiers in Plant Science*, 8: 1–13.

Upadhyay, S. & Sinha, A. 2018. Role of microorganisms in Permeable Reactive Bio-Barriers (PRBBs) for environmental clean-up: A review. *Global NEST Journal*, 20(2): 269–280.

Viamajala, S., Peyton, B.M. & Petersen, J.N. 2003. Modeling chromate reduction in Shewanella oneidensis MR-1: Development of a novel dual-enzyme kinetic model. *Biotechnology and Bioengineering*, 83(7): 790–797.

Vickstrom, K.E., Azizian, M.F. & Semprini, L. 2017. Transformation of carbon tetrachloride and chloroform by trichloroethene respiring anaerobic mixed cultures and supernatant. *Chemosphere*, 182: 65–75. http://dx.doi.org/10.1016/j.chemosphere.2017.04.139.

Villacís-García, M., Villalobos, M. & Gutiérrez-Ruiz, M. 2015. Optimizing the use of natural and synthetic magnetites with very small amounts of coarse Fe(0) particles for reduction of aqueous Cr(VI). *Journal of Hazardous Materials*, 281: 77–86. http://dx.doi.org/10.1016/j.jhazmat.2014.07.007.

Wall, J.D. & Krumholz, L.R. 2006. Uranium reduction. *Annual Review of Microbiology*, 60: 149–166.

Wang, S. & Choi, H.J. 2013. Simulating fate and transport of chromium in saturated sediments. *Applied Mathematical Modelling*, 37(1–2): 102–111.

Wang, Y.T. & Shen, H. 1997. Modelling Cr(VI) reduction by pure bacterial cultures. *Water Research*, 31(4): 727–732.

Wani, P.A., Wahid, S., Khan, M.S.A., Rafi, N. & Wahid, N. 2019. Investigation of the role of chromium reductase for Cr (VI) reduction by Pseudomonas species isolated from Cr

(VI) contaminated effluent. *Biotechnology Research and Innovation*, 3(1): 38–46. https://doi.org/10.1016/j.biori.2019.04.001.

Wei, X.Z., Gan, Z.Q., Shen, Y.J., Qiu, Z.L., Fang, L.F. & Zhu, B.K. 2019. Negatively-charged nanofiltration membrane and its hexavalent chromium removal performance. *Journal of Colloid and Interface Science*, 553: 475–483. https://doi.org/10.1016/j.jcis.2019.06.051.

Witek-Krowiak, A. 2013. Application of beech sawdust for removal of heavy metals from water: Biosorption and desorption studies. *European Journal of Wood and Wood Products*, 71(2): 227–236.

Wu, J., Zhang, J. & Xiao, C. 2016. Focus on factors affecting pH, flow of Cr and transformation between Cr(VI) and Cr(III) in the soil with different electrolytes. *Electrochimica Acta*, 211: 652–662. http://dx.doi.org/10.1016/j.electacta.2016.06.048.

Xia, S., Song, Z., Jeyakumar, P., Shaheen, S.M., Rinklebe, J., Ok, Y.S., Bolan, N. & Wang, H. 2019. A critical review on bioremediation technologies for Cr(VI)-contaminated soils and wastewater. *Critical Reviews in Environmental Science and Technology*, 49(12): 1027–1078. https://doi.org/10.1080/10643389.2018.1564526.

Xu, R., Wu, K., Han, H., Ling, Z., Chen, Z., Liu, P., xiong, J., Tian, F., Zafar, Y., Malik, K. & Li, X. 2018. Co-expression of YieF and PhoN in Deinococcus radiodurans R1 improves uranium bioprecipitation by reducing chromium interference. *Chemosphere*, 211: 1156–1165. https://doi.org/10.1016/j.chemosphere.2018.08.061.

Xu, W., Jian, H. & Liu, Y. 2015. Bioreduction of Chromate by an Isolated Bacillus anthracis Cr-4 with Soluble Cr (III) Product. *Water, Air, & Soil Pollution*, 226(82).

Xue, F., Yan, Y., Xia, M., Muhammad, F., Yu, L., Xu, F., Shiau, Y.C., Li, D. & Jiao, B.

