# A STEADY-STATE MODEL FOR HEXAVALENT CHROMIUM REDUCTION IN SIMULATED BIOLOGICAL REACTIVE BARRIER: MICROCOSM ANALYSIS

**PHALAZANE JOHANNA MTIMUNYE**

A dissertation submitted in partial fulfilment of the requirement for the degree of

**MASTER OF SCIENCE: APPLIED SCIENCE**
**WATER UTILIZATION**

In the
Faculty of Engineering, Built Environment and Information Technology
Department of Chemical Engineering University of Pretoria, Pretoria, South Africa

**April 2011**

# Declaration

I, **PHALAZANE JOHANNA MTIMUNYE,** hereby declare that the work provided in this dissertation is to the best of my knowledge original (except where cited) and that this work has never been submitted for another degree at this or any other tertiary education institution.

_____

Signature of candidate

This …….. day of ……. 2011

# Dedication

This dissertation is dedicated to

*My family*

*My late father who always believed in me and encouraged me to pursue my studies*

*My mother for her ongoing support, endless love, understanding, for believing in me always and for always telling me that the will of God will never take me where the grace of the Lord will not guide me*

*My brothers and sisters who supported me every step of the way both emotionally and financially*

*My friends for their presence in my life, who were always there when I needed to talk*

# Acknowledgements

I would like to express my sincere gratitude to the following persons without who this dissertation would not be possible:

Professor Evans Chirwa my study leader for the guidance, mentorship, motivation and advice he gave me throughout the study. May the good Lord bless him.

Professor Fanus Venter from Department of Microbiology for assistance with the characterization of bacterial isolates.

National Research Foundation of South Africa (NRF) for financial assistance throughout the study.

Colleagues and friends who provided invaluable advice that contributed to this study.

My family and friends for helping me keep my feet on the ground by teaching me never to forget that from my Lord cometh my help and through whom all my blessings flow.

I would like to thank God almighty and my Saviour Jesus Christ for the many blessings that he has bestowed upon me since birth and without him I can not achieve anything.

# ABSTRACT

Biological remediation of Cr(VI) contaminated soil and groundwater is an emerging field. In this study, the *in situ* bioremediation technology for treating Cr(VI) contaminated groundwater aquifers was evaluated using a laboratory microcosm system. The study was conducted using columns with five equally spaced intermediate sampling ports along the length to facilitate finite difference modelling of the Cr(VI) concentration profile within the column. Cr(VI) concentration was continuously measured in the influent, in five equally spaced intermediate ports within the column and in the effluent port. The change or the shift in microbial community within the inoculated column was also monitored due to exposure to toxic conditions after seven weeks of operation using the 16S rRNA genotype fingerprinting method.

The effect of introducing a natural carbon source (sawdust) in inoculated columns in comparison with the performance of sterile controls under various loading conditions was also evaluated. Near complete Cr(VI) removal was achieved in an inoculated carbon source reactor, whereas only 69.5% of Cr(VI) removal was achieved in an inoculated column without an added carbon source after 4 days of operation at 20 mg/L. In a sterile control reactor less than 2% of Cr(VI) was removed after 4 days of operation at 20 mg/L. Experimental cores demonstrated a successful Cr(VI) reduction process in the simulated microbial barrier system that was evaluated internally.

The model that simulates Cr(VI) removal and transport in the subsoil environment was developed. The Cr(VI) mass balance model across the reactor that accounts for the flow characteristics and biological removal mechanism successfully captured the trends of Cr(VI) response profiles under quasi-steady state conditions for different loading conditions. This study demonstrate the potential of applying effective Cr(VI) reducers in the reactive barrier systems to contain or attenuate the spread of Cr(VI) contaminant in groundwater aquifer systems. The finite difference model developed in this study to evaluate the behaviour of Cr(VI) in the reactor could contribute towards improved designs of future *in situ* bioremediation systems that can be implemented for remediation of Cr(VI) on site.

# Table of Contents

Title……page
Declaration……ii
Dedication……iii
Acknowledgement……iv
Abstract……v
List of Figures……x
List of Tables……xii
List of Abbreviations……xiii
Symbol Nomenclature……xiv
**CHAPTER 1:INTRODUCTION**……1
1.1 Background……1
1.2 Methodology and Objectives of the Study……3
1.3 Outline of Dissertation……4
1.4 Significance of Research……4

**CHAPTER 2:LITERATURE REVIEW**……5
2.1 Chromium Occurrences in the Environment……5
2.1.1 Chromium in Water……5
2.1.2 Chromium in Soil……6
2.1.3 Chromium in Air……6
2.2 Production of Chromium and Its Use……7
2.3 Potential Health Effects of Exposure to Chromium……9
2.3.1 Nutrition and Toxicity: Risk to Human and Animal Health……9
2.3.2 Toxicity to Microorganisms……10
2.4 Remediation Strategy……11
2.4.1 Physicochemical Methods……11
2.4.2 Bioremediation Method……13

2.4.3 Biological System Engineering.....14
2.5 Cr(VI) Reducing Organisms.....15
2.6 Cr(VI) Reduction Pathways.....18
2.6.1 Intracellular Processes.....19
2.6.2 Extracellular Processes.....19
2.6.3 Membrane-bound Processes.....20
2.7 Carbon Source.....20
2.8 Summary.....21

**CHAPTER3: MATERIALS AND METHODS**.....22
3.1 Bacterial Culture.....22
3.1.1 Sources of Cr (VI) Reducing Bacteria.....22
3.1.2 Culture Isolation.....22
3.2 Growth Media.....23
3.2.1 Basal Mineral Media.....23
3.3.2 Commercial Broth and Agar.....23
3.3 Reagents.....23
3.3.1 Standard Solutions and Chemicals.....23
3.3.2 DPC Solution.....23
3.3.3 Chemicals.....24
3.4 Culture Characterization.....24
3.4.1 General Characterization of Aerobic Cultures using 16S rRNA ID's.....24
3.4.2 Culture Storage.....25
3.4.3 General Classification of Anaerobic Cultures using 16S rRNA ID's.....25
3.5 Batch Reactor Studies.....27
3.5.1 Aerobic Cr(VI) Reduction Experiments.....27
3.5.2 Anaerobic Cr(VI) Reduction Experiments.....27
3.5.3 Abiotic Experiments.....28
3.6 Biomass Analysis.....28
3.6.1 Total Biomass.....28
3.6.2 Viable Biomass.....28

3.7 Microcosm Studies....29
3.7.1 Mineral Composition of Aquifer Media....29
3.7.2 Reactor Setup....29
3.7.3 Start up culture....30
3.7.4 Reactor Start up....30
3.7.5 Reactors Operation....31
3.8 Analytic Methods....31
3.8.1 Cr(VI) Analysis....31

**CHAPTER 4:CR(VI) REDUCTION BATCH KINETICS STUDIES**....32
4.1 Preliminary Studies....32
4.2 Performance Validation in Cultures....32
4.2.1 Individual Pure Cultures versus Reconstituted Consortium Culture....32
4.2.2 Reconstituted Culture versus Natural/Original Consortium Culture....32
4.3 Cr(VI) Reduction Kinetics....34
4.3.1 Cr(VI) Reduction Kinetics Under Aerobic Conditions....34
4.3.2 Cr(VI) Reduction Under Anaerobic Conditions....36
4.4 Total Biomass Evaluation....38
4.4.1 Evaluation of Total Biomass at the initial Cr(VI) concentration of 100mg/L....38
4.4.2 Evaluation of Total Biomass in the initial Cr(VI) concentration of 400 mg/L....38
4.5 Kinetic Modelling Theory....39
4.5.1 Enzyme Kinetics....39
4.5.2 Anaerobic Batch Cultures Modelling....41
4.6 Parameter Estimation....42
4.6.1 Kinetic Parameter Estimation under Anaerobic Conditions....43
4.7 Sensitivity Analysis....44
4.7.1 Sensitivity Analysis of Anaerobic Batch Culture Kinetics....44
4.8 Summary....45

**CHAPTER 5: MICROCOSM Cr(VI) REDUCTION KINETIC STUDIES**…….....46
5.1 Conceptual Basis of Microcosm Studies.....46
5.2 Performance Evaluation.....46
5.2.1 Reconstituted Consortium Culture versus Native Soil Culture.....46
5.3 Microcosm Kinetic Studies.....47
5.3.1 Cr(VI) Removal Kinetics at Various Time Intervals.....47
5.3.1 Cr(VI) Removal Kinetics at Various Lengths.....49
5.4 Microbial Culture Dynamics in the Aquifer System.....52
5.4.1 Characteristic of Initial Inoculated Consortium Culture.....52
5.4.2 Characterization of Inoculated Columns after Operation.....52
5.5 Kinetic Modelling of Cr(VI) Reduction in the Microcosm Columns.....55
5.5.1 Model Description.....55
5.5.2 Model Validation.....59
5.5.3 Parameter Optimization.....59
5.5.4 Cr(VI) Removal Kinetics at Lower and Higher Concentrations.....59
5.5.5 Summary of Parameters.....60
5.6 Steady State Performance Model.....65
5.6.1 Model Formulation.....65
5.6.2 Steady State Spatial Simulation.....67
5.6.3 Summary of Steady State Kinetic Parameters…….....71
5.7 Summary…….....71

**CHAPTER 6: SUMMARY AND CONCLUSION**…….....73
APPENDIX A: AQUASIM Version 2.0…….....75
APPENDIX B: Octave Version 3.0…….....87
APPENDIX C: Target Site…….....88
**REFERENCES:** ……..…….89

# List of Figures


| | | |
|---|---|---|
| Figure2-1 | Cr cycle in environment..... | 7 |
| Figure2-2 | World chrome ore reserves..... | 8 |
| Figure2-3 | World chromium ferroalloy production..... | 8 |
| Figure2-4 | World production of chrome ore..... | 8 |
| Figure2-5 | Industrial usage of chromium ……..... | 9 |
| Figure3-1 | Laboratory set up microcosm columns……..... | 30 |
| Figure4-1 | Cr(VI) reduction between individual potential pure isolates and reconstituted consortium culture at the initial Cr(VI) concentration of 100 mg/L under aerobic conditions……..……..... | 33 |
| Figure4-2 | Comparison between reconstituted and natural consortium culture at the initial Cr(VI) concentrations of 100 mg/L under aerobic conditions……. | 33 |
| Figure4-3 | Aerobic Cr(VI) reduction in pure isolates at 50 mg/L..... | 35 |
| Figure4-4 | Aerobic Cr(VI) reduction in pure isolates at 100 mg/L..... | 35 |
| Figure4-5 | Cr(VI) reduction in aerobic reconstituted consortium culture at (50-400 mg/L)……..... | 36 |
| Figure4-6 | Anaerobic Cr(VI) reduction in pure isolates at 50 mg/L..... | 37 |
| Figure4-7 | Cr(VI) reduction in anaerobic reconstituted consortium (50-200 mg/L)..... | 37 |
| Figure4-8 | Total biomass of aerobic reconstituted consortium culture at 100 and 400 mg/L..... | 38 |
| Figure4-9 | Anaerobic batch culture model validation at (50-200 mg/L)..... | 43 |
| Figure4-10 | Sensitivity test for the initial Cr(VI) concentration of 100 mg/L with respect to optimized parameters in anaerobic batch culture..... | 44 |
| Figure5-1 | Performance of comparison between inoculated reactors with carbon source and without carbon source at the initial Cr(VI) concentration of 20 mg/L..... | 48 |
| Figure5-2 | Performance of inoculated column without carbon source in comparison with sterile-control column reactor at 30 mg/L …….. | 48 |

Figure5-3 Performance of inoculated reactor amended with carbon source at 40 mg/L……. 49

Figure5-4 Performance of a sterile control reactor in removing Cr(VI) feed concentration of 20 mg/L across the column..... 50

Figure5-5 Performance of native soil culture column in removing Cr(VI) feed concentration of 20mg/L across the column..... 50

Figure5-6 Performance of inoculated reactors in removing Cr(VI) feed concentration of 20 mg/L in (A) C-source reactor, and (B) non C-source reactor……... 51

Figure5-7 Phylogenetic tree of persistent bacterial cells in inoculated reactor columns after operation derived from the 16S rRNA gene sequence, *Bacillus species*..... 53

Figure5-8 Phylogenetic tree of persistent bacterial cells in inoculated reactor columns after operation derived from the 16S rRNA gene sequence, *Enterococcus species*..... 54

Figure5-9 Phylogenetic tree of persistent bacterial cells in inoculated reactor columns after operation derived from the 16S rRNA gene sequence. Possible Cr(VI) reducing species were detectable including, *Enterobacter species*, and *E. coli*..... 55

Figure5-10 Simulation of Cr(VI) effluent at 20 mg/L in a (A) sterile control column, (B)carbon source and non-carbon source reactor..... 63

Figure5-11 Simulation of Cr(VI) effluent at (A) 30 mg/L in a non carbon source reactor, (B) 40 mg/L in a carbon source reactor..... 64

Figure5-12 Simulation of Cr(VI) effluent in a carbon source reactor at various lengths..... 69

Figure5-13 Simulation of Cr(VI) effluent in a non-carbon source reactor over length. The experimental data is the average values of the last three sampling times..... 70

# List of Tables


| | | |
|---|---|---|
| Table 2-1 | Cr(VI) Reducing bacteria reported in literature..... | 17 |
| Table 3-1 | Characterization of Cr(VI) reducing bacteria under aerobic conditions……..... | 25 |
| Table 3-2 | Characterization of Cr(VI) reducing bacteria under anaerobic conditions…..... | 26 |
| Table 3-3 | Mineral composition of aquifer soil media……..... | 29 |
| Table 4-1 | Optimum kinetic parameter in anaerobic batch cultures…..... | 44 |
| Table5-1 | Summary of performance of Cr(VI) reduction after column operation…….….....  | 47 |
| Table5-2 | Optimum kinetic parameter values obtained for the biofilm in a carbon source reactor...….....  | 61 |
| Table5-3 | Optimum kinetic parameter values obtained for the biofilm in a non-carbon source reactor……..…….....  | 62 |
| Table5-4 | Optimum kinetic parameter values for the biofilm at steady state in a carbon source and a non-carbon source reactor..... | 68 |

# List of Abbreviations

| | |
|---|---|
| AAS | Atomic adsorption spectrophotometer |
| APHA | American public health agency |
| BLAST | Basic Logical Alignment Search Tool |
| ChrR | Cr(VI) reductase |
| Cr | Chromium |
| Cr(VI) | Hexavalent chromium |
| Cr(III) | Trivalent chromium |
| CRB | Cr(VI) reducing bacteria |
| +CS | With carbon source |
| -CS | Without carbon source |
| CRL | Control |
| CFU | Colony forming units |
| DNA | Deoxyribonucleic acid |
| ETC | Electron transport chain |
| INC | Inoculated |
| MSM | Mineral salt medium |
| NADH | Nicotinamide adenine dinucleotide |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NTV | Native soil culture |
| pH | Potential hydrogen |
| ppm | Parts per million |
| PVC | Polyvinyl chloride |
| RT-PCR | Reverse transcriptase- Polymerase chain reaction |
| rDNA | Ribosomal deoxyribonucleic acid |
| rRNA | Ribosomal Ribonucleic acid |
| rpm | Rotation per minute |
| TCA | Tricarboxylic acid |
| US EPA | United States Environmental Protection Agency |
| UV | Ultraviolet |
| WHO | World Health Organization |

# Symbol Nomenclature

| | |
|---|---|
| $A_f$ | biofilm surface area ($L^2$) |
| $A$ | cross-sectional area of a reactor column ($L^2$) |
| $C$ | Cr(VI) concentration at time, t ($ML^{-3}$) |
| $C$ | state variable ($ML^{-3}$) |
| $C_S$ | Cr(VI) concentration at the surface ($ML^{-3}$) |
| $C_b$ | Cr(VI) concentration in the bulk flow ($ML^{-3}$) |
| $C_r$ | Cr(VI) toxicity threshold concentration ($ML^{-3}$) |
| $C_{eq}$ | equilibrium concentration at surface area ($ML^{-3}$) |
| $D_w$ | dispersion coefficient ($L^2T^{-1}$) |
| $j$ | mass transport rate ($LT^{-1}$) |
| $j_c$ | Cr(VI) flux rate ($ML^{-2}T^{-1}$) |
| $k$ | reaction rate coefficient ($LM^{-3}T^{-1}$) |
| $K_i$ | inhibition coefficient ($ML^{-3}$) |
| $K_C$ | half velocity constant ($ML^{-3}$) |
| $k_{ad}$ | adsorption rate coefficient ($T^{-1}$) |
| $k_d$ | cell death rate ($T^{-1}$) |
| $k_m$ | maximum specific Cr(VI) reduction rate($T^{-1}$) |
| $L_w$ | stagnant film thickness ($L$) |
| $L$ | length of the reactor ($L$) |
| $\rho_c$ | soil particle density ($ML^{-3}$) |
| Q | inflow rate ($L^{-3}T^{-1}$) |
| $q_c$ | adsorption rate ($ML^{-3}T^{-1}$) |
| $r_c$ | Cr(VI) reduction rate ($ML^{-3}T^{-1}$) |
| $R_c$ | Cr(VI) reduction capacity coefficient ($MM^{-1}$) |
| $t$ | time *(T)* |
| $u$ | flow velocity ($LT^{-1}$) |
| V | volume of the reactor ($L^3$) |
| $\Delta V$ | differential volume ($L^3$) |
| $X_o$ | initial biomass concentration ($ML^{-3}$) |
| X | biomass concentration at time, $t$ ($ML^{-3}$) |

## Subscripts

| | |
|---|---|
| $C$ | chromium |
| $f$ | in biofilm |
| $in$ | influent |
| $w$ | in water |
| o | initial |

CHAPTER 1

# INTRODUCTION

## 1.1 Background

Groundwater is usually of excellent quality, being naturally filtered in its passing through the ground. Unfortunately, a threat is now posed by an ever-increasing number of soluble chemicals from industrial activities. These chemicals are not completely removed by filtration as groundwater passes through the aquifer. The principal pathway by which these metal ions may enter groundwater systems includes leakage from the storage ponds, storm water run-on/off and uncontrolled leaching from landfills (Moncur *et al*., 2005). The rate at which these metal-effluents enter the environment alters the natural flow of materials in the environment and also cause potential hazard to the health of human and other life forms.

Chromium [Cr] is one of the most important chemical contaminant of concern which has been classified as a priority pollutant by the United States Environmental Protection Agency (USEPA) (Smith *et al*., 2002). It is the seventh most abundant element in the earth's crust, which was initially discovered by Nicolasa-Louis Vauquelin in 1797. Cr is detectable in the earth crust in small quantities associated with other metals, particularly iron (Fe). The average concentration of Cr in the continental crust has been reported as 125 mg/kg (National Academy of Science (NAS), 1974). Cr exists in a series of oxidation states ranging from (-II) to (+VI), (Fendorf, 1995; Smith *et al*., 2002). However, only trivalent chromium [Cr(III)] and hexavalent chromium [Cr(VI)] are of environmental significance as a result of their most stable oxidation state in the natural environment.

Cr(III) and Cr(VI) display contrasting toxicity, mobility and bioavailability in the environment. Cr(VI) is a potential soil, surface and groundwater contaminant that readily spreads beyond the site of initial concentration through aquatic and groundwater systems (Cervantes *et al*., 2001; Kamaludeen *et al*., 2003). It is rated as the third most abundant pollutant from anthropogenic sources only superseded by organic pollutant species. Cr(VI) is also known to be mutagenic to most organisms and carcinogenic to humans (Francisco *et al*., 2002; Caglieri *et al*., 2006). Due to Cr(VI) toxicity, stringent regulations are imposed on the discharge of Cr(VI) to surface water to

below 0.05 mg/L by the U.S. EPA (Kiilunen, 1994; Baral *et al*., 2002; Kobya, 2004), while the total Cr, including Cr(III), Cr(VI) and its other forms are regulated to below 2 mg/L (Zayed and Terry, 2003).

On the other hand, the reduced form of chromium, Cr(III), is less toxic, less soluble, and forms insoluble precipitates at higher pH (5.5-10). Cr(III) is also essential (in low concentrations) for human and animal nutrition (Zayed and Terry, 2003; Viamajala *et al*., 2004). Therefore the strong impact of Cr(VI) on the environment and also to human and animal health has increased the demand for suitable technologies to neutralize the hazardous Cr(VI) to the less toxic Cr(III). Currently, most of the Cr(VI) contaminated sites around the world are conventionally treated using the pump-and-treat or dig-and-treat method which involves pumping or digging out the contaminated material, adding of chemical reductants, precipitation followed by the sedimentation or adsorption steps (Nyer, 1992; Eid and Zahir, 1996; Watts, 1998). These methods, however, are not suitable for large scale wastewater treatment especially in developing countries as they may be cost intensive and environmentally unfavorable. Additionally, chemical products used for treatment generate harmful residuals and by-products that are difficult to treat.

Among the most recent alternative technologies for remediation of Cr(VI), microbial reduction of Cr(VI) to Cr(III) as a normal function of their metabolism offers promise as a technology that could play an important role in the decontamination of polluted sites. A wide array of bacterial strains are capable of reducing Cr(VI) to Cr(III) under both aerobic and anaerobic conditions (Guha *et al.*, 2001; Zouboulis *et al*., 2004; Dermou *et al*., 2005; Zakaria *et al*., 2007; Congeevaram *et al*., 2007; Zahoor and Rehman, 2009; Ahmad *et al*., 2010; Tekerlekopoulou *et al*., 2010). However, most of the studies considering the effectiveness of microbial Cr(VI) reduction for the treatment of wastewater under various environmental conditions were performed in the laboratory using suspended cell systems (Chen and Hao, 1998; Shakoori *et al*., 2000; Megharaj *et al*., 2003). *In situ* bioremediation technology using permeable reactive barriers is a relatively new application which has been tested and sometimes implemented for organic pollutants but not for toxic metals detoxification/removal (Liu *et al*., 2006). Only recently, a detailed analysis on *in situ* Cr(VI) biological treatment focusing on the remediation of spillage of Cr(VI) waste on the ground was conducted at the laboratory level (Molokwane and Chirwa, 2009). However, in this study smaller laboratory scale columns (22-30 cm long) with experimental data available only for the inlet and the outlet ports were used for this purpose.

The current study focuses on using larger laboratory scale columns to internally evaluate barrier system. In this study the experimental data was collected from equally space longitudinal sampling ports across each reactor to facilitate the finite difference modelling of Cr(VI) concentration profiles along the column and to completely understand Cr(VI) reduction kinetics within the reactor system. Fundamental knowledge and understanding of kinetic processes within the reactor system responsible for Cr(VI) transformation will be valuable in developing the appropriate biological systems that could be used to effectively treat Cr(VI) at contaminated sites as well as predicting the microbial impact on the long term stewardship of the contaminated sites.

### 1.2 Methodology and Objectives of the Study

The initial step towards the methodology of this study was to collect as much information as possible related to the impacts of Cr(VI) pollution and current treatment practices from literature.

The primary objective of the research was to evaluate the prospect of pollution control in groundwater aquifers using Cr(VI) reducing bacteria isolated from the local environments. In order to achieve the primary objective, different experimental tasks were conducted on the Cr(VI) reduction process, viz:

- Investigation of Cr(VI) reduction kinetics in indigenous Cr(VI) reducing bacteria grown both aerobically and anaerobically in batch reactors over a wide range of initial Cr(VI) concentrations.

- Evaluation of Cr(VI) reduction in aquifer microcosm reactors over a range of Cr(VI) feed concentrations.

- Investigation of the microbial culture shift in a microcosm system after operation.

- Development of a mathematical model that simulates the contaminant movement across the microcosm reactor at a transient state.

- Development of the mathematical model that simulates the contaminant movement across the reactor at a steady-state.

### 1.3 Outline of Dissertation

The outline of this dissertation is subdivided into three main parts:

*Literature Review*– contains the background information of the study and the records of recent developments on the Cr(VI) bioremediation process. The information is focused on the occurrence of chromium in the environment, impact of Cr on human health, animals and microorganisms, remediation strategies, Cr(VI) reducing microorganisms, and biological Cr(VI) reduction pathways.

*Materials and Methods*– illustrate all the materials and methods used during the study.

*Cr(VI) Reduction Kinetic Studies*– contains the performance evaluation studies and the kinetic modelling of the batch system and continuous-flow bioreactor system.

### 1.4 Significance of Research

The introduction of Cr(VI) reducing bacterial species isolated from the sand drying beds could be used in the formulation of biological permeable barriers for protection against the spread of Cr(VI) contamination in groundwater systems. The model developed in this study under both transient and steady-state is suitable for simulation of the contaminant movement in the porous aquifer media under a range of Cr(VI) feed concentrations and it can be easily modified for application in engineered biological systems for treating wastewater with higher concentrations of toxic metals.

CHAPTER 2

# LITERATURE REVIEW

## 2.1 Chromium Occurrences in the Environment

### 2.1.1 Chromium in Water

Trivalent chromium (Cr(III)) in water originates from natural sources, such as the weathering of rock constituents, wet precipitation and dry fallout from the atmosphere, and run-off from the terrestrial systems. Cr(III) can form both anionic ($Cr(OH)_4^-$, $CrCl_6^{3-}$) or cationic ($Cr(H_2O)_6^{3+}$, $Cr(OH)_2^+$) compounds, which are considered to be non-labile, inert species in the environment. The main aqueous Cr(III) species are $Cr^{3+}$, $Cr(OH)_2^+$, $Cr(OH)_3$ and $Cr(OH)_4^-$. Cationic Cr(III) is regarded as relatively nontoxic, and above pH 5.5 it precipitates virtually as insoluble oxides and hydroxides, $Cr(OH)_3$ in soil and water systems (McGrath and Smith, 1990).

Hexavalent chromium (Cr(VI)) on the other hand is rarely naturally occurring in the environment. Only 0.001% is attributed to natural geological processes (Merian, 1984). Cr(VI) enters environmental water almost exclusively as a result of anthropogenic activities. Cr(VI) compounds are highly soluble in water and forms chromates ($CrO_4^{2-}$, $HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$) which are thermodynamically stable over a large pH range in the environment (Uyguner and Bekbolet, 2003). The equilibria of the Cr(VI) oxygenated species favours extremely high solubility and is pH dependent. The following equations describe the distribution of Cr(VI) species in aqueous solution:

$$H_2CrO_4 \Leftrightarrow HCrO_4^- + H^+ \quad (2\text{-}1)$$

$$2HCrO_4^- \Leftrightarrow Cr_2O_7^{2-} + H_2O \quad (2\text{-}2)$$

$$HCrO_4^- \Leftrightarrow CrO_4^{2-} + H^+ \quad (2\text{-}3)$$

The $Cr_2O_7^{2-}$ anion is dominant in acidic solution while the $CrO_4^{2-}$ prevails in basic or slightly acidic Cr(VI) aqueous solution (Jain *et al*., 2005).

### 2.1.2 Chromium in Soil

The main source of Cr in natural soils is the weathering of the parent materials. The concentration of Cr(III) and Cr(VI), in natural soil ranges from 7-220 mg/kg (McBride, 1994). However, in most soils Cr exists as Cr(III) and occurs within mineral structures or as mixed Cr(III) and Fe(III) oxide. The compounds of Cr(III) in soil are considered to be stable due to their slight mobility in acid media and their precipitation at pH 5.5.

In neutral to alkaline soils, Cr(VI) exists mostly in moderately to sparingly soluble chromates (e.g. $CaCrO_4$, $BaCrO_4$, and $PbCrO_4$) (James, 1996). In more acidic soils, (pH<6), $HCrO_4^-$ becomes a dominant form. The $CrO_4^{2-}$ and $HCrO_4^-$ ions are the most mobile forms of Cr in soils that can be easily taken up by plants into the deeper soil layers, causing ground and surface water pollution (James *et al.*, 1983b). Oxidation and reduction reactions in soil can convert Cr(III) to Cr(VI) and Cr(VI) to Cr(III) (Makino *et al.*, 1998). However these oxidation and reduction processes are highly depend on pH, oxygen concentration, the presence of appropriate reducers, and mediators acting as ligands or catalysts.

### 2.1.3 Chromium in Air

About 60% -70% of Chromium (Cr) present in the atmosphere originates from anthropogenic sources and the remaining 30% - 40% is from the natural sources (Seigneur *et al.*, 1995). The main human activities contributing to the increase of Cr in the atmosphere are: ferrochrome production, electroplating, pigment production, and tanning plus burning of fossil fuels, stainless steel welding and waste incineration, while the natural sources of air-chromium are forest fires and, volcanic eruptions, sea salt particles, erosion of soils and rocks (Pacyna *et al.,* 1988).

Chromium compounds in the air are present mainly as fine dust particles that eventually settle over the land and water. Cr compounds can also occur in the air of non-industrialized areas in concentrations of less than (0.1 $\mu g/m^3$). The chemical forms of chromium in the air are not known, but it is assumed that part of the air-chromium exists in the hexavalent form, especially that is derived from high-temperature combustion. Chromium trioxide ($CrO_3$) may be the most important compound of Cr in the air (Sullivan, 1969).

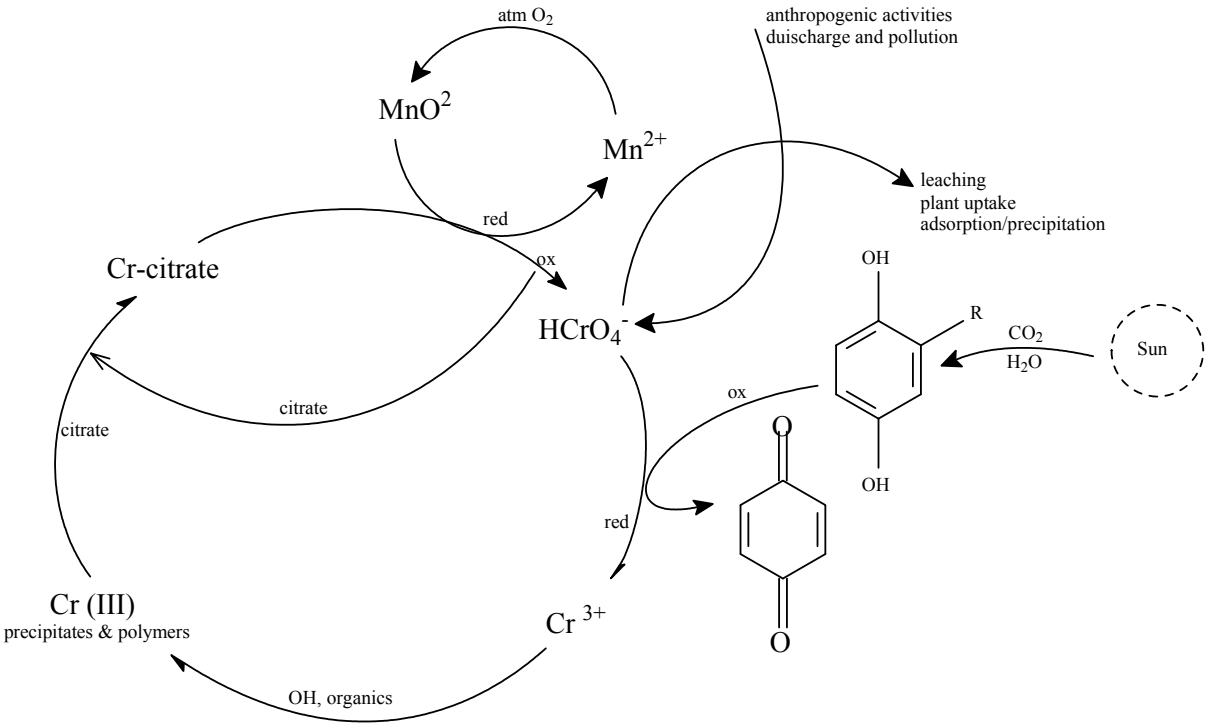


Figure 2-1: Cr cycle in environment (Bartlett, 1991; Yassi and Nieboer, 1988)

## 2.2 Production of Chromium and Its Use

Elemental Cr does not occur in nature, but is present in ores. Cr occurs in more than fifty different ores such as barbertonite, brezinaite, chromite, chromitite and nichromite in nature. Among the above mentioned Cr ores, chromite is the most important economical form of ore and the two main products of the refined chromite ore are ferrochromium and metallic chromium.

Cr production in the world is in the order of 10,000,000 tons per year (Cervantes *et al.*, 2001). About 72% of the mined chromium is used for metallurgical purposes, 12% for refractory purposes and 17% for chemical purposes (Figure 2.5) (Papp, 1999). South Africa has produced (since the 1940's) 72% of the worlds Cr ore, with majority of the ore being mined in the North Eastern region of the country (Mintek, 2004). South Africa is also the largest exporter of chromite ore to the U.S., where chromite is not mined (Barnhart, 1997). Other countries with exploitable Cr ore reserves include Philippines, Southern Zimbabwe, and Turkey (Armitage, 2002) (Figure 2.2). Most of these chrome reserves come from the bushveld igneous complex (BIC) ores and represent 44% of the world's chromium ore (Figure 2.3) and 47% of the world's ferrochrome (Figure 2.4).

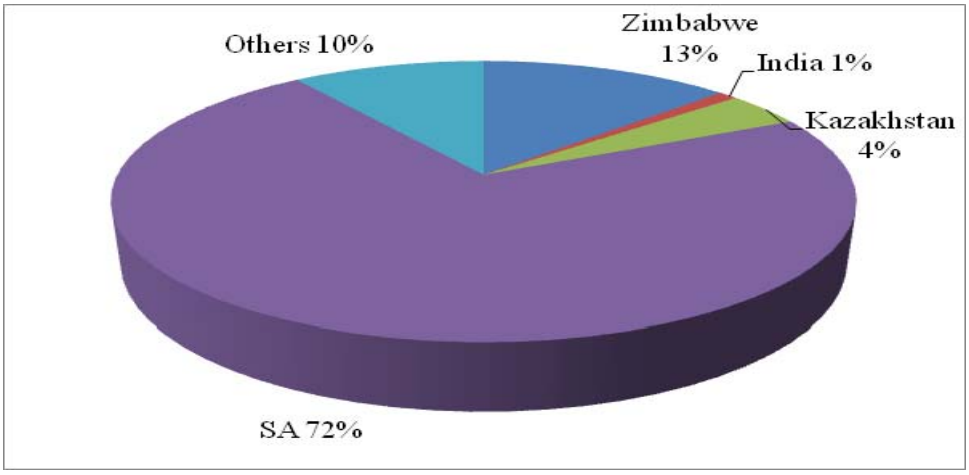


Figure 2-2: World chrome ore reserves (Armitage, 2002)

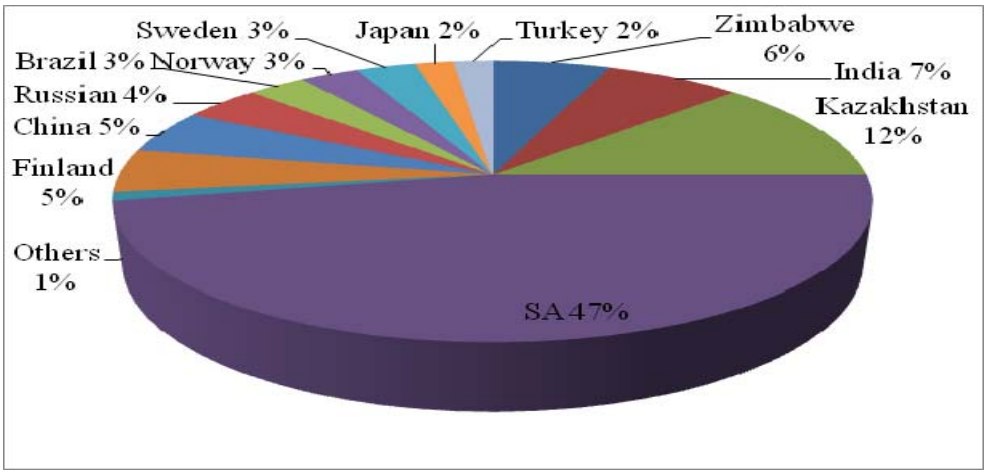


Figure 2-3: World chromium ferroalloy production (Armitage, 2002)

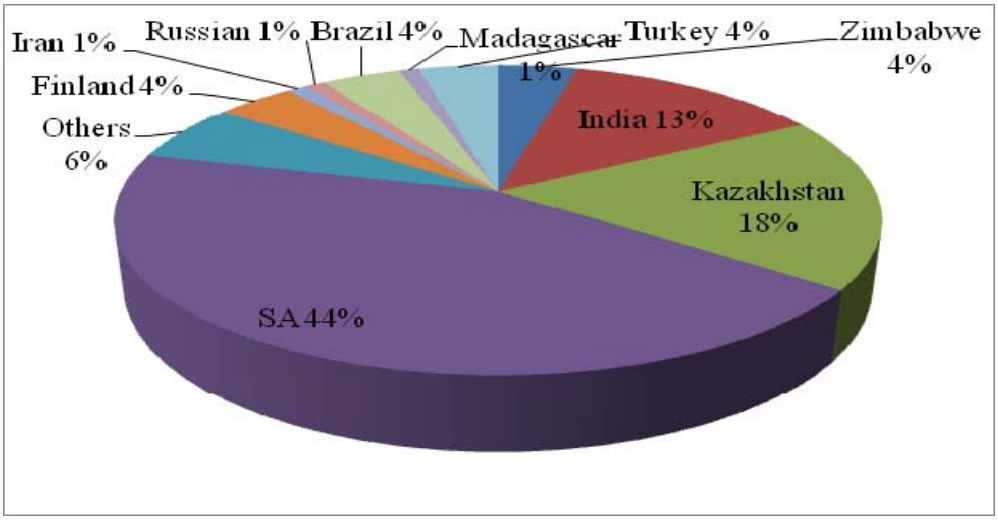


Figure 2-4: World production of chrome ore (Armitage, 2002)

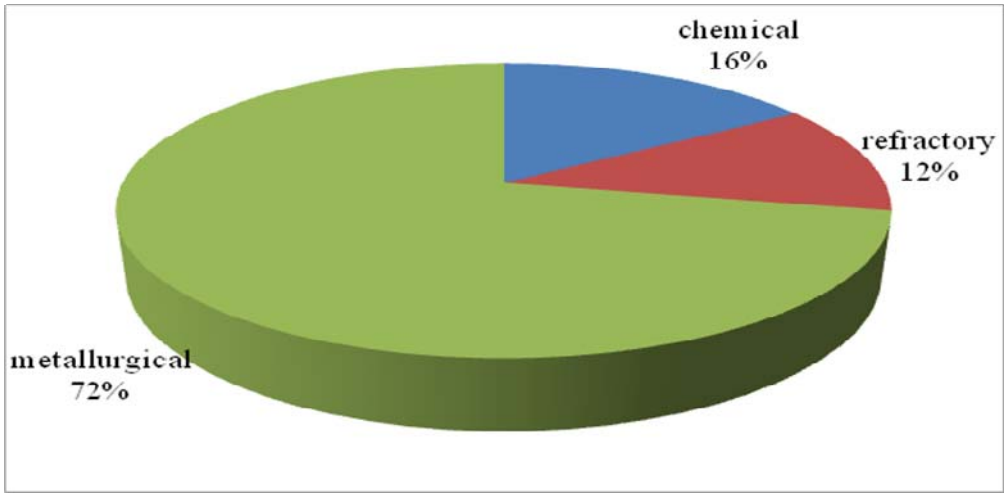


Figure 2-5: Industrial usage of chromium (Papp, 1999)

Cr is used in metallurgy to manufacture ferrous and non-ferrous alloys. It is used in chemical industries for pigment production, electroplating, leather tanneries, fungicides production and wood preservation (Ryan *et al*., 2002; Middleton *et al*., 2003). Also, Cr serves as a catalyst in the synthesis of many organic chemicals. The manufacture of chromite and chrome magnetite bricks accounts for its use in the refractory (Palmer and Wittbrodt, 1991; Opperman and van Heerden, 2007). As a result of its high corrosion, resistance and hardness, Cr can be extensively utilized in manufacturing stainless steel.

The widespread use of chromium by metal and chemical industries (Kotas and Stasicka, 2000; Das and Mishra, 2008; Shai *et al*., 2009) produces wastes which are often very difficult to treat. The Cr(VI)-bearing wastes may enter groundwater through improper disposal of industrial effluent or through leakage due to improper handling and faulty storage containers (accidental spills). Cr(VI), being highly mobile transport quickly into groundwater aquifers, any of which may serve as direct water supply source for animal and human consumption in communities that can not afford advance treatment of water (Krishna and Philip, 2005).

## 2.3 Potential Health Effects of Exposure to Chromium

### 2.3.1 Nutrition and Toxicity: Risk to Human and Animal Health

Chromium can enter the human body through ingestion or dermal contact. Relying on the chemical, toxicological, and epidemiological evidence, regulation of Cr(VI) concentration is different from that of Cr(III). Cr(III) is nutritionally required in trace amounts for normal

carbohydrates and lipid metabolism (Viamajala *et al*., 2004). When Cr(III) is at least taken up through food and drinking water it may also even improve health and cure neuropathy and encephalopathy. Deficiency to Cr(III) may increase the risk factors associated with diabetes and cardiovascular diseases including elevated circulating insulin, glucose, and total cholesterol (Zayed and Terry, 2003; Viamajala *et al*., 2004). However, long term exposure to high concentration of Cr(III) may also lead to health problems such as cancer (Zhitkovich *et al.,* 1996).

Cr(VI) compounds on the other hand, have been found to be carcinogenic, mutagenic and teratogenic to mammals (Flores *et al.,* 1999; Francisco *et al*., 2002; Caglieri *et al*., 2006). The toxicity of Cr(VI) on living organisms is associated to easy diffusion of Cr(VI) compounds specifically chromate across the barrier of the cells via sulphate transport pathways as it bears structural similarity with $SO_4^{2-}$ (Pattanapipitpaisal *et al.*, 2002). Once in the cell, chromate can oxidatively damage the DNA via the production of free radicals and cause illness such as cancer within the living cell.

Short-term inhalation of high levels of Cr(VI) can cause adverse effects on human including ulcers, irritation of nosal mucosa, allergic and asthmatic reactions, and nasal septum perforation. Long-term exposure to high levels of Cr(VI) can cause kidney and liver damage, stomach ulcers, irritation of the gastrointestinal tract, diarrhea, stomach and intestinal bleeding, and death. Apart from its contact toxicity and carcinogenicity, Cr(VI) also causes birth defects and decrease reproduction health in mammals (Losi *et al.,*1994b). The resulting complications may result in death of the organism (Zayed and Terry, 2003). As a result of these and other toxic effects, the World Health Organization (WHO) has set the maximum acceptable chromium concentration in drinking water at 0.05 mg/L (Kiilunen, 1994; Lu and Yang, 1995; Baral and Engelken, 2002).