2017. Electro-kinetic remediation of chromium-contaminated soil by a three-dimensional electrode coupled with a permeable reactive barrier. *RSC Advances*, 7(86): 54797–54805.

Yadav, K.K., Gupta, N., Kumar, V. & Singh, J.K. 2017. Bioremediation of Heavy Metals From Contaminated Sites Using Potential Species : A Review Bioremediation of Heavy Metals From Contaminated Sites Using Potential Species : A Review. *Indian Journal of Environmental Protection*, 37(1): 65–84.

Yang, X., Zhao, Z., Yu, Y., Shimizu, K., Zhang, Z., Lei, Z. & Lee, D.J. 2020. Enhanced biosorption of Cr(VI) from synthetic wastewater using algal-bacterial aerobic granular sludge: Batch experiments, kinetics and mechanisms. *Separation and Purification Technology*, 251(June): 117323. https://doi.org/10.1016/j.seppur.2020.117323.

Yang, Y., Ge, Y., Zeng, H., Zhou, X., Peng, L. & Zeng, Q. 2017. Phytoextraction of cadmium-contaminated soil and potential of regenerated tobacco biomass for recovery of cadmium. *Scientific Reports*, 7(1): 1–10. http://dx.doi.org/10.1038/s41598-017-05834-8.

Yao, Y., Hu, L., Li, S., Zeng, Q., Zhong, H. & He, Z. 2020. Exploration on the bioreduction mechanisms of Cr(VI) and Hg(II) by a newly isolated bacterial strain Pseudomonas umsongensis CY-1. *Ecotoxicology and Environmental Safety*, 201(February): 110850. https://doi.org/10.1016/j.ecoenv.2020.110850.

Yin, W., Li, Y., Wu, J., Chen, G., Jiang, G., Li, P., Gu, J., Liang, H. & Liu, C. 2017. Enhanced Cr(VI) removal from groundwater by Fe0-H2O system with bio-amended iron corrosion. *Journal of Hazardous Materials*, 332: 42–50. http://dx.doi.org/10.1016/j.jhazmat.2017.02.045.

Zakaria, Z.A., Zakaria, Z., Surif, S. & Ahmad, W.A. 2007. Hexavalent chromium reduction by Acinetobacter haemolyticus isolated from heavy-metal contaminated wastewater. *Journal of Hazardous Materials*, 146(1–2): 30–38.

Zayed, A.M. & Terry, N. 2003. Chromium in the environment: Factors affecting biological remediation. *Plant and Soil*, 249(1): 139–156.

Zeng, Q., Hu, Y., Yang, Y., Hu, L., Zhong, H. & He, Z. 2019. Cell envelop is the key site for Cr(VI) reduction by Oceanobacillus oncorhynchi W4, a newly isolated Cr(VI) reducing bacterium. *Journal of Hazardous Materials*, 368(January): 149–155. https://doi.org/10.1016/j.jhazmat.2019.01.031.

Zhang, C., Ren, H.X., Zhong, C.Q. & Wu, D. 2020. Biosorption of Cr(VI) by immobilized waste biomass from polyglutamic acid production. *Scientific Reports*, 10(1): 1–8. http://dx.doi.org/10.1038/s41598-020-60729-5.

Zhang, X., Yan, J., Luo, X., Zhu, Y., Xia, L. & Luo, L. 2020. Simultaneous ammonia and Cr (VI) removal by Pseudomonas aeruginosa LX in wastewater. *Biochemical Engineering Journal*, 157(February): 107551. https://doi.org/10.1016/j.bej.2020.107551.

Zhao, Y., Kang, D., Chen, Z., Zhan, J. & Wu, X. 2018. Removal of chromium using electrochemical approaches: A review. *International Journal of Electrochemical Science*, 13(2): 1250–1259.

Zheng, Y., Liu, S., Dai, C., Duan, Y. & Makhinov, A.N. 2020. Study on the influence mechanism of underground mineral element Fe(II) on Cr(VI) transformation under subsurface and groundwater interaction zones. *Environmental Sciences Europe*, 32(62).