### 2.3.2 Toxicity to Microorganisms

Cr(VI) is toxic to most microorganisms even at low concentrations, due to its ability to inhibit enzyme activity, ‘poison’ cells non-specifically by blocking essential functional groups, displacing essential metal ions and modifying the conformations of the biological molecules, or induce mutations (Ehrlich, 1986). Cr(VI) ions are known to have inhibitory and mutagenic effects on most microorganisms such as *Escherichia coli*, *Bacillus subtilis*, and *Salmonella*

*typhimurium* (Venitt and Levy, 1974; Petrilli and DeFlora, 1977; Ross *et al.,* 1981; Zibilske and Wanger, 1982; Aislabie and Loutit, 1984; Ajmal *et al.,* 1984). The visible mutagenic effects reported in bacterial species include cell elongations, cell enlargement, and inhibited cell division, which eventually lead to cell growth inhibition (Coleman and Paran**,** 1983). These mutagenic effects were reported to be only effective when Cr(VI) ions diffuse across the cell membrane of a bacterial species. The subsequent reduction of these Cr(VI) ions within the cell may alternatively result in the formation of free radicals which may generate DNA alterations as well as toxic effects (Arslan *et al*., 1987; Kadiiska *et al*., 1994; Lui *et al*.,1995; Molokwane, 2010). The genotoxic effects of bacterial cells include frame shift mutations and base pair substitutions (Petrilli and DeFlora, 1977). Changes in morphologies of gram-positive and gram-negative bacteria were also observed (Bondarenko *et al.,* 1981).

## 2.4 Remediation Strategy

### 2.4.1 Physico-chemical Methods

Cr(VI) is currently extracted and treated from the contaminated environment using the conventional methods including pump and treat, iron exchange, and electrochemical precipitation method.

#### Pump and Treat Remediation

It is one of the most common approaches for contaminated groundwater remediation. This method relies on pumps to bring polluted groundwater to the surface where it can be treated efficiently and released back or reintroduced into the groundwater environment. The pump and treat method may be considered as a best option in cases where the contaminant have seeped into the groundwater. It can also be used to help to keep polluted groundwater from spreading to drinking water wells. However this technique fails to attend to source of the contamination in vadose zone and also create the problem of lowering the water table, leaving behind residual contamination in new vadose zone. Also in new areas of low permeability, residual levels of Cr will be missed, thereby creating future sources of contamination (Bayer and Finkel, 2006).

#### Electrochemical Precipitation

This method utilizes an electrical potential to maximize the removal of heavy metal from contaminated wastewater over the conventional chemical precipitation method (Kurniawan *et*

*al*., 2006). It is the most common method for removing toxic heavy metals up to parts per million (ppm) levels from water. Electrochemical Cr(VI) reduction process is often employed in combination with the pump and treat method and uses consumable iron electrodes and electrical current to generate ferrous ions that react with Cr(VI) to Cr(III) as given:

$$3Fe^{2+} + CrO_4^{2-} + 4H_2O \rightarrow 3Fe^{3+} + Cr^{3+} + 8OH^- \qquad (2\text{-}4)$$

The efficiency of this method is affected by low pH and the presence of other salts (ions). Additionally, this process takes a long period of time to reach the regulatory level for remediation of contaminated sites and also it result in increased quantity of toxic sludge.

**Ion Exchange**

Ion exchange is a unit process by which ions of given species are displaced from an insoluble exchange material by ions of a different species in a solution. In the ion exchange equipment Cr-containing solution enters one end of the column under pressure, passes through the resin bed and then Cr is removed from the solution. When the resin capacity is exhausted, the column is backwashed to remove the trapped solids and then regenerated. Commonly used matrices for ion exchange are synthetic organic ion exchange resins. The disadvantage of an ion exchange method for Cr removal is that ion exchange resins are very selective (Lin and Kiang, 2003). Additionally, ion exchange equipment can be quite expensive. Incomplete removal of Cr in the salt solution is likely in the ion exchange method (Cabatingan *et al*., 2001; Camargo, 2003). Furthermore, ion exchange equipment can not handle concentrated metal solution as the matrix gets easily fouled by organics and other solids in wastewater and also it is highly sensitive to pH of the solution.

The major drawbacks of these existing conventional treatment methods for Cr(VI) contaminated soil and groundwater includes high energy expenditure in the process, use of expensive toxic chemical reductants that result in the production of large quantity of toxic sludge which is also difficult to treat (Komori *et al*.,1990; Blowes, 2002; Gonzalez *et al*., 2003). This indicates that physicochemical methods are less effective in addressing the final waste disposal problem. Bioremediation on the other hand is more attractive opinion as it offers a potential of treating the waste under near neutral conditions and produces a minimum or no toxic sludge.

### 2.4.2 Bioremediation Method

The term bioremediation has been used to describe the process of using living organisms; primarily microorganisms to detoxify, degrade or destroy hazardous pollutants from the environment (Glazer and Nikaido, 1995). Microbial Cr(VI) reduction have appeared to be ubiquitous in nature as the consortia culture isolated from both Cr(VI) contaminated and uncontaminated sites were able to reduce Cr(VI) (Turick *et al*., 1996; Chen and Hao, 1998; Schmieman *et al*., 1998; Sani *et al*., 2002; Camargo *et al*., 2003). However the indigenous microorganisms were more preferred over foreign isolates, as they displayed the best characteristics for the remediation process (Vadali, 2001) and also their release in the environment did not result into microbial diversity shift and yielding of new dominant species in the environment.

The emerging technologies for bioremediation of Cr(VI) includes an *in situ* and an *ex situ* technology. The *in situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance to the surrounding environment (Krishna and Philip, 2005). Conversely *ex situ* techniques are defined as those that are applied to soil and groundwater at an alternative site, in which case the contaminant is removed from the actual site via excavation and pumping. *In situ* bioremediation technology is considered as the most advantageous technique over the *ex situ* bioremediation technique as a result of its low installation cost its potential to minimize the risk associated with waste transportation. *In situ* bioremediation technology can therefore be applied to circumvent the limitation of physicochemical methods.

Biological processes include (i) biotransformation (Shashidar *et al*., 2007; Molokwane *et al*., 2008) which is the transformation of contaminated molecule into less or non-hazardous molecules, (ii) biosorption (Juwarkar and Jambulkar, 2008) which involves the detoxification/removal of hazardous substance instead of transferring them from one medium to another by means of microbes and plants. It also defined as a metabolic passive process (i.e. It does not require energy), and (iii) bioaccumulation is process similar to biosorption process, but it differs in a way that it is an active metabolic process driven by energy from a living organism and requires respiration (Velasquez and Dussan, 2009). In practise the application of biological Cr(VI) reduction processes may be limited by high initial concentrations of Cr(VI) which can cause a significant deactivation of the introduced

microorganisms; the presence of other metals and/or toxic organic compounds in the growth medium which may severely inhibit the reduction activity of Cr(VI); the electron donor, redox potential, pH and temperature (Shen and Wang, 1994a; Fulladosa *et al*., 2006; Wu *et al*., 2010; Ye *et al*., 2010). Therefore efforts has been made by several authors to circumvent the problem of limited biological Cr(VI) reduction capacity in contaminated environments by isolating potential Cr(VI) reducing organisms that can survive the contaminated environment and also by developing an appropriate biological reactor system that can effectively detoxify Cr(VI) wastes both aerobically and anaerobically in the presence of other toxic compounds (Mazerski *et al*., 1994; Shen and Wang, 1995; Chirwa and Wang, 2001).

### 2.4.3 Biological Systems Engineering

The principal biological systems used for environmental treatment can be divided into two main categories: suspended and attached growth systems. The successful design and operation of these systems requires full understanding of the types of microorganisms' involved, specific reaction they perform, their nutritional needs and their reaction kinetics.

#### Suspended Growth System

In a suspended culture system the microorganisms which are responsible for the remediation process are maintained in liquid suspension by appropriate mixing methods. The remediation process in a suspended culture system may be operated under both aerobic and anaerobic conditions with sufficient contact time provided for mixing the waste effluent with the microbial suspension. Studies on suspended culture systems have been investigated by several authors (Mazerski *et al*., 1994; Shen and Wang, 1994a; Wang *et al*., 2000). It was observed that suspended culture were more susceptible to Cr(VI) toxicity. It was also observed that shock loadings of Cr(VI) in a suspended culture reactor leads to excessive loss in biomass (Wang *et al*., 2000; Molokwane, 2010). This implies that for effectives Cr(VI) reduction in a suspended culture reactor cells re-inoculation is required. The other drawback of suspended growth process is that during the actual treatment of highly Cr(VI) concentrated influent stream dilution of highly Cr(VI) concentrated influent to lower Cr(VI) concentration is required before treatment in the suspended culture reactor. This relatively indicates that larger reactor volume are required for treatment of relatively low concentrations of Cr(VI).

### Attached Growth (Biofilm) System

In a biofilm system the microorganisms responsible for the treatment process are attached to an inert packing material. The packing material used in attached growth processes includes gravel, soil, rocks and a wide range of plastic and other synthetic materials. Attached growth processes can be operated as an open or a closed system. In the open system aeration occurs while in the closed system no air penetration is allowed in or out of the vessel. In these processes the microorganisms responsible for the treatment process forms a biofilm on the packing material. The pollutants are removed by passing the waste effluent through the biofilm at an optimum flow rate and hydraulic retention time to allow sufficient contact time between the attached cells and the distributed contaminant over the packed material. Studies on biofilm systems have been investigated by (Wanner *et al*., 1995; Nelson *et al*., 1996; Beaudoin *et al*., 1998; Chirwa and Wang, 2001; Molokwane, 2010). Biofilm systems are preferred over suspended culture systems as they enable biomass to be retained in the reactor at flow rates greater than the washout flow rates during the operation. Higher removal kinetics of Cr(VI) were also observed in the biofilm system than in the suspended one as a result of culture acclimatization and mass transport resistance across the biofilm layer on cell exposure to toxicity (Wang and Chirwa, 2001). This indicates that the exposure of Cr(VI) toxicity to bacterial cells decreases with the increasing biofilm depth.

## 2.5 Cr(VI) Reducing Organisms

Cr(VI) is toxic to biological systems as a result to its strong oxidizing potential that can damage cells (Kotas and Stasicka, 2000). However some microorganisms are able to reduce toxic Cr(VI) to less toxic Cr(III) in the presence or absence of oxygen (Francisco *et al*., 2002; Polti *et al*., 2007). The microorganisms that are able to reduce Cr(VI) to Cr(III) are known as chromium reducing bacteria (CRB). Although Cr(VI) can be reduced by algae and other plants in soil, bacteria has been demonstrated to be the most efficient microorganism in Cr(VI) reducing process (Basu *et al*., 1997; Cervantes *et al*., 2001; Ganguli and Tripathi, 2002; Francisco *et al*., 2002). Bacteria can reduce Cr(VI) to Cr(III) either aerobically or anaerobically and in each case the process of reduction differs (Ackerley *et al*., 2004; Molokwane, 2010).

There are multiple reports of mesophilic bacteria capable of reducing Cr(VI) under various conditions. These include both gram-positive and gram-negative bacterial species (Pal and

Paul, 2005; Horton *et al*., 2006). Several authors have reported that gram-positive bacteria are more chromate tolerant than gram-negative bacteria (Ross *et al*., 1981; Baldi *et al*., 1990; Francis *et al*., 2000; Viti and Giovannetti, 2001; Viti and Giovannetti, 2005, Molokwane, 2010). These bacterial strains have been promoted for Cr(VI) contaminated environment as they are able to protect themselves from toxic substances in the environment by transforming toxic compounds through oxidation, reduction or methyliation into more volatile, less toxic or readily precipitating form (Dermou *et al*., 2005). The sensitivity of gram-negative bacterial species to Cr(VI) toxicity may be associated with their lack of true cell wall (Ross *et al*., 1981).

The reduction of Cr(VI) by bacterial consortia culture isolated from the natural environment have also been observed (Chirwa and Wang, 2000; Stasinanakis *et al*., 2004; Dermou *et al*., 2005; Chen and Gu, 2005; Chang and Kim, 2007; Molokwane *et al*., 2008). The consortium culture from the natural environment have been utilized for Cr(VI) reduction process in order to obtain a close or a clear picture of what really happens in the environment when the microorganisms do not live in pure cultures. The consortia culture have been found to be very much effective in degrading and detoxifying a wide variety of pollutants in the environment due to their diversity of metabolic pathways in the community (Sharma, 2002).

Microbial Cr(VI) reduction often results in the elevation of pH background. The increased pH facilitates the precipitation of the reduced form of chromium as chromite oxide $Cr(OH)_3$.In general, microbial reduction of Cr(VI) and consequence precipitation of Cr(III) can be illustrated as follows (Brock and Madigan, 1991; Zakaria *et al*, 2007):

$$CrO_4^{2-} + 8H^+ + 3\bar{e} \xrightarrow{CRB} Cr^{3+} + 4H_2O \xrightarrow{neutralpH} Cr(OH)_3 + 3H^+ + H_2O \qquad (2\text{-}5)$$

where: CRB represent Cr(VI) reducing bacteria or enzyme. It can be seen in Equation (2-5) that $CrO_4^{2-}$ needs to accept three electrons to be converted to Cr(III).

In the case of electron donor being acetate, microbial Cr(VI) reduction under anaerobic conditions can be expressed as:

$$3CH_3COO^- + 8CrO_4^{2-} + 17H_2O \xrightarrow{CRB} 8Cr(OH)_3\,(s) + 6HCO_3^- + 13OH^- \qquad (2\text{-}6)$$

Table 2- 1: Cr(VI) Reducing bacteria reported in literature

| **Name of a species** | **Isolation condition or system/Carbon source** | **References** |
|---|---|---|
| *Achromobacter sp.*CH1 | Anaerobic/LB broth medium, glucose, acetate | Zhu *et al*., 2008 |
| *Acinetobacter haemolyticus* | Packed bed bioreactor/Liquid pineapple wastewater | Ahmad *et al*., 2010 |
| *Activated sludge* | Batch/Cheese whey, lactose, glucose, acetate, citrate | Ferro Orozco *et al*., 2010 |
| *Agrobacterium radiobacter* EPS-916 | Aerobic-Anaerobic/glucose mineral salt medium | Llovera *et al*., 1993 |
| *Aspergillus sp.* | Batch/Potato dextrose broth and nutrient broth | Congeevaram *et al*., 2007 |
| *Bacillus sp.* | Aerobic/sodium acetate | Zahoor and Rehman, 2009 |
| *Bacillus sp.*E29 | Aerobic/LB broth medium | Camargo *et al*., 2003 |
| *Bacillus subtilis* | Cell suspension (Aerobic-Anaerobic)/glucose | Garbisu *et al*., 1998 |
| *Candida lipolytica* | Batch/glucose | Ye *et al.*,2010 |
| *E. coli* ATCC 33 456 | Aerobic-Anaerobic/Nutrient broth medium, glucose, acetate, glycerol and propionate | Shen and Wang, 1994b |
| *Enterobacter cloacae* HO1 | Anaerobic/KSC medium,sadium acetate | Wang *et al.*,1989 |
| *Ocherobacterium* | Aerobic/glucose | Zhigou *et al*., 2009 |
| *Pentoea agglomerans* SP1 | Anaerobic/acetate | Francis *et al.*, 2000 |
| *Pseudomanas putida* MK1 | Anaerobic/LB broth-citric acid | Park *et al*., 2000 |
| *Pseudomonas sp.* | Batch/Anaerobic/VB broth medium, D-glucose, Lactate or dextrose | McLean and Beveridge, 2001 |
| *Pseudomonas aeruginosa* | Aerobic/Nutrient broth medium, LB broth medium | Auguilera *et al.*,2004 |

## 2.6 Cr(VI) Reduction Pathways

Heterogeneous organisms obtain their energy for metabolism by participating in several oxidation-reduction reactions. In the environment where the photosynthesis does not occur the transfer of electron is a driving force that governs all the microbial process. Depending on the environment, microorganisms have adapted and evolved the ability to be able to mediate various oxidation-reduction couples to conserve energy.

Depending on the microbial species the reduction of Cr(VI) can be explained by two prevalent models: (i) direct enzymatic reduction, and (ii) indirect reduction. Direct enzymatic reduction refers to the reduction by the metal reductase system whereas indirect mechanism refers to Cr(VI) reduction mainly by conditions provided by bacterial source such as the redox potential, or the bacterial metabolites ($H_2S$).

### Direct Enzymatic Reduction

It has been shown in various instances that Cr(VI) is fortuitously reduced by enzymes and other primary physiological functions (Garbisu *et al*. 1998; Opperman and van Heerden, 2007). Direct enzymatic Cr(VI) reduction by bacterial species has been documented by several researchers under both aerobic and anaerobic conditions (Fujie *et al*., 1996; Garbisu *et al*., 1998; Guha *et al*., 2000; Yang *et al*., 2009; Molokwane, 2010). Microbial Cr(VI) reduction under aerobic conditions has been reported to be generally associated with soluble proteins using NADH as an electron donor or either as a requirement or for enhance activity (Suzuki *et al*., 1992; Shen and Wang, 1993; Garbisu *et al*., 1998). In the absence of electron donor, Cr(VI) reducing organisms may utilize endogenous reserves for reduction of Cr(VI) through the activity of soluble reductase. Under anaerobic condition Cr(VI) reduction can be carried out through energy yielding dissimilatory respiratory process in which Cr(VI) serves as a terminal electron acceptor. The reduction of Cr(VI) under anaerobic conditions has been reported to be generally associated with soluble reductase, a membrane-bound or both reductase with the possibility of involving hydrogenase or cytochrome $C_3$ (Tebo and Obraztsova, 1998; Michel *et al*., 2001).

### Indirect Enzymatic Reduction

Sulfate and iron-reducing bacteria are the two well known bacterial species which are able to reduce Cr(VI) indirectly via their anaerobic metabolic end products, hydrogen sulphide ($HS^-$)

and Fe (II), respectively (Pettine *et al*., 1994, 1998; Sedlak and Chan, 1997; Patterson *et al*., 1997). The metabolic end products produced by these bacterial species act as a reducing agent for the Cr(VI) in the medium.

### 2.6.1 Intracellular Processes

Transport of the metal across the cell membrane yields intracellular accumulation, which is depended on the microbial activity (Asku *et al*., 1991). Earlier studies on microbial Cr(VI) reduction indicate that bacteria such as *P.putida* PRS200, *P. ambigua* G-1 and *E.coli* ATCC33456, produced a soluble reductase enzyme capable of catalyzing the reduction of Cr(VI) to Cr(III). Experimentation with supernatant samples of cell extract and the intact cells has been reported to show almost the same Cr(VI) reduction activity, indicating a largely soluble reductase activity as a result of co-metabolism in cells (Shen and Wang, 1993).

In intracellular processes, Cr(VI) is reduced in the cytosol using cytoplasmic soluble reductase enzymes. These enzymes play an intermediate role between associated biological electron donors. The electron donors implicated with Cr(VI) reduction are NADH and/or NADPH, which are active within a wide range of temperature (40-70°C) and pH (6-9). According to Suzuki et al. (1992), NADH in the cell protoplasm donates an electron to Cr(VI) and generates Cr(V) that accepts two electrons from two molecules of the same co-enzyme to produce Cr(III).

$$Cr^{6+} + \bar{e} \rightarrow Cr^{5+} \qquad (2\text{-}7)$$

$$Cr^{5+} + 2\bar{e} \rightarrow Cr^{3+} \qquad (2\text{-}8)$$

### 2.6.2 Extracellular Processes

Attached growth systems can influence the removal of metal species through adsorption or extracellular polymeric substances and cellular excretions, which indicates that extracellular processes are facilitated by viable microorganisms. In this process Cr(VI) reducing enzyme are deliberately released into the media from the cell cytoplasm when Cr(VI) is detected in the media to reduce Cr(VI). The evidence of extracellular Cr(VI) reduction has been presented by few researchers (Shen and Wang, 1993; Chirwa and Wang, 1997b; Molokwane

*et al*., 2008) through a mass balance of Cr(VI) and the reduced Cr species in the supernatant and cells. Extracellular Cr(VI) reduction is beneficial to the cell as the cell does not require transport mechanism transfer chromate and dichromate into the cell and export Cr(III) into the media. As a result, such reduction mechanism protect the cell from DNA damaging and from Cr(VI) toxicity.

### 2.6.3 Membrane-bound Processes

The membrane-bound process may be facilitated by using dead or viable microorganisms. Membrane bound Cr(VI) reductase has been revealed with *P. fluorescens* LB300, *E. cloacae* HO1 (Bopp and Ehrlich, 1988; Wang *et al*., 1989). Mechanism of membrane-bound reductases may ensure the occurrence of Cr(VI) reduction on the cell surface, forming insoluble $Cr(OH)_3$ in the external medium. As a result, such a reduction mechanism protects cells from Cr(VI) toxicity.

## 2.7 Carbon Source

Chromium reducing bacteria may utilize a number of organic compounds to serve as electron donors for the Cr(VI) reducing process. Although Cr(VI) reducing bacteria may utilize a variety of organic compounds as electron donors for Cr(VI) reduction. Early studies have shown that the majority of organic compounds which serves as electron donors are generally limited to natural aliphatic, mainly low molecular weight carbohydrates, amino acids and fatty acids (Wang and Shen, 1995). The selection of these organic compounds was dependent on the growth condition and the type of Cr(VI) reducing microorganism. Addition of organic compounds which are widely available and easily biodegradable (glucose, sodium acetate) may support the growth of certain species under varies conditions, cause a dramatic increase in the rate of Cr(VI) reduction of some species or protect the reducing enzyme from inactivating. The effective role of brown sugar, in particular the glucose component, to reduce Cr(VI) was also demonstrated by (Chirwa and Wang, 1997) as follows:

$$C_6H_{12}O_6 + 8CrO_4^{2-} + 34H^+ \rightarrow 8Cr^{3+} + 6HCO_3^- + 20H_2O \qquad (2\text{-}9)$$

This equation indicates that complete brake down of 1 mol glucose would yield sufficient energy to reduce 8 mol of Cr(VI). The effectiveness of glucose as a carbon source for the reduction of Cr(VI) may be associated to the fact that glucose directly enters into glycolysis,

TCA cycle and ETC mechanism to donate energy/electrons for Cr(VI) reduction, whereas other carbon sources need intermediate conversion process to form glucose.