Zheng, Z., Li, Y., Zhang, X., Liu, P., Ren, J., Wu, G., Zhang, Y., Chen, Y. & Li, X. 2015. A

Bacillus subtilis strain can reduce hexavalent chromium to trivalent and an nfrA gene is involved. *International Biodeterioration and Biodegradation*, 97: 90–96. http://dx.doi.org/10.1016/j.ibiod.2014.10.017.

Zhitkovich, A., Voitkun, V. & Costa, M. 1996. Formation of the amino acid-DNA complexes by hexavalent and trivalent chromium in vitro: Importance of trivalent chromium and the phosphate group. *Biochemistry*, 35(22): 7275–7282.

Zhou, M., Xu, J., Zhu, S., Wang, Y. & Gao, H. 2018. Exchange electrode-electrokinetic remediation of Cr-contaminated soil using solar energy. *Separation and Purification Technology*, 190(October 2016): 297–306. http://dx.doi.org/10.1016/j.seppur.2017.09.006.

Zhu, G., Guo, Q., Yang, Junxing, Zhang, H., Wei, R., Wang, C., Peters, M., Zhou, X. & Yang, Jun. 2015. Washing out heavy metals from contaminated soils from an iron and steel smelting site. *Frontiers of Environmental Science and Engineering*, 9(4): 634–641.

Zhu, Y., Yan, J., Xia, L., Zhang, X. & Luo, L. 2019. Mechanisms of Cr(VI) reduction by Bacillus sp. CRB-1, a novel Cr(VI)-reducing bacterium isolated from tannery activated sludge. *Ecotoxicology and Environmental Safety*, 186(July): 109792. https://doi.org/10.1016/j.ecoenv.2019.109792.

Zolfaghari, G. & Kargar, M. 2019. Nanofiltration and microfiltration for the removal of chromium, total dissolved solids, and sulfate from water. *MethodsX*, 6(January): 549–557.

APPENDIX A: AQUASIM SIMULATION

BATCH STUDIES

****

AQUASIM Version 2.0 (win/mfc) - Listing of System Definition

****

Date and time of listing: 01/19/2022 18:48:22

****

Variables

****

C: Description: Cr(VI) concentration at time t

Type: Dyn. Volume State Var.

Unit: ML-1

Relative Accuracy: 1e-006

Absolute Accuracy: 1e-006

C_0: Description: initial Cr(VI) concentration

Type: Constant Variable

Unit: ML-3

Value: 50

Standard Deviation: 1

Minimum: 0

Maximum: 55

Sensitivity Analysis: inactive

Parameter Estimation: inactive

C_meas: Description: Cr(VI) concentration measured

Type: Real List Variable

Unit: ML-3

Argument: t

Standard Deviations: global

Rel. Stand. Deviat.: 0

Abs. Stand. Deviat.: 1

Minimum: 0

Maximum: 1e+009

Interpolation Method: linear interpolation

Sensitivity Analysis: inactive

Real Data Pairs (9 pairs):

0 45.733607

1	32.243169
2	17.489071
3	7.2909836
4	3.1789617
5	0
6	0
7	0
24	0

K_c: Description: Half velocity constant

Type: Constant Variable

Unit: ML-3

Value: 1072.7685

Standard Deviation: 1

Minimum: 0

Maximum: 1500

Sensitivity Analysis: inactive

Parameter Estimation: active

---

k_m: Description: maximum specific Cr(VI) reduction rate coefficient

Type: Constant Variable

Unit: T-1

Value: 0.62412878

Standard Deviation: 1

Minimum: 0

Maximum: 10

Sensitivity Analysis: inactive

Parameter Estimation: active

R_c: Description: finite Cr(VI) reduction capacity

Type: Constant Variable

Unit: ML-3

Value: 26.593488

Standard Deviation: 1

Minimum: 0

Maximum: 50

Sensitivity Analysis: inactive

Parameter Estimation: active

---

t: Description: time

Type: Program Variable

Unit: h

Reference to: Time

X_0: Description: initial active cell concentration

Type: Constant Variable

Unit: ML-3

Value: 994.87565

Standard Deviation: 1

Minimum: 0

Maximum: 1500

Sensitivity Analysis: inactive

Parameter Estimation: active

****

****

Processes

****

Cr_reduction: Description: Cr(VI) reduction

Type: Dynamic Process

Rate: (k_m*C/(K_c+C))*(X_0-((C_0-C)/R_c))