## 2.8 Summary

Literature survey of this study illustrates that both physico-chemical and biological methods have been utilized for the remediation of contaminated environments. The latter method appeared to be of great interest compare to the traditional physico-chemical methods. The microbial Cr(VI) reduction process has been investigated further by several authors under various conditions using different systems. Although much research has been conducted on Cr(VI) reduction processes, the problem of Cr(VI) pollution still remain a matter of concern strongly affecting soil and groundwater systems. As an endeavor to solve the problem of soil and groundwater contamination, this study evaluates the *in situ* bioremediation process as a strategy for effective Cr(VI) pollution control in soil and groundwater systems.

CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Bacterial Culture

### 3.1.1 Sources of Cr (VI) Reducing Bacteria

The microbial culture consortium was collected from the sand drying beds at the Brits Wastewater Treatment Works (North West Province, South Africa). The samples were collected in sterile containers and stored at 4°C in the refrigerator until used.

### 3.1.2 Culture Isolation

Bacteria cultures were isolated from the samples collected from sand drying beds at Wastewater treatment plant using the enrichment culture technique. A grain (0.2 g) of sludge was inoculated in the sterilized media (100 mL, Luria-Bettani (LB) broth amended with 75 mg/L of Cr(VI)) for culturing. The inoculum was incubated for 24 hours at 30 ± 2°C under shaking at 120 rpm in a Rotary Environmental Shaker (Labotec, Gauteng, South Africa). Aerobic cultures were grown in cotton plugged 250 mL Erlenmeyer flasks whereas anaerobic cultures were grown in 100 mL serum bottles purged with pure (nitrogen) $N_2$ gas (99% pure grade) and sealed with silicon rubber stoppers and aluminium seals prior to incubation. After 24 hours enriched bacteria strains were isolated by serial dilution of the cultivated culture.

Pure cultures were prepared by depositing 1 mL of serially diluted sample from the $7^{th}$ to the $10^{th}$ tube in the petri dishes containing LB agar using the spread method. The plates were then incubated for 24-48 hours at 30 ± 2°C to develop separate identifiable colonies. Individual colonies based on their colour and morphology were transferred into 100 mL sterile LB broth amended with 150 mg/L of Cr(VI) using a heat sterile wire loop. Cells were allowed to grow for 24 hours and then again 1mL of 24 hours grown culture was serially diluted and 1mL from the $7^{th}$ to the $10^{th}$ tube was deposited into a LB agar plate and incubated for 24-48 hours at 30 ± 2°C. The persistent colonies from the third isolation of 200 mg/L Cr(VI) were used for detailed Cr(VI) reduction experiments.

## 3.2 Growth Media

### 3.2.1 Basal Mineral Media

Basal Mineral Medium (BMM) was prepared by dissolving: 10 mM $NH_4Cl$, 30 mM $Na_2HPO_4$, 20 mM $KH_2PO_4$, 0.8 mM $Na_2SO_4$, 0.2 mM $MgSO_4$, 50 µM $CaCl_2$, 25 µM $FeSO_4$, 0.1 µM $ZnCl_2$, 0.2 µM $CuCl_2$, 0.1 µM NaBr, 0.05 µM $Na_2MoO_2$, 0.1 µM $MnCl_2$, 0.1 µM KI, 0.2 µM $H_3BO_3$, 0.1 µM $CoCl_2$, and 0.1 µM $NiCl_2$ into 1 L of distilled water and then amended with 5 g of glucose to act as a carbon and energy source for the bacteria. The prepared medium was sterilized before use by autoclaving at 121°C at 115 kg/cm$^2$ for 15 minutes.

### 3.2.2 Commercial Broth and Agar

The first three media, Luria-Bettani (LB) broth, Luria-Bettani (LB) agar, and Plate count (PC) agar (Merck, Johannesburg, South Africa) was prepared by respectively dissolving 25 g, 45 g, and 23 g in 1000 mL of distilled water. The LB and PC agar media were cooled at room temperature after sterilization at 121°C at 115 kg/cm$^2$ for 15 minutes and then dispensed into petri dishes to form agar plates for colony development.

## 3.3 Reagents

### 3.3.1 Standard Solutions and Chemicals

Cr(VI) stock solution (1000 mg/L) was prepared by dissolving 3.74 g of 99% pure $K_2CrO_4$ (Analytical grade) in 1 L deionised water. This stock solution was used through out the experiments to serve as Cr(VI) source. The standard solutions of Cr(VI) were prepared from the Cr(VI) stock solutions in a 10 mL volumetric flask by diluting certain volume of Cr(VI) stock solution with distilled water to give desirable final concentrations ranging from 0-8 mg/L. From these data points (absorbance against concentration) a linear graph/calibration curve with the regression of 99% was obtained.

### 3.3.2 DPC Solution

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

### 3.3.3 Chemicals

Sodium chloride solution (0.85% NaCl) was prepared by dissolving 1.85 g of sodium chloride salt in 100 mL distilled water and sterilized by autoclaving at 121°C for 15 minutes. All chemicals used were of analytical grade obtained from Sigma Aldrich, Johannesburg, South Africa.

### 3.4 Culture Characterization

The phylogenetic characterization of cells was performed on isolated individual colonies of bacteria from the $7^{th}$ to the $10^{th}$ tube in the serial dilution preparation. In preparation for the 16S rRNA (16 Svedburg unit *ribosomal* Ribo-Nucleic-Acid) fingerprint method which is used to obtain DNA sequences of pure isolated cultures, the colonies were first classified based on morphology. Seven different morphologies were identified for the aerobic cultures. These cultures were streaked on LB agar plates followed by incubating at 30 ± 7°C for 18 hours.

Genomic DNA was extracted from the pure cultures using a DNeasy tissue kit (QIAGEN Ltd, West Sussex, UK). The 16S rRNA genes of isolates were amplified by a reverse transcriptase-polymerase chain reaction (RT-PCR) using primers pA and pH1 (Primer pA corresponds to position 8-27; Primer pH to position 1541-1522 of the 16S gene. An internal primer pD was used for sequencing (corresponding to position 519 - 536 of the 16S gene). The resulting sequences were deposited in the GenBank to be compared to known bacteria using a basic BLAST tool search of the National Centre for Biotechnology Information (NCBI, Bethesda, MD).

### 3.4.1 General Characterization of Aerobic Cultures using 16S rRNA ID's

In preparation to 16S rRNA sequence identification, the colonies were first classified based on their morphology. The 16S rRNA resulted in a total of seven aerobic isolates. At 99% identity results indicated the predominance of four aerobic phenotypes. Partial sequences of 16S rRNA matched the following bacterial species:

- *Bacillus cereus* ATCC 10987, *Bacillus cereus* 213 16S,
- *Bacillus thuringiensis* (serovar finitimus), Bacillus mycoides
- *Microbacterium foliorum* and *Microbacterium sp*. S15-M4.

Table 3- 1: Characterization of Cr(VI) reducing bacteria under aerobic conditions (Molokwane *et al*., 2008; Molokwane, 2010)

| Pure Culture | Species Identified | % Identity |
|---|---|---|
| X1 | *Bacillus cereus strain* 213 16S, *Bacillus thuringiensis* 16S | 99 |
| X2 | *Bacillus sp*. ZZ2 16S, *Bacillus cereus* ATCC 10987, *Bacillus thuringiensis strain* Al Hakam | 99 |
| X3 | *Bacillus sp*. 32-661 16S, *Bacillus cereus* 16S | 99 |
| X4 | *Bacillus mycoides strain* BGSC 6A13 16S, *Bacillus thuringiensis serovar finitimus strain* BGSC 4B2 16S | 99 |
| X5 | *Bacillus mycoides strain* BGSC 6A13 16S, *Bacillus thuringiensis serovar finitimus strain* BGSC 4B2 16S | 99 |
| X6 | *Bacillus mycoides strain* BGSC 6A13 16S, *Bacillus thuringiensis serovar finitimus strain* BGSC 4B2 16S | 99 |
| X7 | *Microbacterium sp*. S15-M4, *Micribacterium foliorum* | 99 |

### 3.4.2 Culture Storage

To 80 mL of bacterial culture, 20 mL of sterile glycerol was added (final glycerol concentration: 20%, v/v). The culture was then vortexed to ensure that the glycerol was evenly dispersed and then transferred into a screw cap tube, labelled and stored at –70°C. In order to utilize the pure stored bacterial isolates, the frozen cultures were allowed to melt at room temperature for approximately 10-15 minutes. The cultures were then streaked onto the surface of an LB agar plate using a sterile inoculating loop. The labelled LB plates were then incubated for 18-24 hours at 30 ± 2°C.

### 3.4.3 General Classification of Anaerobic Cultures using 16S rRNA ID's

A total of eighteen different morphologies were identified in anaerobically grown cultures using the method, with only ten colonies partially identified by the BLAST results. The results obtained indicated the predominance of seven facultative anaerobic phenotypes.

Table 3- 2: Characterization of Cr(VI) reducing bacteria under anaerobic conditions (Molokwane *et al*., 2008; Molokwane, 2010)

| **Pure culture** | **Colour on plate** | **Blast results** | **% Identity** |
|---|---|---|---|
| X1 | Light brown/cream | Could not subculture/amplify | |
| X2 | Off-white | *Enterococcus avium, Enterococcus pseudoavium* | 99 |
| X3 | Cream | Uncultured bacterium clone Y2, *Acinetobacter sp.* ANT9054 | 97 |
| X4 | Coral | Could not subculture/amplify | |
| X5 | Yellow | Could not subculture/amplify | |
| X6a | Yellow | *Arthrobacter sp.* Sphe3, uncultured soil bacterium clone TA12 | 93,94 |
| X6b | | *Arthrobacter sp*. AK-1 | 99 |
| X7 | Cream and yellow rings | *Bacillus drentensis, B. drentensis* | 96,97 |
| X8 | Light brown | Could not subculture/amplify | |
| X9 | Light brown | Could not subculture/amplify | |
| X10 | Light brown | *Oceanobacillus sp.* JPLAk1, *Virgibacillus necropolis* | 99,98 |
| X11 | Off-white | *Enterococcus faecium strain* R0026, *Rumen bacterium* R4-4 | 99 |
| X12 | Coral | *Paenibacillus pabuli, Paenibacillus xylanilyticus strain* XIL14 | 99 |
| X13 | Yellow | Could not subculture/amplify | |
| X14 | Orange | Could not subculture/amplify | |
| X15 | Cream | [*Brevibacterium] frigoritolerans, Bacillus sp.* R21S | 99 |
| X16 | Yellow | Could not subculture/amplify | |
| X17 | Cream | Uncultured *bacterium, Bacillus sp*. BS19 | 93 |

## 3.5 Batch Reactor Studies

### 3.5.1 Aerobic Cr(VI) Reduction Experiments

The pure cultures were grown aerobically in a 1 L Erlenmeyer flask containing 400 mL LB broth for a period of 24 hours. Cells were then collected by centrifuging at 6000 rpm (2820 g) at 4°C for 10 minutes. The supernatant was decanted and the remaining pellet was washed three times in a sterile saline solution (0.85% NaCl) while centrifuging. Aerobic Cr(VI) reduction experiments were conducted in 250 mL Erlenmeyer flasks by adding Cr(VI) stock solution into 100 mL BMM to give the desirable effective final Cr(VI) concentration ranging between 50-400 mg/L. Prior inoculating the flask with harvested cells, 1 mL of a sample was initially withdrawn from the Erlenmeyer flask to determine the absorbance of Cr(VI) before introducing the cells in each flask. The flasks containing the suspended cells were then plugged with cotton wool to allow aeration while filtering away microorganisms from the air and then incubated at 30 ± 2°C with continuous shaking on a lateral shaker (Labotec, Gauteng, South Africa) at 120 rpm. All experiments were duplicated and performed at a stationary phase. To monitor Cr(VI) reduction 1 mL of the samples were withdrawn at regular time intervals. The withdrawn samples were then centrifuged using a 2 mL eppendorf tubes at 6000 rpm (2820 *g*) for 10 minutes and the supernatant was used for Cr(VI) concentration analysis.

### 3.5.2 Anaerobic Cr(VI) Reduction Experiments

The pure cultures were grown anaerobically in a 1 L Erlenmeyer flask containing 400 mL LB broth for a period of 24 hours. Cells were then collected under anaerobic conditions by centrifuging at 6000 rpm (2820 *g*) at 4°C for 10 minutes. The supernatant was decanted and the remaining pellet was washed three times in a sterile saline solution (0.85% NaCl) under an anaerobic glove bag purged with 99% $N_2$ gas. Anaerobic Cr(VI) reduction experiment were conducted in 100 mL serum bottles by adding Cr(VI) stock solution into the BMM to give the desirable effective final Cr(VI) concentration ranging between 50-200 mg/L. Prior inoculating the serum bottles with the harvested cells under anaerobic conditions, 1 mL of a sample was withdrawn from each serum bottle at various Cr(VI) concentration to determining the absorbance of Cr(VI) before inoculating the bottles with viable cells. The cells were then transferred into 100 mL serum bottles under an anaerobic glove bag purged with

99% $N_2$ gas. The samples in the bottles were then directly purged with 99% $N_2$ gas for about 10 minutes to expel any oxygen gas before sealing with silicon rubber stopper and aluminium seals. The samples were then incubated at 30 ± 2°C with continuous shaking on a lateral shaker (Labotec, Gauteng, South Africa) at 120 rpm. Cr(VI) reduction was monitored by withdrawing 1 mL of the sample at regular time intervals using a sterile syringe. The withdrawn samples were then centrifuged using a 2 mL Eppendorf tube at 6000 rpm (2820 *g*) for 10 minutes in a Minispin® Microcentrifuge (Eppendorf, Hamburg, Germany) before Cr(VI) analysis to remove suspended cells.

### 3.5.3 Abiotic Experiments

Heat killed cultures were used to determine the abiotic Cr(VI) reduction in the batch experiments. Overnight grown cells were heat killed by autoclaving at 121°C for 20 minutes. Cultures were collected by centrifuging at 6000 rpm (2820 *g*) for 10 minutes and then washed three times in a sterile saline solution (0.85% NaCl), while centrifuging. The pellet collected from centrifuge were then used for Cr(VI) reduction processes.

## 3.6 Biomass Analysis

### 3.6.1 Total Biomass

Samples 5 mL were withdrawn at regular time intervals 0-24 hours, centrifuged for 10 minutes at 6000 rpm (2820 *g*). The supernatant was used to analyse Cr(VI) concentration and the settled pellet was used for biomass analysis. The pellet was resuspended in 1 mL distilled water and filtered through a pre-weight Whatman filter paper No.1. The filter with the microorganism was dried in the oven at 75-80°C to get a constant weight. The difference between the dried filter paper with cells and the empty filter paper was considered as a biomass.

### 3.6.2 Viable Biomass

Samples (1 mL) were withdrawn from experimental batches at regular time intervals of 0-24 hours for the analysis of viable cell concentration. Samples were then serially diluted into a 9 mL sterile 0.85% NaCl solution and from the $7^{th}$ to the $10^{th}$ tube 0.1 mL of the diluted sample were transferred into a PC agar plate using the spread method. The PC agar plates were then incubated for 18-24 hours at 30 ± 2◦C.

Colonies were counted after incubation and multiplied by a dilution factor. The bacterial count was reported as colony forming units (CFU) per mL of sample.

## 3.7 Microcosm Studies

### 3.7.1 Mineral Composition of Aquifer Media

Samples were collected the aquifer zone at the depth of 3m below ground surface and 200-300 m from the hot spots. The mineral composition of the aquifer media was quantitatively measured by inductively-coupled plasma mass spectrometry (ICP-MS). This was done to reveal the source of possible interference and levels of background of chromium in the aquifer media. The presence of other elements such as iron, manganese and nitrates in the soil which can act as electron sinks and accept electron from reactive organic and inorganic sources in the reduction process of Cr(VI) are expected to course interference in the Cr(VI) reduction process and elements such as calcium and magnesium are expected to course interference in the spectrometric analysis of Cr(VI). The background Cr concentration in the soil sample was detected as 50 µg/kg. Table (3-3) shows elementary soil composition of significant presence.

Table 3- 3: Mineral composition of aquifer soil media (Molokwane, 2010)

| Element | Symbol | Mass concentration (µg/kg) |
|---|---|---|
| Aluminium | Al | 4003 |
| Calcium | Ca | 2868 |
| Magnesium | Mg | 542 |
| Sodium | Na | 248 |
| Iron | Fe | 15145 |
| Manganese | Mn | 543 |
| Zinc | Zn | 367 |

### 3.7.2 Reactor Setup

Four columns constructed from a Plexiglas (PVC glass), (60 cm long, 5 cm internal diameter) were installed in a laboratory as continuous flow columns as shown in Figure (3-1). Each column consisted of an influent port, five intermediate ports and an effluent port. The columns were then packed with aquifer media from the target site. Prior closing the columns on both ends with PVC caps, one of the packed columns was sterilized by autoclaving at 121°C for 20 minutes and the other two columns were

amended with an organic electron donor (sawdust) to represent the heterogeneous carbon source from decaying vegetation. The four packed columns were then capped on both ends with PVC caps and installed vertically on the board by clapping. Each column was vertically interconnected to a 500 mL reservoir that gravimetrically transfers the contaminants into the packed bed reactor through the interconnecting tubes at the vertical height of 58 cm. Reservoirs were also interconnected to one another horizontally from the main feed container through out the re-circulation point to maintain the continuous flow process.

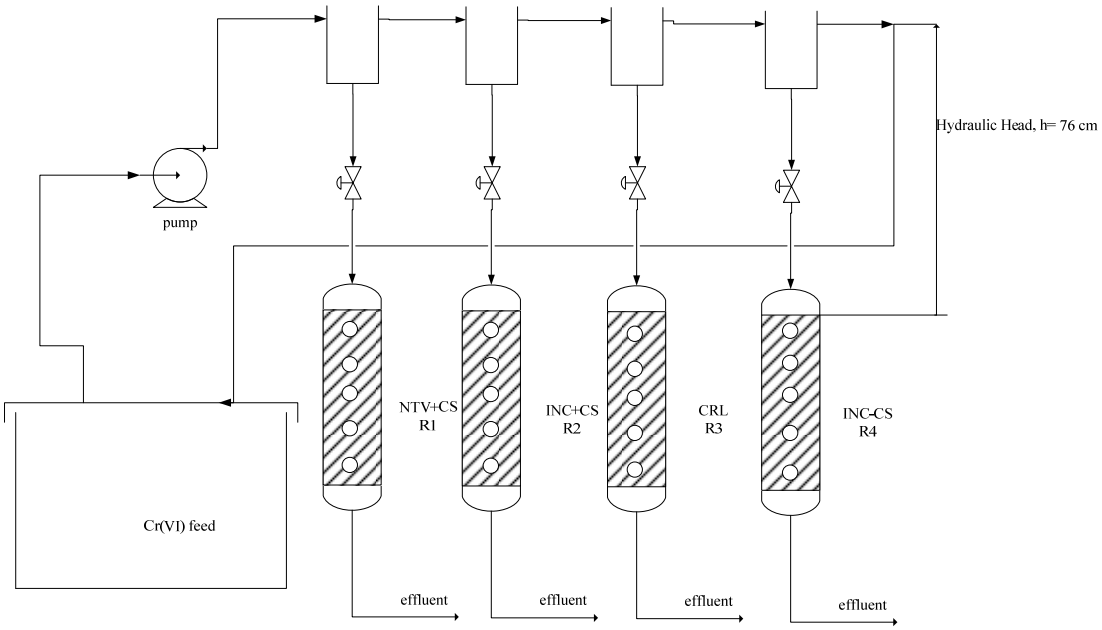


Figure 3- 1: Laboratory set up microcosm columns

### 3.7.3 Start up culture

Reconstituted consortium culture isolated from the dried sludge was cultivated for 24 hours in the LB broth medium amended with 75 mg/L of Cr(VI). The cultivated cells were then harvested by centrifuging at 6000 rpm (2820 *g*) for 10 minutes and then thoroughly mixed with a BMM.

### 3.7.4 Reactor Start up

Prior experimental run, distilled water was fed through each column in order to saturate pores with water. Flow rates were measured and adjusted to establish the hydraulic retention time (HRT) of approximately 24 hours in each reactor. Two

reactor columns were then inoculated with viable cells for 24 - 48 hours, enough time to allow uniform distribution and attachment of cells to soil particles in the reactor.

### 3.7.5 Reactors Operation

Cr(VI) solution from the main feed container was continuously fed into the receiving feed containers (reservoirs) through the peristaltic pump to maintain the feed level in the reservoirs. Cr(VI) feed from the reservoirs was then gravimetrically transferred to the packed bed reactors as in the case of open aquifers at the actual contaminated site. The microcosm reactors were operated as packed beds at different Cr(VI) concentrations of (20, 30, 40 and 50 mg/L) respectively. The experiments were conducted for seven weeks under micro-aerobic condition at an ambient pH and temperature. Prior running new feed of Cr(VI) concentration in each column, distilled water was passed through the aquifer media in each column to remove or to wash out the traces or the residual Cr(VI) in the aquifer media from the previous run. Samples were then withdrawn from each sampling port for analysis.