Stoichiometry:

Variable : Stoichiometric Coefficient

C : -1

****

****

Compartments

****

Batch100: Description: Batch reactor with 100 mg/L Cr(VI)

Type: Mixed Reactor Compartment

Compartment Index: 0

Active Variables: C

Active Processes: Cr_reduction

Initial Conditions:

Variable(Zone) : Initial Condition

C(Bulk Volume) : C_0

Inflow: 0

Loadings:

Volume: 1

Accuracies:

Rel. Acc. Q: 0.001

Abs. Acc. Q: 0.001

Rel. Acc. V: 0.001

Abs. Acc. V: 0.001

****

****

Definitions of Calculations

****

CrReduction: Description: Cr(VI) reduction

Calculation Number: 0

Initial Time: 0

Initial State: given, made consistent

Step Size: 0.01

Num. Steps: 12500

Status: active for simulation

inactive for sensitivity analysis

****

****

Definitions of Parameter Estimation Calculations

****

fit1: Description:

Calculation Number: 0

Initial Time: 0

Initial State: given, made consistent

Status: active

Fit Targets:

Data : Variable (Compartment,Zone,Time/Space)

C_meas : C (Batch100,Bulk Volume,0)

****

****

Plot Definitions

****

Cr: Description: Cr(VI) reduction

Abscissa: Time

Title: Cr(VI) reduction

Abscissa Label: Time (h)

Ordinate Label: Cr(VI) concentration (mg/L)

Curves:

Type : Variable [CalcNum,Comp.,Zone,Time/Space]

Value : C_meas [0,Batch100,Bulk Volume,0]

Value : C [0,Batch100,Bulk Volume,0]

****

****

Calculation Parameters

****

Numerical Parameters: Maximum Int. Step Size: 1

Maximum Integrat. Order: 5

Number of Codiagonals: 1000

Maximum Number of Steps: 1000

Fit Method: simplex

Max. Number of Iterat.: 100

****

****

Calculated States

****

Calc. Num. Num. States Comments

0 9 Range of Times: 0 - 24

****

CONTINUOUS FLOW STUDIES

****

AQUASIM Version 2.0 (win/mfc) - Listing of System Definition

****

Date and time of listing: 03/15/2022 17:08:47

****

Variables

****

A: Description: Cross-sectional area

Type: Constant Variable

Unit: m2

Value: 0.0165

Standard Deviation: 1

Minimum: 0

Maximum: 10

Sensitivity Analysis: inactive

Parameter Estimation: inactive

Af: Description: Biofilm surface area

Type: Constant Variable

Unit: m2

Value: 0.095

Standard Deviation: 1

Minimum: 0

Maximum: 10

Sensitivity Analysis: inactive

Parameter Estimation: inactive

Alpha: Description:

Type: Formula Variable

Unit:

Expression: 0.5

C: Description: Cr(VI) concentration

Type: Dyn. Volume State Var.

Unit:

Relative Accuracy: 1e-006

Absolute Accuracy: 1e-006

Cin: Description: Influent Concentration

Type: Real List Variable

Unit: mg/L

Argument: t

Standard Deviations: global

Rel. Stand. Deviat.: 0

Abs. Stand. Deviat.: 1

Minimum: 0

Maximum: 1e+009

Interpolation Method: linear interpolation

Sensitivity Analysis: inactive

Real Data Pairs (82 pairs):

0	43.103825
1	46.027322
2	45.754098
3	46.57377
4	45.918033
.	.
.	.
86	62.47541
87	60.808743
88	63.903825
89	65.754098
90	62.885246

Cout: Description: Effluent concentration

Type: Real List Variable

Unit: mg/L

Argument: t

Standard Deviations: global

Rel. Stand. Deviat.: 0

Abs. Stand. Deviat.: 1

Minimum: 0

Maximum: 1e+009

Interpolation Method: linear interpolation

Sensitivity Analysis: inactive

Real Data Pairs (81 pairs):