## 3.8 Analytic Methods

### 3.8.1 Cr(VI) Analysis

Cr(VI) reduction was determined colorimetrically using UV/vis spectrophotometer (WPA, Light Wave II, and Labotech, South Africa). The measurement was carried out using the DPC method according to the following procedure: In a 10 mL volumetric flask, 0.2 mL of a sample was acidified with 1mL of 1N $H_2SO_4$, then followed by distilled water and 0.2 mL of 1,5 DPC up to the mark (APHA, 2005). The mixture was then agitated thoroughly for about 15-30 seconds and let to stand for about 3 minutes for full colour development. The red-violet purple colour formed was then measured at wavelength of 540 nm (10 mm light path) using the calibrated instrument. Total Cr was measured at a wavelength of 359.9 nm using a Varian AA–1275 Series Flame Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, CA) equipped with a 3 mA chromium hollow cathode lamp. Samples were digested with concentrated nitric acid ($HNO_3$) before analysis. Cr(III) was determined as the difference between total Cr and Cr(VI) concentration. AAS was calibrated prior total Cr analysis using 1-5 mg/L Cr(VI) concentration prepared from the Cr(VI) stock solution and 2.5% nitric acid.

CHAPTER 4

# CR(VI) REDUCTION BATCH KINETICS STUDIES

## 4.1 Preliminary Studies

Primarily, different cultures of bacteria from the dried sludge were tested for effectiveness in reducing Cr(VI). This initial test was conducted at a low initial Cr(VI) concentration of 10 mg/L. It was observed from this test that four aerobic isolates (X2, X4, X5, X6) and three anaerobic isolates (X6b, X12, X15) were able to remove Cr(VI) in the medium. Each of the four aerobic isolates achieved complete Cr(VI) removal within 4 hours of incubation, whereas the anaerobic isolates achieved near complete Cr(VI) removal at a longer period of 12 hours. The highest performing cultures were then used in future investigations that lead to the development of a batch kinetic model and aquifer model.

## 4.2 Performance Validation in Cultures

### 4.2.1 Individual Pure Cultures versus Reconstituted Consortium Culture

Analysis under aerobic conditions were conducted to determine the rate of Cr(VI) reduction in individual pure isolates and compare to performance with Cr(VI) removal by a reconstituted consortium. Reconstituted consortium culture was obtained by culturing specific colonies of the Cr(VI) reducing culture (X1+X2+X3+X4+X5+X6+X7). Results in Figure (4-1) show that no species acting alone can achieve the same level of Cr(VI) reduction rate as the reconstituted consortium culture. The reconstituted consortium culture out performed the individual pure isolates acting alone. The two potential isolates acting alone (X5 and X6) achieved 85% of Cr(VI) removal at 24 hours of incubation while reconstituted consortium culture achieved a complete Cr(VI) removal within the same time of incubation at the initial Cr(VI) concentration of 100 mg/L. These results indicate that CRB from the dried sludge may provide a robust Cr(VI) reduction model.

### 4.2.2 Reconstituted Culture versus Natural/Original Consortium Culture

Figure (4-2) shows that the rate of Cr(VI) reduction in the reconstituted consortium was much faster than the rate in the natural consortium culture. Hypothetically the rate of Cr(VI) reduction was expected to be more faster in the natural consortium culture compared to the reconstituted consortium culture. These unexpected results may be associated to higher

percentage of Cr(VI) reducing organisms in the reconstituted consortium culture due to pre-selection. The sterile control reactor on the other hand showed no Cr(VI) removal over time.

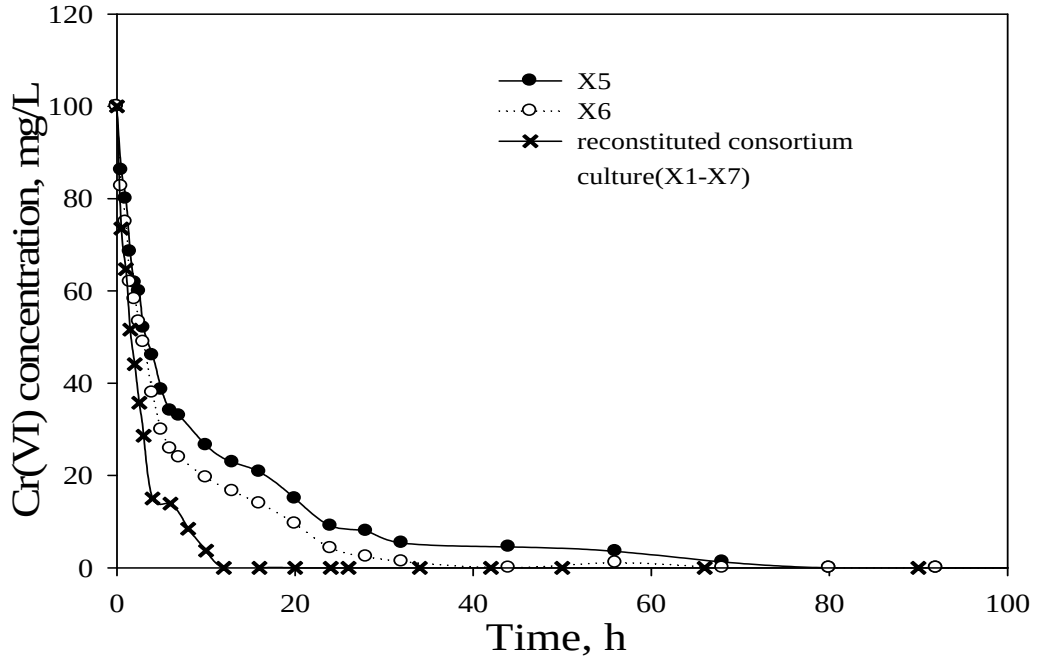


Figure 4- 1: Cr(VI) reduction between individual potential pure isolates and reconstituted consortium culture at the initial Cr(VI) concentration of 100 mg/L under aerobic conditions

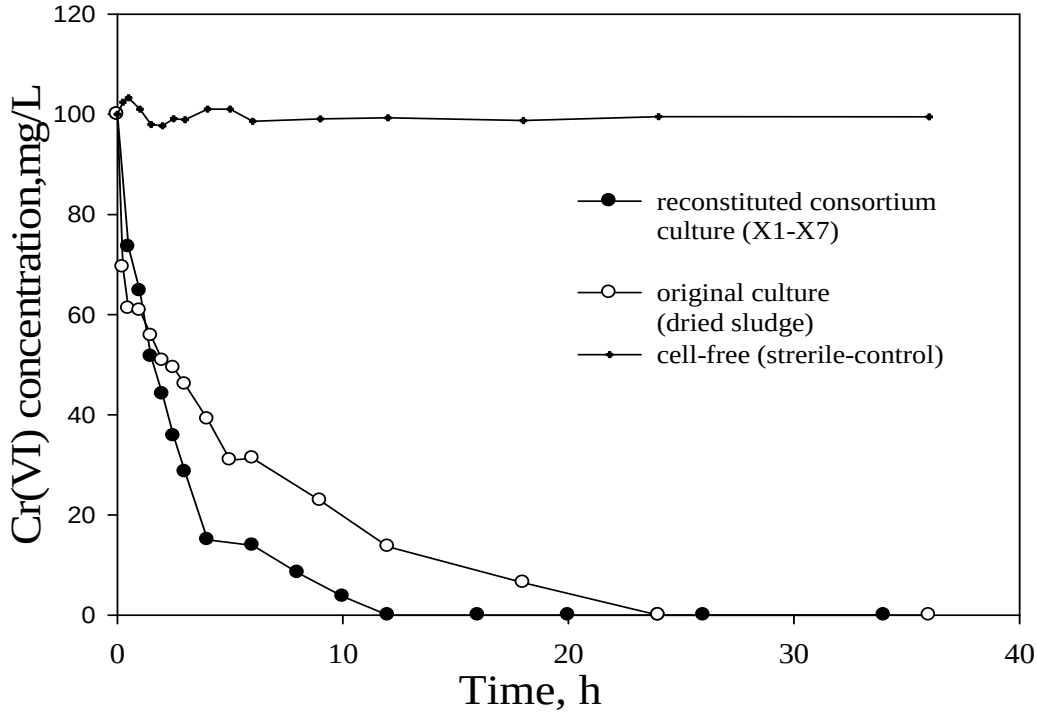


Figure 4- 2: Comparison between reconstituted and natural consortium culture at the initial Cr(VI) concentrations of 100 mg/L under aerobic conditions

## 4.3 Cr(VI) Reduction Kinetics

### 4.3.1 Cr(VI) Reduction Kinetics Under Aerobic Conditions

Individual pure isolates X2, X4, X5, X6 acting alone achieved complete reduction of Cr(VI) in batch with initial Cr(VI) concentration up 100 mg/L. Figure (4-3 and 4-4) shows that X6 was the highest performing isolate in reducing Cr(VI).

The reconstituted consortium, on the other hand, achieved a complete removal of Cr(VI) in all batches under initial Cr(VI) concentrations up to 200 mg/L. For instance, 50 mg Cr(VI)/L was completely removed within 4 hours of incubation, 100 mg Cr(VI)/L was completely removed within 12 hours, and 200 mg Cr(VI)/L was completely removed within 50 hours of incubation. The reduction activity of Cr(VI) at higher initial Cr(VI) concentrations of 300 and 400 mg/L was very slow compared to the reduction at relatively lower initial Cr(VI) concentrations. Up to 82% of Cr(VI) was reduced after 120 hours of incubation at 300 mg/L and further incubation to 200 hours increased the removal efficiency to 85%, approximately 60% of Cr(VI) was reduced within 200 hours of incubation at 400 mg Cr(VI)/L. Figure (4-5) shows that at higher initial Cr(VI) concentrations (300 and 400 mg/L), the finite reduction capacity of cells which is attributed to Cr(VI) toxicity on cells was reached after 60 hours of exposure to Cr(VI). It also is observed in Figure (4-5) that once the finite reduction capacity is reached, Cr(VI) reduction cease regardless of continued metabolic activity or cell synthesis.

The aerobic experiments in this study clearly show that Cr(VI) reduction facilitated by cells is inhibited by high initial Cr(VI) concentration in the medium. These observations are consistent with early studies (Shen and Wang, 1994a; Shen and Wang, 1995; Wang and Shen, 1997; Chirwa and Wang, 1997a) in which high levels of Cr(VI) inhibited both the growth and Cr(VI) reduction capacity in both pure and mixed culture of bacteria. The autoclave control on the other side exhibits no significant Cr(VI) reduction with time (Figure 4-5). Only 15% Cr(VI) removal efficiency was observed in heat-killed cells after the first 24 hours of incubation. The 15% removal in the heat-killed cells may be associated to the reductase released into the medium from heat-lysed cells and regrwoth of cells that escaped heat destruction. Therefore the insignificant Cr(VI) removal in the heat-killed cells over time implies that the abiotic processes are negligible.

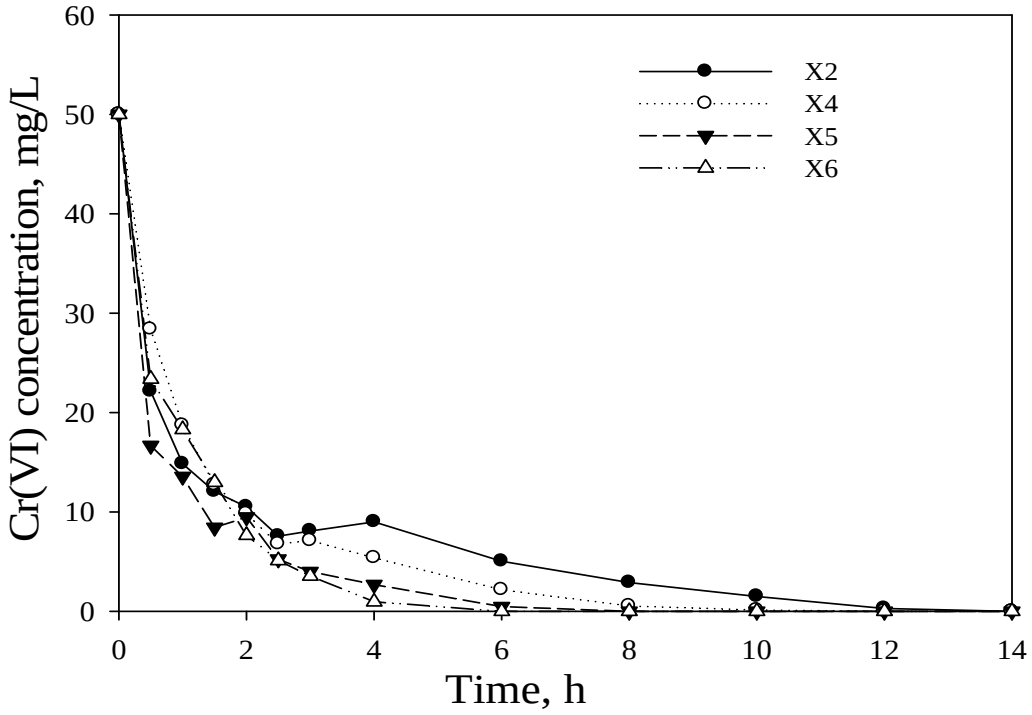


Figure 4- 3: Aerobic Cr(VI) reduction in pure isolates at 50 mg/L

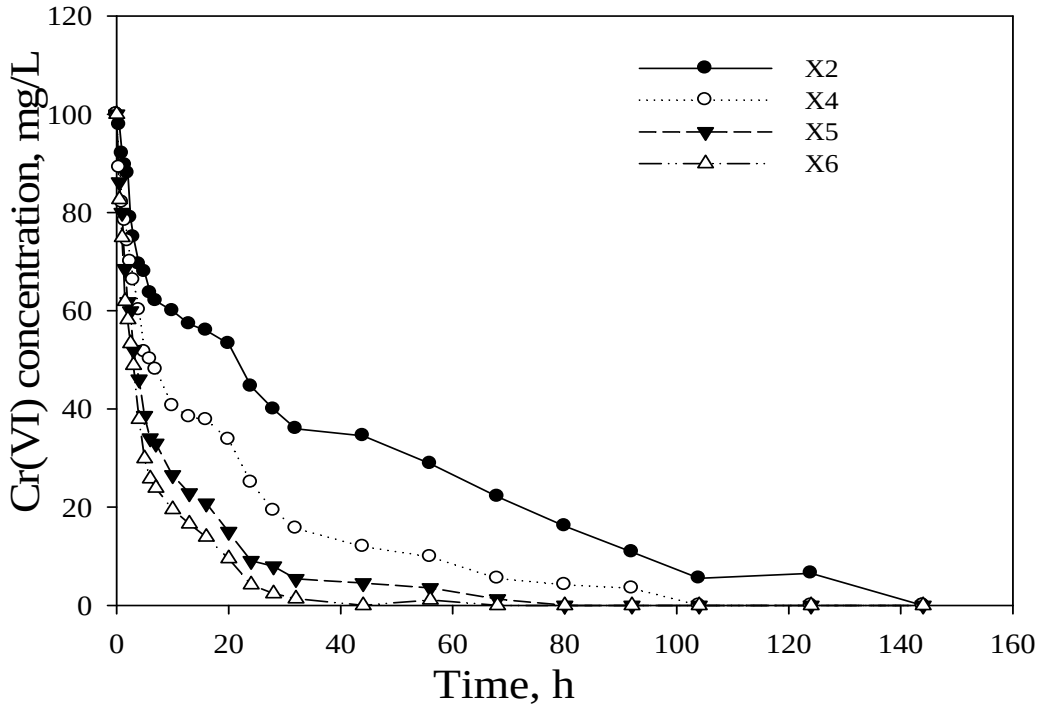


Figure 4- 4: Aerobic Cr(VI) reduction in pure isolates at 100 mg/L

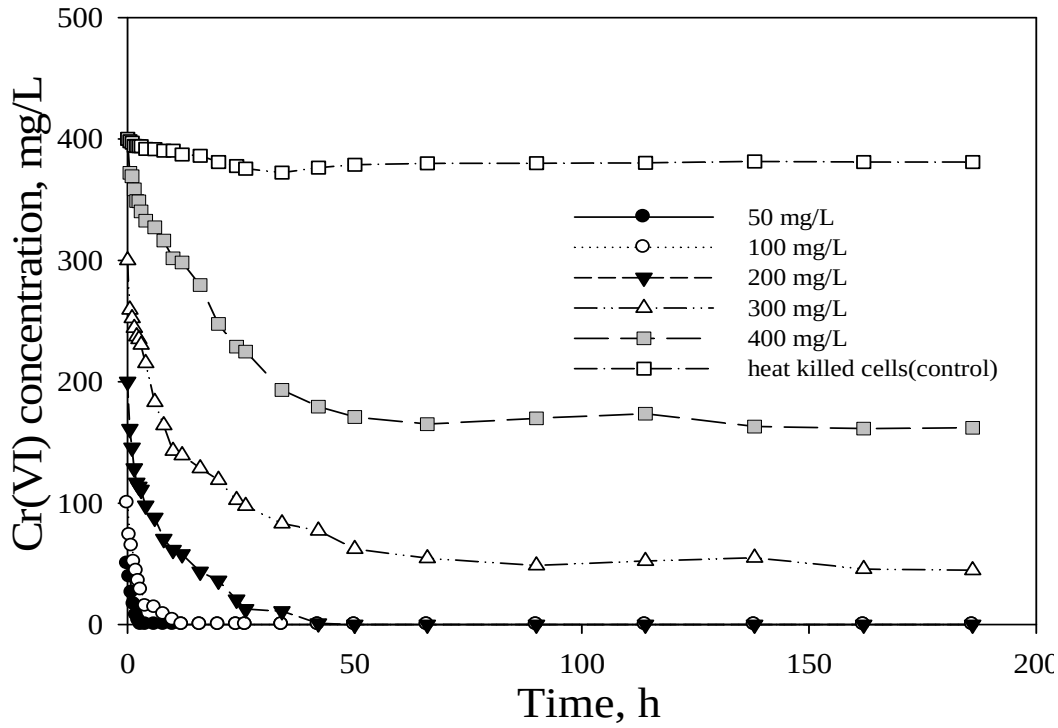


Figure 4- 5: Cr(VI) reduction in aerobic reconstituted consortium culture at (50-400 mg/L)

### 4.3.2 Cr(VI) Reduction Under Anaerobic Conditions

Cr(VI) reduction under anaerobic conditions was investigated due to its significant engineering applications, since most of the environments where the *in situ* bioremediation process could occur are closed systems such as sediment zones and under ground environments whereby the direct contact with the atmosphere does not occurs. Additionally, anaerobic biological processes are determined to be passive in nature with no excessive energy input requirements for aeration. The experiments under anaerobic conditions were conducted over initial Cr(VI) concentrations of (50–200 mg/L) at temperature of 30 ± 2°C and pH 7 ± 0.2. Evaluation of Cr(VI) reduction at lower initial Cr(VI) concentration compared to the aerobic Cr(VI) reduction experiments was associated to slower growth observed in the anaerobic cultures. Figure (4-6) shows Cr(VI) reduction in individual pure isolates X6b, X12 and X15. It is shown in this figure that the species (X6b, X12 and X15) completely reduce 50 mg/L of Cr(VI) within 90 hours of incubation, whereas the reconstituted anaerobic consortium culture achieved 78% removal efficiency at the initial Cr(VI) concentration of 200 mg/L in 200 hours of incubation Figure (4-7). Results obtained in anaerobic batch cultures showed a lower Cr(VI) reduction rate than in the aerobic culture.

Lower reduction rates of Cr(VI) in the anaerobic culture may be associated to slower anaerobic bacterial activity.

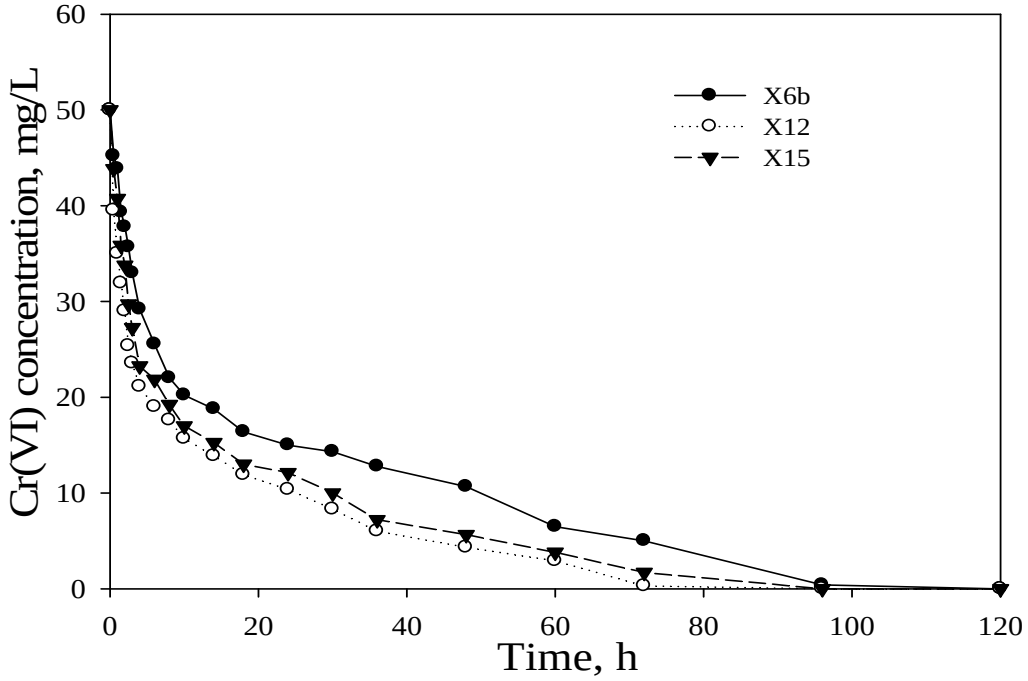


Figure 4- 6: Anaerobic Cr(VI) reduction in pure isolates at 50 mg/L

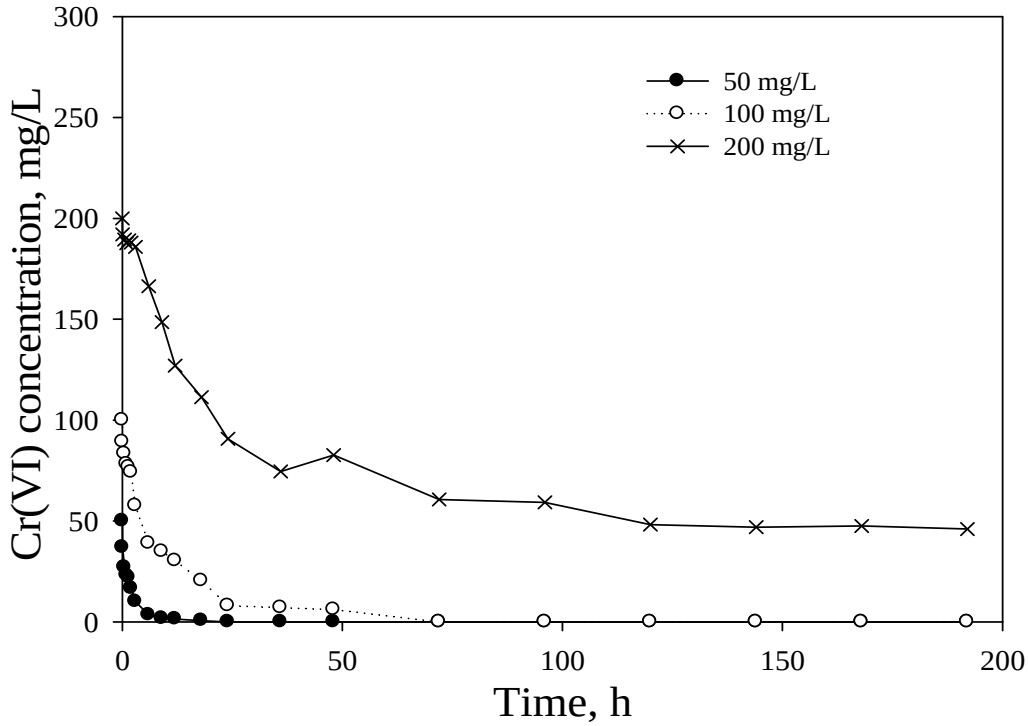


Figure 4- 7: Cr(VI) reduction in anaerobic reconstituted consortium (50-200 mg/L)

## 4.4 Total Biomass Evaluation

### 4.4.1 Evaluation of Total Biomass at the initial Cr(VI) concentration of 100 mg/L

The rapid increase in the biomass between 0–6 hours of exposure to Cr(VI) (Figure 4-8) showed that the reconstituted consortium culture was not just surviving but also growing rapidly at relatively high Cr(VI) concentration of 100 mg/L. A decline in biomass was observed after 6 hours of incubation. The reason for this decline is not clear at the moment.