0	0
1	0
2	0
3	0
4	0
.	.
.	.
86	2.2295082
87	6.3278689
88	7.9672131
89	11.874317
90	21.765027

Cr: Description: Cr(VI) toxicity threshold

Type: Constant Variable

Unit:

Value: 10

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: inactive

Parameter Estimation: inactive

K: Description: Dimensionless Cr(VI) inhibition con
stant

Type: Constant Variable

Unit:

Value: 1

Standard Deviation: 1

Minimum: 0

Maximum: 1000

Sensitivity Analysis: inactive

Parameter Estimation: inactive

---

kad: Description:

Type: Constant Variable

Unit:

Value: 0.41640783

Standard Deviation: 1

Minimum: 0

Maximum: 1000

Sensitivity Analysis: inactive

Parameter Estimation: active

Kc: Description: Saturation concentration

Type: Constant Variable

Unit:

Value: 0.85661238

Standard Deviation: 1

Minimum: 0

Maximum: 1000

Sensitivity Analysis: inactive

Parameter Estimation: active

---

kL: Description: Dispersion coeffient

Type: Constant Variable

Unit:

Value: 3e-006

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: inactive

Parameter Estimation: active

---

km: Description: Cr(VI) specific reduction rate

Type: Constant Variable

Unit:

Value: 1.1482897

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: inactive

Parameter Estimation: active

Lw: Description: Biofilm length

Type: Constant Variable

Unit: m

Value: 0.15

Standard Deviation: 1

Minimum: 0

Maximum: 10

Sensitivity Analysis: inactive

Parameter Estimation: inactive

---

p: Description: Density of sand

Type: Constant Variable

Unit: kg/m3

Value: 10

Standard Deviation: 1

Minimum: 0

Maximum: 3000

Sensitivity Analysis: inactive

Parameter Estimation: inactive

Q: Description: flow rate

Type: Constant Variable

Unit: m3/day

Value: 0.0048

Standard Deviation: 1

Minimum: 0

Maximum: 10

Sensitivity Analysis: inactive

Parameter Estimation: inactive

Rc: Description: Cell reduction capacity

Type: Constant Variable

Unit:

Value: 0.05

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: inactive

Parameter Estimation: active

S: Description: Adsorbed Cr(VI) conc

Type: Dyn. Volume State Var.

Unit:

Relative Accuracy: 1e-006

Absolute Accuracy: 1e-006

S_eq: Description:

Type: Constant Variable

Unit:

Value: 0.01

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: inactive

Parameter Estimation: inactive

---

t: Description: time

Type: Program Variable

Unit: days

Reference to: Time

---

theta: Description: Porosity

Type: Constant Variable

Unit:

Value: 0.42

Standard Deviation: 1

Minimum: 0

Maximum: 10

Sensitivity Analysis: inactive

Parameter Estimation: inactive

V: Description: reactor volume

Type: Formula Variable

Unit: m3

Expression: 0.00195

X0: Description: Initial biomass concentration

Type: Constant Variable

Unit:

Value: 0.14248886

Standard Deviation: 1

Minimum: 0

Maximum: 1000

Sensitivity Analysis: inactive

Parameter Estimation: active

****

****

Processes

****

Reduction: Description: Cr(VI)_biological _reduction

Type: Dynamic Process

Rate: km*C*(X0-((Cout-C)/Rc))/((C+Kc))

Stoichiometry:

Variable : Stoichiometric Coefficient

C : -1

Sorption: Description: Cr(VI) adsorption

Type: Dynamic Process

Rate: kad*(S_eq-S)

Stoichiometry:

Variable : Stoichiometric Coefficient

S : 1

****

****

Compartments

****

BRP_reactor: Description: Cr(VI) reduction compartment

Type: Soil Column Compartment

Compartment Index: 0

Active Variables: C, S

Active Processes: Reduction, Sorption

Initial Conditions:

Variable(Zone) : Initial Condition

C(Advective Zone) : 0

Inflow: Q

Loadings:

Variable : Loading

C : Q*Cout

Lateral Inflow: 0

Start Coordinate: 0

End Coordinate: 1

Cross Section: A

Adv. Vol. Fract.: theta

Dispersion: kL

Parallel Zones:

Num. of Grid Pts: 52 (low resolution)

Accuracies:

Rel. Acc. Q: 0.0001

Abs. Acc. Q: 1e-006

Rel. Acc. D: 1e-006

Abs. Acc. D: 1e-006

****

****

Definitions of Calculations

****

calc1: Description:

Calculation Number: 0

Initial Time: 0

Initial State: given, made consistent

Step Size: 0.01

Num. Steps: 9000

Status: active for simulation

active for sensitivity analysis

****

****

Definitions of Parameter Estimation Calculations

****

fit1: Description:

Calculation Number: 0

Initial Time: 0

Initial State: given, made consistent

Status: active

Fit Targets:

Data : Variable (Compartment,Zone,Time/Space)

Cout : C (BRP_reactor,Advective Zone,0)

****

****

Plot Definitions

****

plot1: Description:

Abscissa: Time

Title: Cr(VI) reduction

Abscissa Label: Time (days)

Ordinate Label: C (mg/L)

Curves:

Type : Variable [CalcNum,Comp.,Zone,Time/Space]

Value : C [0,BRP_reactor,Advective Zone,0]

Value : Cin [0,BRP_reactor,Advective Zone,0]

Value : Cout [0,BRP_reactor,Advective Zone,0]

****

****

Calculation Parameters

****

Numerical Parameters: Maximum Int. Step Size: 1

Maximum Integrat. Order: 5

Number of Codiagonals: 1000

Maximum Number of Steps: 1000

Fit Method: secant

Max. Number of Iterat.: 100

****

****

Calculated States

****

Calc. Num. Num. States Comments

0 9001 Range of Times: 0 - 90

****

APPENDIX B: STANDARD CURVE

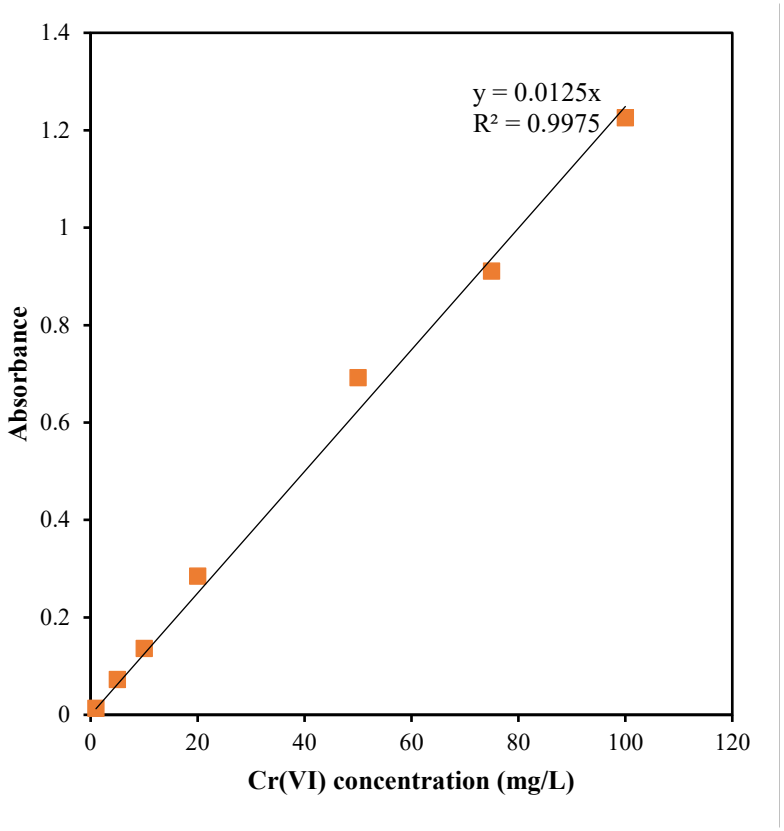


Figure B-0-1: Standard curve for absorbance variation with Cr(VI) concentration at 540 nm wavelength