### 4.4.2 Evaluation of Total Biomass at the initial Cr(VI) concentration of 400 mg/L

The slight increase in biomass concentration between 0–12 hours was observed in the bacterial consortium at 400 mg/L. The slow increase of suspended cell concentration within the first 6 hours of exposure to high Cr(VI) concentration may be associated to cell acclimatization to highly toxic environment. After 12 hours of incubation, the decline in bacterial population was observed. These results suggest the inhibitive effect of Cr(VI) was responsible for a slower growth rate.

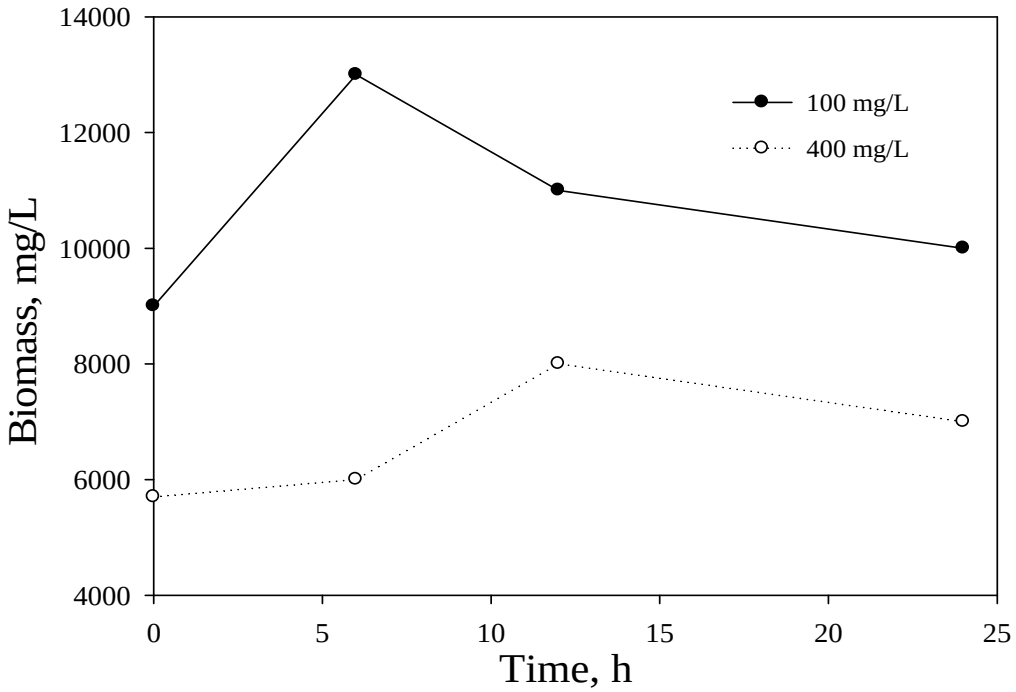


Figure 4- 8: Total biomass of aerobic reconstituted consortium culture at 100 and 400 mg/L

## 4.5 Kinetic Modelling Theory

### 4.5.1 Enzyme Kinetics

Cr(VI) reduction in living cells is assumed to be linked to cellular metabolism as it was illustrated in the early studies (Wang *et al*., 1989; Ohtake *et al*., 1990; Shen and Wang, 1993 and others). Biochemical studies of enzymatic Cr(VI) reduction suggest that Cr(VI) reducing mechanisms may be coupled to the membrane-electron transport system in Cr(VI) reducing bacteria (Horitsu *et al*., 1989; Wang *et al*., 1989; Ishibashi *et al*., 1990; Srinath *et al.*, 2002). Following further research, Viamajala (2003) and others proposed that Cr(VI) reduction is mediated by enzymes that are not substrate specific for Cr(VI) and that "chromate reductases" may be serendipitous contributors to Cr(VI) reduction while engaged in other primary physiological functions. The action of many different enzymes acting together can have a net effect of one characteristic enzyme for a consortium culture, thus this effect can be represented by one composite enzyme, $E_T$.

The single enzyme kinetic model is based on the following reaction scheme:

$$\text{Cr(VI)} + \text{E} \underset{k2}{\overset{k1}{\rightleftharpoons}} \text{E*Cr(VI)} \xrightarrow{k3} \text{E} + \text{Cr(III)} \qquad (4\text{-}1)$$

where: E = enzyme, E*Cr(VI) = enzyme-Cr(VI) complex , $k_1$ = rate constant for complex formulation, $k_2$ = rate constant for reverse complex formulation, $k_3$ = rate constant for Cr(III) formation.

Let Cr(VI) = C and E*Cr(VI) = E*

Therefore the rates of the above equation would be as follows:

$$\left.\frac{dE^*}{dt}\right|_{k=1} = k_1 C E_T \qquad (4\text{-}2)$$

$$\left.\frac{dE^*}{dt}\right|_{k=2} = k_2 E^* \qquad (4\text{-}3)$$

$$-\left.\frac{dE^*}{dt}\right|_{k=3} = \frac{dCr(III)}{dt} = k_3 E^* \tag{4-4}$$

The rate formulation of E* can be represented as:

$$\frac{dE^*}{dt} = k_1 C E_T - k_2(E^*) - k_3(E^*) \tag{4-5}$$

where : $E_T$ (total complexe and un-complex enzyme) = E – E*

As soon as the enzyme and Cr(VI) are mixed ,the concentration of enzyme-Cr(VI) complex will reach constant value so that a steady state can be applied as follows:

$$\frac{dE^*}{dt} = 0 = k_1 C(E - E^*) - k_2 E^* - k_3 E^* \tag{4-6}$$

Solving Equation (4-6) for E* the following equation is obtained:

$$E^* = \frac{k_1 CE}{k_1 C + k_2 + k_3} = \frac{CE}{C + \frac{k_2 + k_3}{k_1}} \tag{4-7}$$

Then the Cr(VI) reduction rate becomes:

$$\frac{-dC}{dt} = \frac{k_3 CE}{C + \frac{k_2 + k_3}{k_1}}$$

Analogous to Monod kinetics, $k_3$ is analogous to maximum specific Cr(VI) reduction rate ($k_m$), $E$ is analogous to biomass concentration (X) and $\frac{k_2 + k_3}{k_1}$ is analogous to half saturation constant ($K_c$).

$$\therefore \frac{-dC}{dt} = \frac{k_m C}{C + K_C} X \tag{4-8}$$

This model was previously derived by several researchers (Shen and Wang, 1994b Mazierski, 1995; Schmieman *et al*., 1998; Guha *et al*., 2001) where the soluble reductase activity was the predominant mechanism of Cr(VI) reduction in bacterial systems. It was

observed as an appropriate expression to cope with both toxicity and mutation effect of Cr(VI) on Cr(VI) reduction. It was also determined from this model that the rate and the extend of Cr(VI) reduction in bacterial system depends on the number of cells in the reactor and the capacity of reduction per cell represented by the term $R_c$. This indicates that the amount of Cr(VI) reduced under resting cells conditions will be proportional to the amount of cells inactivated by Cr(VI), thus the active biomass concentration is assumed to decrease with the increasing amount of Cr(VI) reduced due to toxicity (Shen and Wang, 1994b). Therefore X (biomass concentration) in Equation (4-8) can be represented as:

$$X = X_0 - \frac{C_0 - C}{R_c} \tag{4-9}$$

Equation (4-8) can therefore be represented as follows:

$$\frac{-dC}{dt} = \frac{k_m C}{C + K_c}\left(X_o - \frac{C_0 - C}{R_c}\right) \tag{4-10}$$

where: $k_m$ = maximum specific rate of Cr(VI) reduction ($T^{-1}$); $K_c$ = half-velocity concentration ($M.L^{-3}$); $X_o$ = initial biomass concentration ($ML^{-3}$); $C$ = Cr(VI) concentration at time, $t$, ($ML^{-3}$); $C_0$ = initial Cr(VI) concentration ($ML^{-3}$); and $R_c$ = Cr(VI) reduction capacity of cells ($MM^{-1}$).

### 4.5.2 Anaerobic Batch Cultures Modelling

The biotransformation of Cr(VI) to Cr(III) in batch culture result from the activity of cells. Although the reduction of Cr(VI) to Cr(III) may be limited by reaction kinetics under physiological conditions (Glaze, 1990). The kinetics of Cr(VI) reduction in the anaerobic batch culture may be improved by coupling Cr(VI) reduction to the energy yielding dissimilatory respiration process in which Cr(VI) serves as a terminal electron sink (Lovley and Phillips, 1994). It was observed in the anaerobic batch cultures of this study that Cr(VI) reduction (metabolic process) under low initial Cr(VI) concentration appeared to benefit from the presence of Cr(VI). Therefore in such a case the toxicity threshold concentration had to be reached before Cr(VI) inhibition become effective. The model developed in Equation (4-10) based on enzymatic Cr(VI) reduction kinetics could not describe the kinetics

for Cr(VI) under anaerobic conditions as the kinetic process under anaerobic conditions was more complex as a result of high biodiversity of anaerobic species and slow growing culture that is susceptible to toxic loading of Cr(VI). Therefore the results obtained in Equation (4-10) suggested a Cr(VI) reduction kinetic equation that accounts for Cr(VI) toxicity threshold concentration (Molokwane and Chirwa, 2009). The non-competitive inhibition model that accounts for Cr(VI) toxicity threshold concentration is represented as follows:

$$\frac{-dC}{dt} = \frac{k_m C}{K^{\left(1-\frac{C_r}{C_o}\right)}\left(K_c + C\right)}\left(X_o - \frac{C_o - C}{R_c}\right) \tag{4-11}$$

where: $k_m$ = maximum specific rate of Cr(VI) reduction ($T^{-1}$); $K_c$ = half-velocity concentration ($M.L^{-3}$); $C_r$ = Cr(VI) toxicity threshold concentration ($ML^{-3}$); $X_o$ = initial biomass concentration ($ML^{-3}$); $K$ = limiting constant ($ML^{-3}$); and $R_c$ = maximum Cr(VI) reduction capacity of cells ($MM^{-1}$).

### 4.6 Parameter Estimation

The unknown kinetic parameters in Equation (4-11), $k_m$, $K_c$, $K_i$, $K$ and $R_c$ were determined by performing a nonlinear regression analysis using the Computer Program for Identification and Simulation of Aquatic Systems (AQUASIM 2.0), (Riechert, 1998). For each parameter, a search was carried out through a range of values. Trial values of the unknown parameters were initially guessed. Constrains were also enforced to set upper and lower limits for each parameter so that nonsensical or invalid parameter values were omitted. Whenever optimization converged at/or very close to a constraint, the constraint was relaxed until the constraint no longer forced the model.

The process was repeated until unique values lying away from the constraints but between set limits were found for each parameter. The best fit values were obtained by repetition of parameter estimation of the unknowns. The objective function for parameter optimization was defined as the least sum of the squares between the observed and the modelled concentrations and was computed as follows:

$$\sigma^2 = \frac{1}{n-q}\sum_{i=1}^{i=n}\left(y_i - y\right)^2 \tag{4-12}$$

where: $\sigma$ = average deviation of model from the measured values; $y_i$ = observed variables; $y$ = simulated variables; $n$ = number of observations; and $q$ = degrees of freedom representing the number of parameters being evaluated.

### 4.6.1 Kinetic Parameter Estimation under Anaerobic Conditions

Experimental data with initial Cr(VI) concentration of 100 mg/L was initially used to estimate the kinetic parameters, $k_m$, $K_c$, $R_c$ and $K$. The validation of this model was performed and Figure (4-9) confirms that the kinetics parameter values obtained at 100 mg Cr(VI)/L simulated Cr(VI) reduction data very well for a broader range of Cr(VI) concentrations under anaerobic conditions (50 and 200 mg/L). However, the maximum Cr(VI) reduction capacity was not experimentally observed which implies that a unique value of the model kinetic parameter, $R_c$, can not be obtained. Cr(VI) reduction capacity of cells, $R_c$, in this model was observed to increase with increasing initial Cr(VI) concentration Table 4-1.

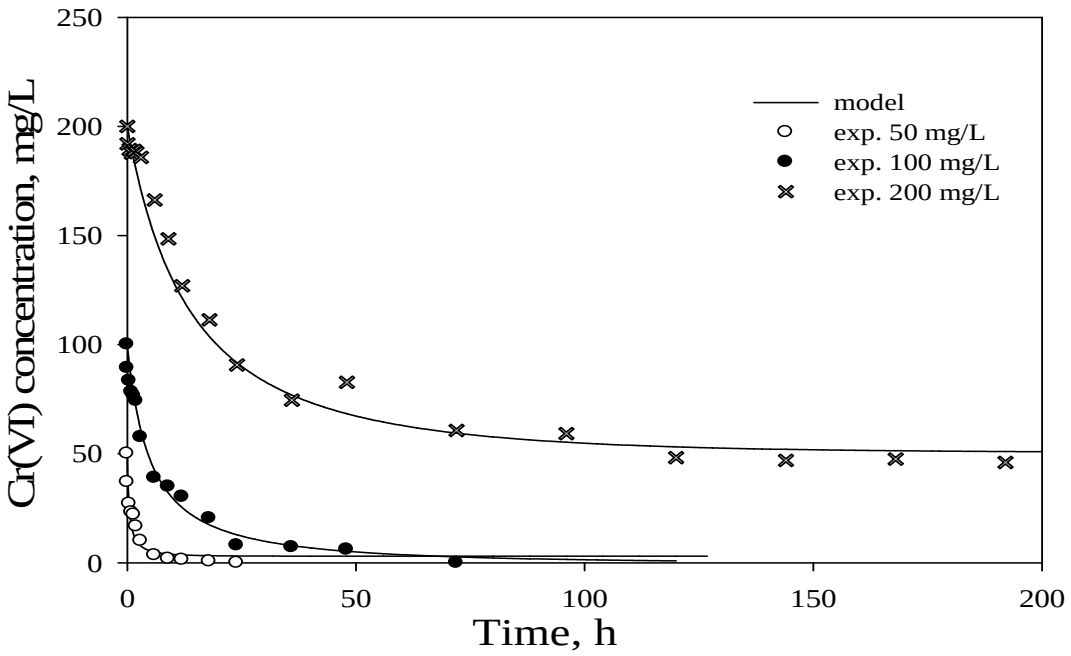


Figure 4- 9: Anaerobic batch culture model validation at (50-200 mg/L)

This indicates that the more viable cells are exposed to higher doses of Cr(VI) the more the population of bacterial species is decreased or the more the cell finite reduction capacity is reached due to Cr(VI) toxicity within cells. Additionally Figure (4-9) shows that both the model predictions and experimental data indicated that the rate of Cr(VI) reduction became

slower with continuous reduction of Cr(VI) and finally ceased when initial Cr(VI) concentration exceeded 100 mg/L.

Table 4- 1: Optimum kinetic parameter in anaerobic batch cultures

| $C_o$ (mg/L) | $k_m$ ($h^{-1}$) | $K_c$ (mg/L) | $K$ (mg/L) | $R_c$ (mg/mg) | $\chi^2$ |
|---|---|---|---|---|---|
| 50 | 0.131816 | 672.09135 | 10 | 0.089395 | 259.1872 |
| 100 | 0.131816 | 672.09135 | 10 | 0.090764 | 268.6806 |
| 200 | 0.131816 | 672.09135 | 10 | 0.124514 | 1306.9093 |

### 4.7 Sensitivity Analysis

#### 4.7.1 Sensitivity Analysis of Anaerobic Batch Culture Kinetics

The sensitivity functions of Cr(VI) concentration under anaerobic condition with respect to $C_o$,, $K_c$, $k_m$, *and* $R_c$ were also analyzed to compare the effect of each parameter on the reduction process.

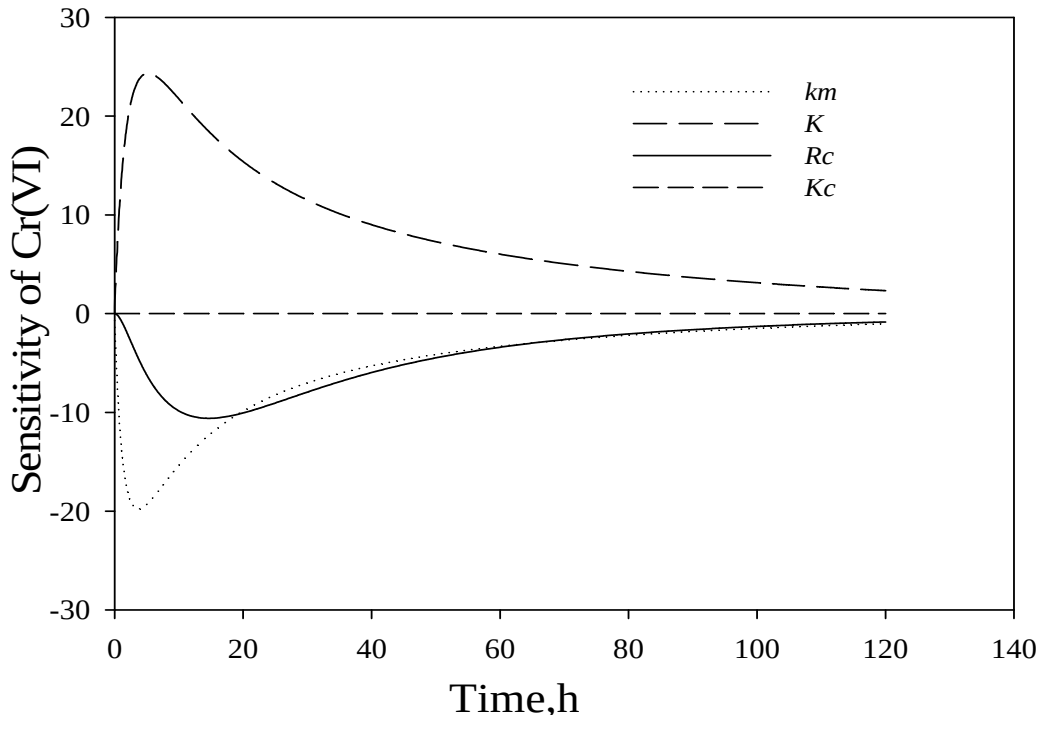


Figure 4- 10: Sensitivity test for the initial Cr(VI) concentration of 100 mg/L with respect to optimized parameters in anaerobic batch culture

It is observed in Figure (4-10) that the anaerobic batch culture model is highly sensitive to $K_c$, $R_c$, and $k_m$ in the first 50 hours of incubation. The response was extremely high in the first

5 hours of incubation which indicates that the cell Cr(VI) reduction activity was high during that period of incubation. The impact of the non-competitive parameter, *K*, was mild at 100 mg/L, since this was just a threshold value when the presence of Cr(VI) was not longer beneficial to the metabolic process.

### 4.8 Summary

This chapter describes Cr(VI) reducing capability in different consortia of bacteria reconstituted in our laboratory from previous isolated pure cultures or CRB's. It is demonstrated in this chapter that, for successful design and operation of suspended growth biological system in wastewater treatment, it is essential to understand the types of microorganisms involved. Batch studies conducted on different configurations indicate enzymatically mediated Cr(VI) reduction in anaerobic cultures. The evaluation of the non-competitive inhibition model with Cr(VI) toxicity threshold concentration for anaerobic batch cultures was necessary as the original model based on pure culture kinetics (Equation 4-10) failed to fit experimental data. The non-competitive inhibition model with Cr(VI) toxicity threshold best represented Cr(VI) reduction in anaerobic cultures with Cr(VI) toxicity threshold concentration of approximately 100 mg/L following mechanism observed by (Molokwane, 2010). The higher Cr(VI) toxicity threshold concentration in this study compared to other studies may be associated to non-toxic carbon source (glucose) which was used in this study. The model predicted well the experimental data at a wide range of Cr(VI) concentrations (50, 100, and 200 mg/L). The Cr(VI) reduction kinetic parameters obtained in this study however were slightly different from those found in earlier studies (Molokwane, 2010) using the same culture. This result may be associated to slightly different experimental conditions such as: (i) the cell age, as time is a crucial parameter when using cultures and thus implies that the duration of culture in the freezer may result into slight loss of cell activity after keeping or storing the cell culture for several weeks prior using; (ii) initial biomass concentration; and other factors which may also involve random errors. On the other hand testing for sensitivity of each kinetic parameter in the model it has been observed that the model is highly sensitive or affected by the change in kinetic parameters ($k_m$, $K_c$, and $R_c$), similar results were also observed by (Molokwane, 2010). This indicates the reliability of the non-competitive inhibitory model with Cr(VI) toxicity threshold concentration in evaluating Cr(VI) reduction under anaerobic conditions. Mathematical representations determined from the anaerobic batch modelling in this study would be used for simulation of Cr(VI) effluent in aquifer environments in the next chapter.

CHAPTER 5

# MICROCOSM Cr(VI) REDUCTION KINETIC STUDIES

## 5.1 Conceptual Basis of Microcosm Studies

Microbial barrier studies were conducted to simulate the behaviour of Cr(VI) across the soil strata into the open aquifer system at the contaminated site. The aquifer soil samples were collected at the depth of 3m below the ground surface of the contaminated site for microcosm study purpose. In this study the impact of carbon source on Cr(VI) reduction and removal in an aquifer system was evaluated, as the aquifer zone is characterized as the high pore volume zone with lower organics content from the decaying vegetation. Microcosms were installed in the laboratory and operated as packed-bed continuous flow bioreactor systems.

The influent loading in the columns was simulated by gravity feeding as in the case of open aquifer at the site. The performance of each column was evaluated based on the influent and the effluent Cr(VI) concentration under sustained hydraulic loading. In order to develop appropriate biological systems that can effectively reduce Cr(VI) at the contaminated sites the kinetic processes within the reactor system influencing Cr(VI) reduction and removal were evaluated. The one dimensional dispersion-reaction model was evaluated in this study to determine the optimum kinetic parameters for microbial barrier system at a transient state. In order to evaluate the spatial modelling of Cr(VI) concentration profiles along the column under quasi-steady state conditions which is referred to as steady-state in this study, the plug-flow reaction model that account for flow characteristics and biological removal mechanism was developed.

## 5.2 Performance Evaluation

### 5.2.1 Reconstituted Consortium Culture versus Native Soil Culture

The Cr(VI) reducing performance between the consortium culture reconstituted from the potential pure isolates of the dried sludge and the native bacterial species in the aquifer medium columns was compared. Table (5-1) summarise the overall performance of different aquifer medium reactors. The results shows that the rate of Cr(VI) reduction was more pronounced in the column inoculated with reconstituted consortium culture than in the non-

inoculated native soil culture column. The absence of Cr(VI) reduction in the native soil culture column may be associated to the absence of Cr(VI) reducing culture in the soil. In addition, majority of the microbial species found in the aquifer soil samples could not be cultured using the conventional methods, this implies that most of the soil species in the soil sample are not identifiable.

Table5- 1: Summary of performance of Cr(VI) reduction after column operation

| **Reactor no.** | **Flow rate Q(mL/min)** | **Cr(VI)concentration (mg/L)** | **Cr(VI) removal efficiency, (%)** |
|---|---|---|---|
| R1(NTV+CS) | 0.233 | 20 | <10 |
| | | 40 | <8 |
| | | 50 | <5 |
| R2 (INC+CS) | 0.218 | 20 | 100 |
| | | 40 | 55 |
| | | 50 | <10 |
| R3 (Control) | 0.248 | 20 | <2 |
| | | 40 | <2 |
| | | 50 | <2 |
| R4 (INC- CS) | 0.225 | 20 | 69.5 |
| | Average (Q)=0.23 | 30 | 55 |

## 5.3 Microcosm Kinetic Studies

### 5.3.1 Cr(VI) Removal Kinetics at Various Time Intervals

Experimentation on Cr(VI) reduction in packed-bed reactors was conducted at various Cr(VI) concentrations ranging from 20-50 mg/L in carbon source and non-carbon source reactors over time. Figure (5-1) shows that the inoculated soil column amended with sawdust as a carbon source outperformed the inoculated soil column without carbon source. Significant amounts of Cr(VI) were removed from the influent feed with maximum observable rates of approximately 2 mg/L/h in the inoculated soil column amended with sawdust. This indicates that the presence of carbon source in the inoculated column greatly enhance the performance of Cr(VI) removal. Figure (5-2 and 5-3) also demonstrate that

Cr(VI) reduction performance is improved by the presence of the carbon source as Cr(VI) removal efficiency in a non-carbon source reactor at 30 mg Cr(VI)/L was observed to be equal to Cr(VI) removal efficiency at 40 mg Cr(VI)/L in a carbon source reactor after one week of operation.

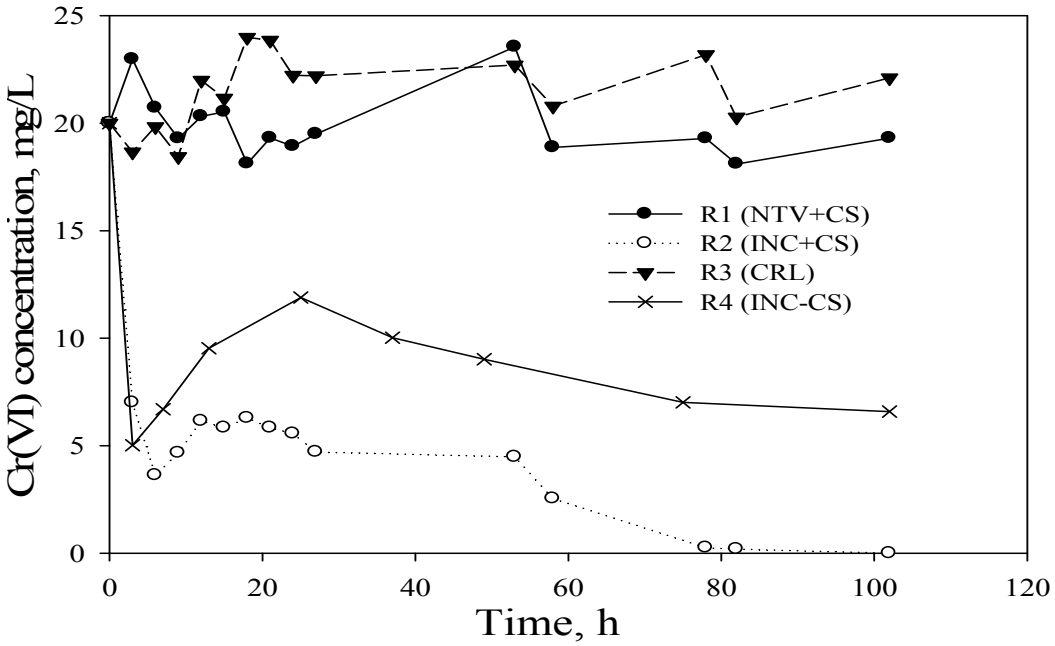


Figure5- 1: Performance of comparison between the inoculated reactors with carbon source and without carbon source at the initial Cr(VI) concentration of 20 mg/L.

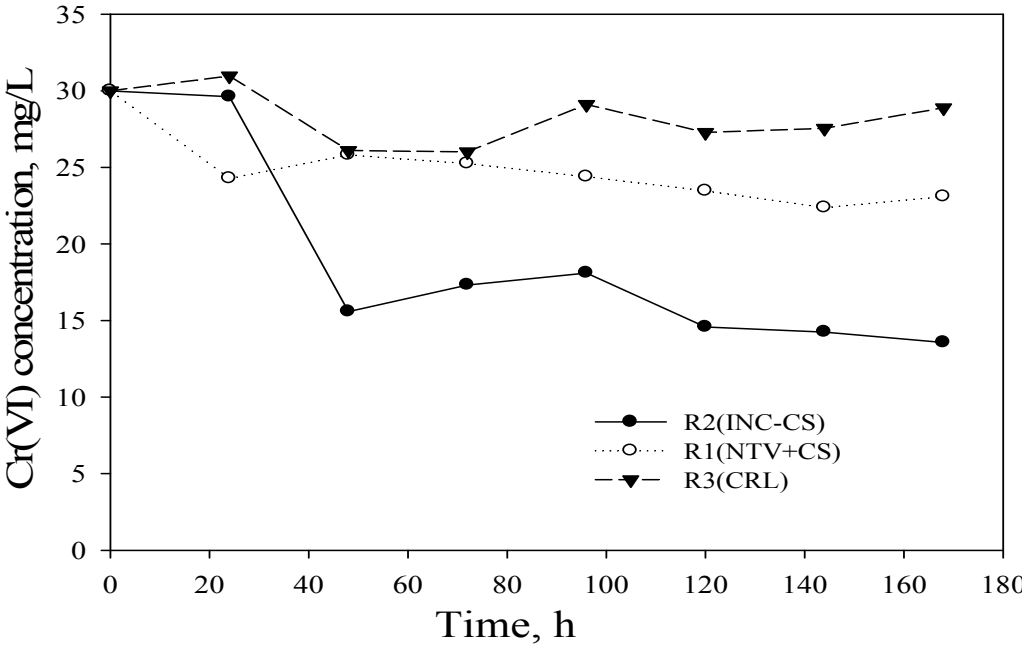


Figure5- 2: Performance of inoculated column without carbon source in comparison with sterile-control column at 30 mg/L.

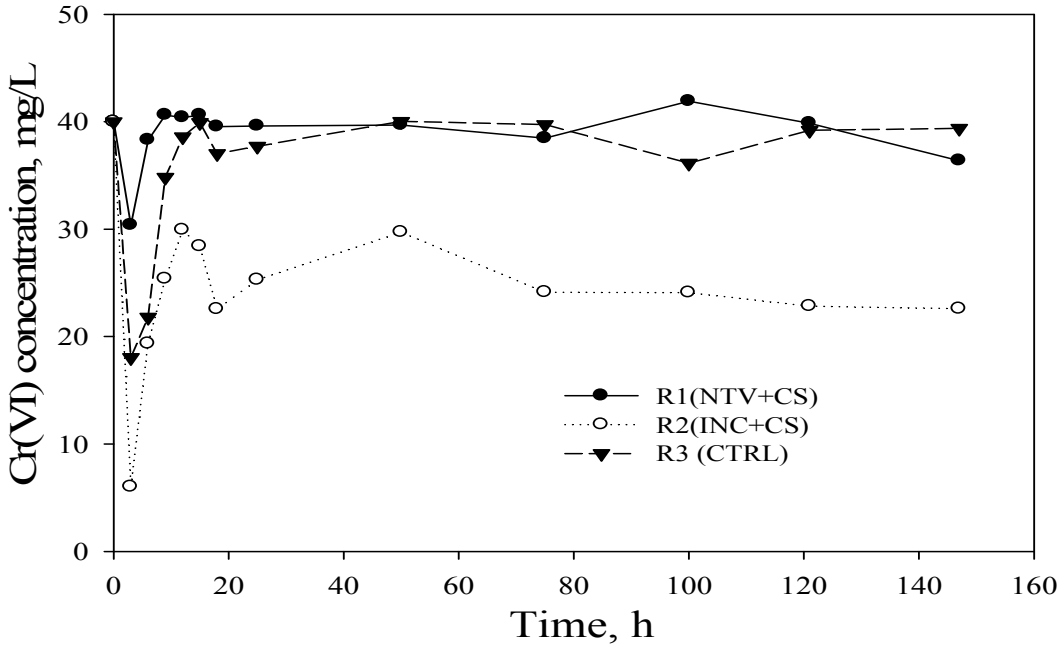


Figure5- 3: Performance of inoculated reactor amended with carbon source at 40 mg/L

### 5.3.2 Cr(VI) Removal Kinetics at Various Lengths

#### Cr(VI) Concentration Profile in a Sterile Control Column

The rate at which Cr(VI) was reduced within the sterile control reactor was shown to be insignificant through out its operation Figure (5-4). This indicates that the abiotic processes are negligible.

#### Cr(VI) Concentration Profile in Native Soil Culture Column

Figure (5-5) shows that the rate of Cr(VI) reduction increases insignificantly with increasing reactor length over time in the native soil culture column amended with sawdust. It is also observed in Figure (5-4 and 5-5) that performance of native soil culture column in Cr(VI) removal is similar to that of a sterile control column. The low performance of the native soil culture in reducing Cr(VI) in a column at both lower and higher initial Cr(VI) concentrations may be associated to the absence of Cr(VI) reducing culture in the soil. Based on the experimental data and the microbial culture dynamics obtained in the native soil culture column at various Cr(VI) feed concentrations it can be postulated that the native species in the soil samples are Cr(VI) resistors as they remained persistent in the column after long period of exposure to Cr(VI) loadings but were unable to reduce Cr(VI) in the reactor.

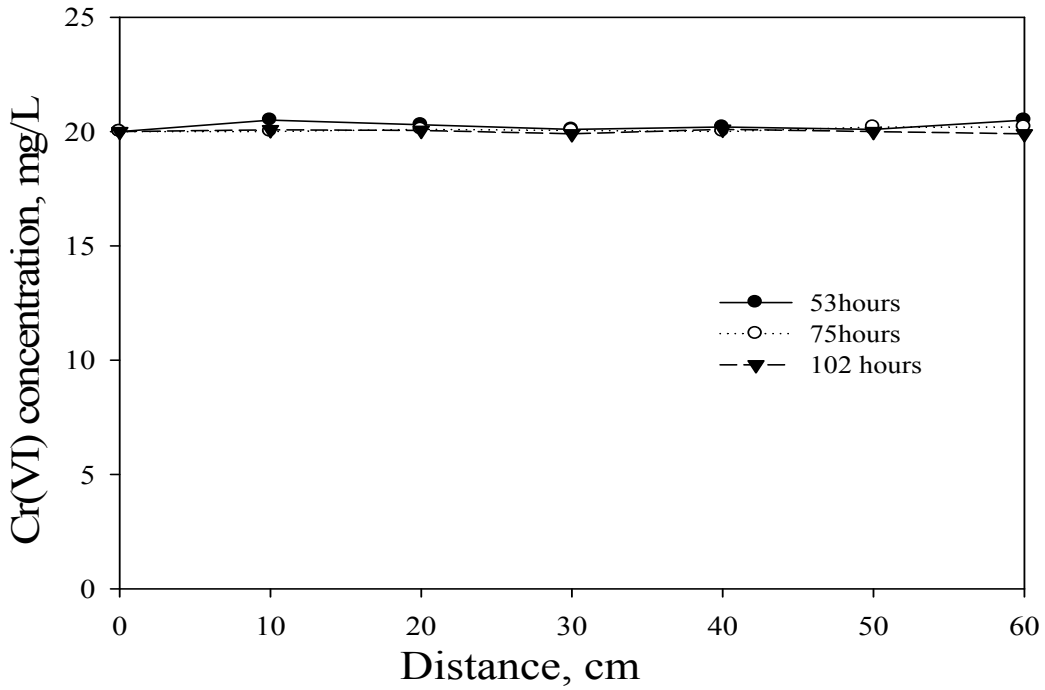


Figure5- 4: Performance of a sterile-control reactor in removing Cr(VI) feed concentration of 20 mg/L across the column

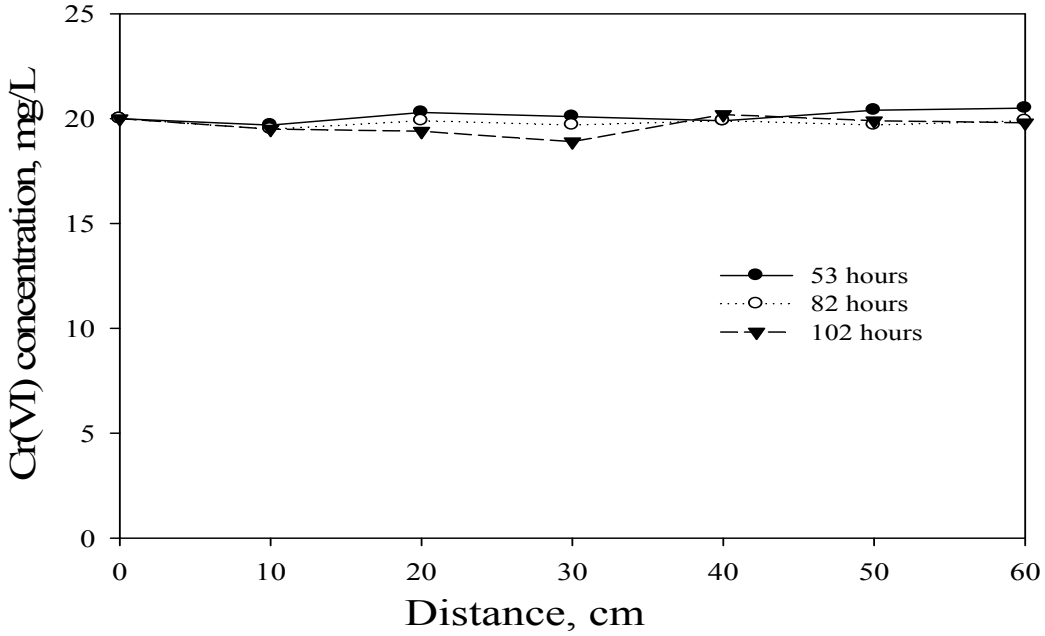


Figure5- 5: Performance of native soil culture column in removing Cr(VI) feed concentration of 20mg/L across the column

**Cr(VI) Concentration Profile in the Inoculated Columns**

Spatial variation of Cr(VI) removal in the inoculated reactors was evaluated over time. Data collected from equally spaced longitudinal sampling ports over random hours of operation

were evaluated for Cr(VI) removal across the reactors. Figure (5-6) demonstrate that the rate of Cr(VI) removal at non-steady state, thus for the first 50-75 hours of operation increases significantly over length. Up to 70% of Cr(VI) removal efficiency was achieved during the first 53 hours of operation across the reactor Figure (5-6A).

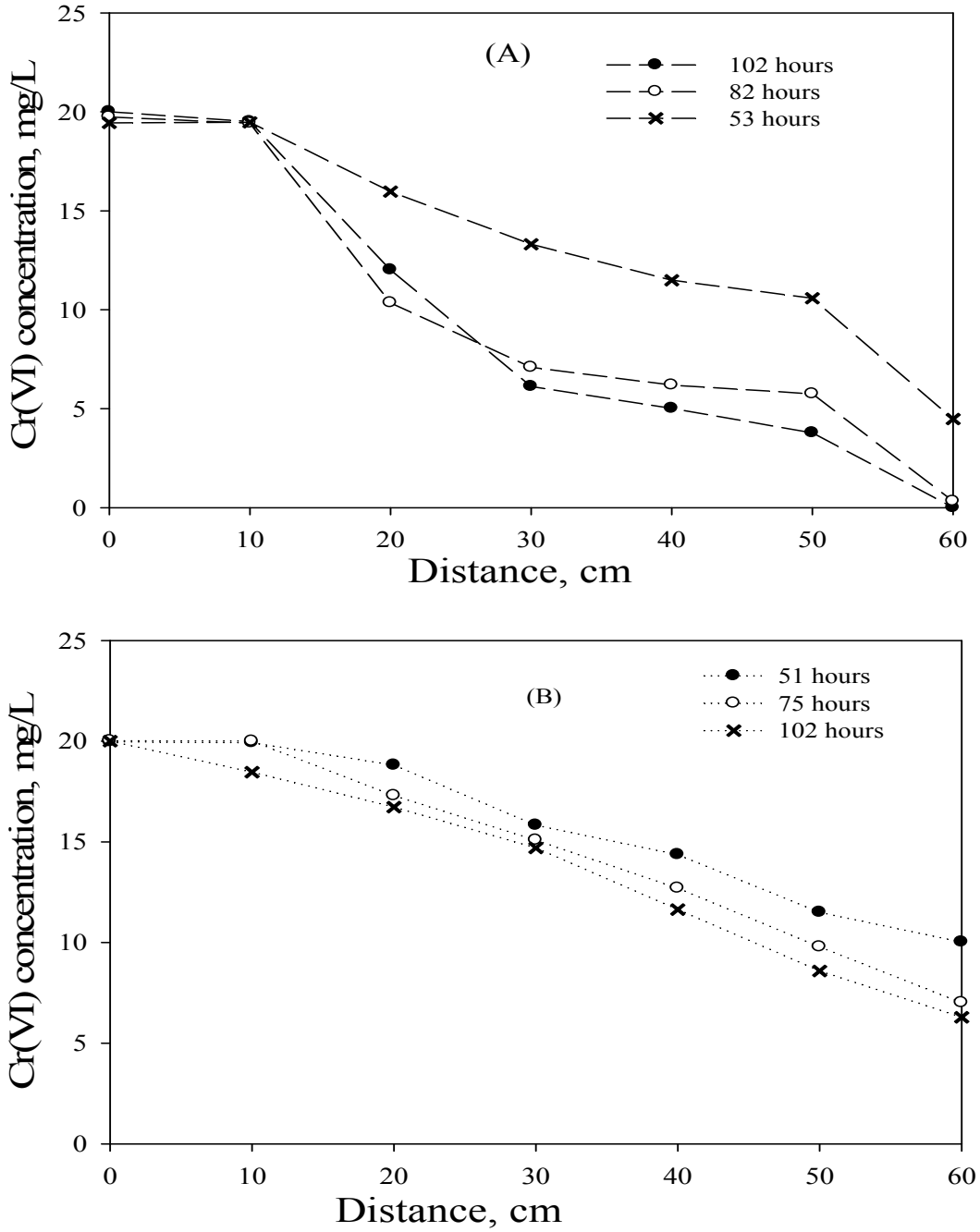


Figure5- 6: Performance of inoculated reactors in removing Cr(VI) feed concentration of 20 mg/L in (A) C-source reactor, and (B) non C-source reactor

Although the rate of Cr(VI) reduction was observed to increase with increasing length, near complete Cr(VI) removal was achieved at the final effluent port over time. It is also observed in Figure 5-6 (A-B) that the effluent Cr(VI) concentration did not stabilize until a quasi-steady state was reached, thus after three to four days of operation depending on the column experimental condition. The rate of Cr(VI) reduction at quasi-steady state became insignificant over time ( less than 5%). The insignificant Cr(VI) removal over time may be associated to various reasons which involves: (i) Cr(VI) inhibitory effects on the Cr(VI) reducing bacteria; (ii) loss of Cr(VI) reducing capacity, and (iii) saturation of the physical chemical processes-adsorption and biosorption in the reactors over time.

## 5.4 Microbial Culture Dynamics in the Aquifer System

### 5.4.1 Characteristic of Initial Inoculated Reconstituted Consortium Culture

The pure cultures isolated from the dried sludge were grown aerobically as a reconstituted consortium culture. Table (3-1), from Chapter 3 shows the pure isolates which were initially inoculated in the specific aquifer media reactors as a reconstituted consortium culture (X1+X2+X3+X4+X5+X6+X7) prior contaminant loading.

### 5.4.2 Characterization of Inoculated Columns after Operation

After operating the columns under oxygen stressed condition, the microcosm columns were opened, to analyse the microbial community shift in the dried sludge culture and native soil culture column. Microbial shift analysis due to exposure to toxic conditions was monitored by 16S rRNA fingerprinting method. The results presented by the Phylogenetic trees in (Figure 5-7) confirms that after microcosm system operation (seven weeks) the well known Cr(VI) reducers, *Bacillus thuringiensis* and *Bacillus cereus* remained persistent in different reactors, R2 (inoculated column + CS) and R4 (inoculated column - CS). In the native soil culture column (R1), the *Bacillus anthracis* remained persistent. Figure (5-8) confirms that *Enterococcus faecium* remained persistent in both (R1) and (R2) and *Enterococcus villorum* remained persistent in (R1).

This indicates that *Bacillus thuringiensis* and *Bacillus cereus* in different inoculated columns (R1 and R4) are less sensitive to Cr(VI) toxicity or their resilience against Cr(VI) toxicity. The presence of diverse bacterial species in the native culture column (R1) which are

*Bacillus anthracis, Enterococcus faecium, Enterococcus villorum* and several unidentified species in the soil after operation indicate that the native bacterial species in the soil sample are most certainly Cr(VI) resistors, but not Cr(VI) reducers as non of the cultured soil bacteria were recognised or reported from the literature as Cr(VI) reducing species.

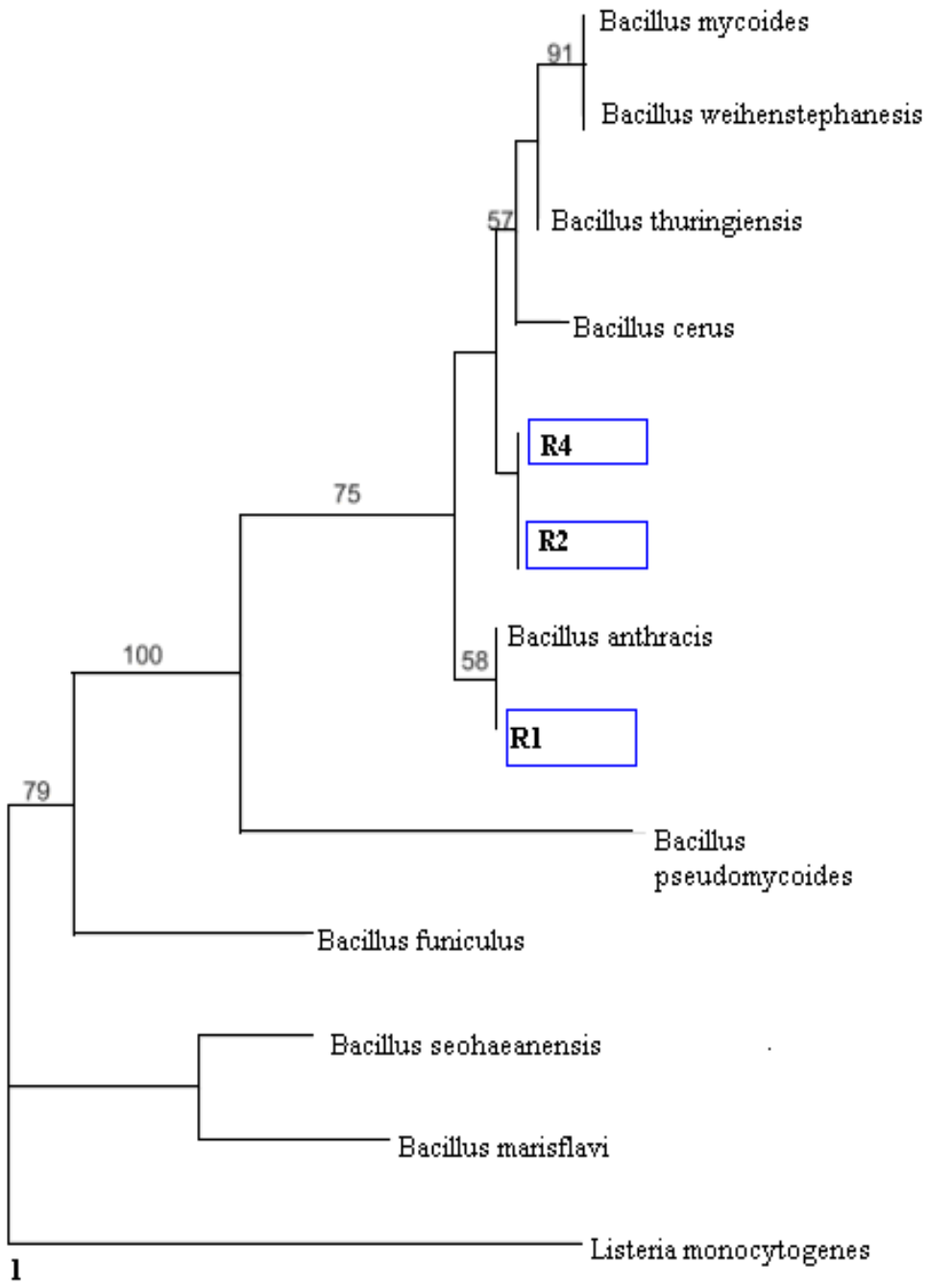


Figure5- 7: Phylogenetic tree of persistent bacterial cells in inoculated reactor columns after operation derived from the 16S rRNA gene sequence, *Bacillus species*

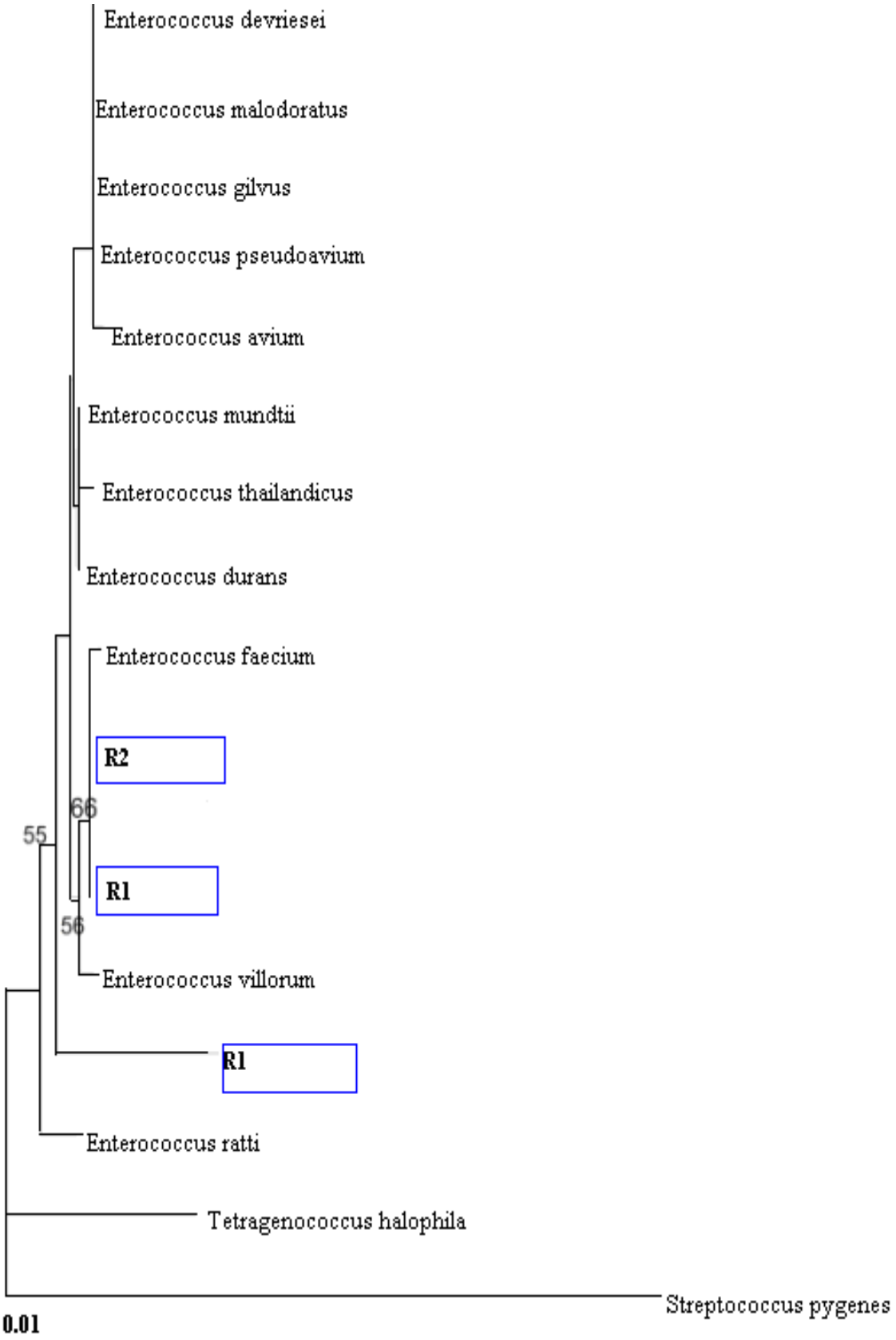


Figure5- 8: Phylogenetic tree of persistent bacterial cells in inoculated reactor columns after operation derived from the 16S rRNA gene sequence, *Enterococcus species*

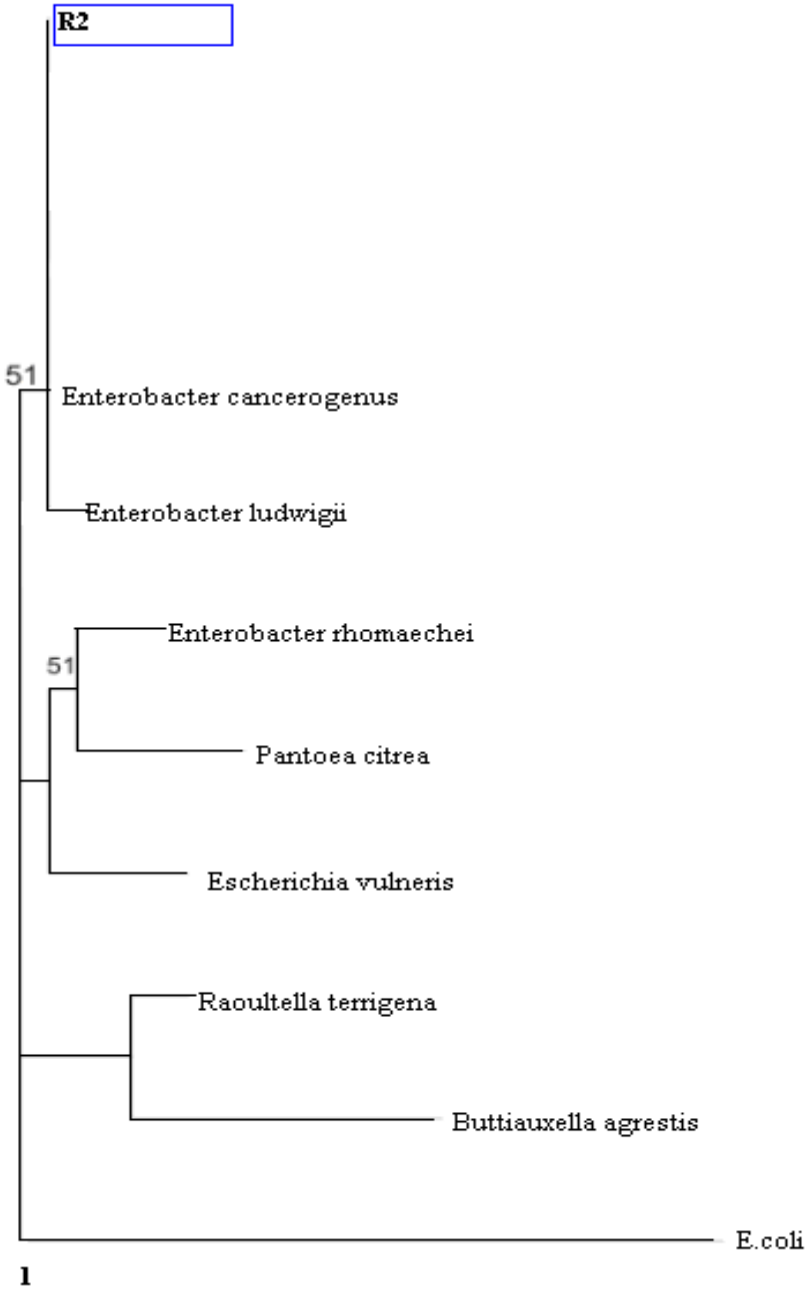


Figure5- 9: Phylogenetic tree of persistent bacterial cells in inoculated reactor columns after operation derived from the 16S rRNA gene sequence. Possible Cr(VI) reducing species were detectable including, *Enterobacter species* and *E. coli*

## 5.5 Kinetic Modelling of Cr(VI) Reduction in the Microcosm Columns

### 5.5.1 Model Description

Cr(VI) transport through saturated porous media is a highly dynamic process that can not be fully define through batch reactor experiments. Anaerobic batch studies were initially

conducted in this study at various initial Cr(VI) concentration prior continuous-flow studies to evaluate the fundamentals of each biological process at various time intervals. The evaluation of batch studies under anaerobic condition prior microcosm column studies is associated to the oxygen stressed condition attained in the microcosm packed-bed reactor after long period of operation. Advection and dispersion are the main modes of transport of Cr(VI) in the groundwater. However, reaction-microbial reduction in this study also significantly influence the fate and transport of Cr(VI) in a saturated porous media. A detailed mathematical model that simulate microbial Cr(VI) removal in the fixed-media reactor system includes a system of coupled differential equations which represent Cr(VI) reduction rate ($r_c$), mass transport rate ($j_c$), adsorption rate ($q_c$) and dispersion:

**Advection**

The transport of dissolved species of Cr(VI) along with bulk fluid flow which is represented as:

$$\frac{-d(CV)}{dt} = Au(C_{in} - C) \tag{5-2}$$

where: $C$ = effluent Cr(VI) concentration ($ML^{-3}$); $V$ = volume of the reactor ($L^3$); $C_{in}$ = influent Cr(VI) concentration ($ML^{-3}$); $Q$ ($Au$) = influent flow rate ($L^3T^{-1}$); $A$ = cross sectional area of reactor ($L^2$); $u$ = velocity of the flow ($LT^{-1}$); and $t$ = time ($T$).

**Flux through attached cell layers on soil particles**

Mass transfer within the attached cell layer is described by *Fick's* law for dispersion. The contaminant flux across the stagnant layer to the biofilm is a function of the contaminant dispersion coefficient and concentration and is represented as:

$$\frac{d(CV)}{dt} = -D_w \frac{dC}{dx} = -\frac{D_w}{L_w} A_f (C_b - C_s) = -j_C . A_f \tag{5-3}$$

where: $D_w$ = dispersion coefficient of Cr(VI) in water ($L^2T^{-1}$); $dC/dx$ = Cr(VI) concentration gradient ($ML^{-3}L^{-1}$); $L_w$ = thickness of stagnant layer ($L$); $C_b$ = bulk liquid Cr(VI)

concentration ($ML^{-3}$); and $C_S$ = Cr(VI) surface concentration ($ML^{-3}$). NB: In most mass transfer-limited reactions $C_b >> C_S$, therefore $C_S$ is negligible.

**Reduction due to reaction**

Since the aquifers were operated under predominately anaerobic conditions, Cr(VI) reduction model with toxicity threshold inhibition was chosen. The kinetic rate parameters obtained from the batch anaerobic cultures were maintained in the continuous flow systems with minor adjustments allowed due culture sensitivity to Cr(VI) toxicity after long a period of operation under oxygen stressed conditions.

$$\frac{-dC}{dt} = \frac{k_m C}{(K_c + C)\left(K^{\left(1-\frac{C_r}{C_0}\right)}\right)}\left(X_0 - \frac{C_0 - C}{R_c}\right) = -r_c \qquad (5\text{-}4)$$

where: $k_m$ = maximum specific rate of Cr(VI) reduction ($T^{-1}$); $K_c$ = half-velocity concentration ($M.L^{-3}$); $C_r$ = Cr(VI) toxicity threshold concentration ($ML^{-3}$); $X_o$ = initial biomass concentration ($ML^{-3}$); $K$ = limiting constant ($ML^{-3}$); and $R_c$ = Cr(VI) reduction capacity of cells ($MM^{-1}$).

**Adsorption**

Removal of Cr(VI) in the reactor depends at the rate at which the Cr(VI) is transported and adsorbed in the biofilm of the reactor and also in the reaction taking place on the surface area. The removal of Cr(VI) by adsorption is represented as:

$$\frac{-dC}{dt} = k_{ad}(C_{eq} - C) = -q_c \qquad (5\text{-}5)$$

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

Total mass balance of the reactor involving all the non-linear ordinary differential equations for modelling the fate and transport of Cr(VI) in a packed-bed reactor at the transient state can be represented as follows:

$$\frac{d(CV)}{dt} = Au(C_{in} - C) - r_c \Delta V - j_C . A_f - q_c \Delta V \qquad (5\text{-}6)$$

where: $C$ = effluent Cr(VI) concentration ($ML^{-3}$); $V$ = volume of the reactor ($L^3$); $C_{in}$ = influent Cr(VI) concentration ($ML^{-3}$); $Q$ = influent flow rate ($L^3T^{-1}$); $r_c$ = reduction rate coefficient ($ML^{-3}T^{-1}$); $A$ = cross sectional area of reactor column at time $t$ ($L^2$); $A_f$ = biofilm surface area ($L^2$); $u$ =velocity of the flow ($LT^{-1}$); and $q_c$ = the rate of Cr(VI) removal by adsorption ($T^{-1}$). N.B: $Au = Q$ (inflow rate, $L^3T^{-1}$).

The coupled mass balance equations were simulated using a forth-order Runge-Kutta routine for solution of simultaneous ordinary and partial differential equations in A Computer Program for Identification and Simulation of Aquatic Systems (AQUASIM 2.0) (Reichert, 1998).

Basic assumptions made to formulate the model are summarised as follows:

- The flow in the column is one-dimensional
- The flow is turbulent and has no radial gradient in velocity (thus a plug flow condition)
- The porous medium is homogeneous
- The rate of nutrient dissolution is greater than the rate of nutrient consumption
- Some microbes are mobile and some are immobile
- The contaminant is toxic and has inhibitory effect on microbial growth rate
- Cr(III) generated due to biotransformation is either precipitated and retained or adsorbed onto the soil matrix almost immediately
- Temperature and pH are constant

### 5.5.2 Model Validation

The model for saturated packed column with dispersion was adapted from AQUASIM 2.0 and tested with a sterile packed-bed reactor (control). This model was used in combination with Cr(VI) kinetic parameters adapted from the anaerobic batch culture studies due to oxygen stressed condition attained in the reactor after long period of operation. The model was initially tested with sterile-control column and then used to simulate time series data at various Cr(VI) concentrations in the microcosm reactors as shown in Appendix A.

### 5.5.3 Parameter Optimization

Kinetic parameters were obtained by performing a nonlinear regression analysis using AQUASIM 2.0. For each kinetic parameter, a search was carried out through a range of values which were initialized by guessed values and values from batch studies. To ascertain that the optimized parameters obtained using the mathematical model were dependable, upper and lower constrains were set for each parameter to allow the omission of invalid parameter values. Whenever optimization converged at or very close to a constraint, the constraint was relaxed until the constraint no longer forced the model. This process was repeated until unique values lying away from the constraints, but between the set limits were found for each parameter.

### 5.5.4 Cr(VI) Removal Kinetics at Lower and Higher Concentrations

The optimum kinetic parameters summarized in Table (5-3 and 5-4), shows that the dispersion coefficient in the inoculated column amended with sawdust as a carbon source is much higher than the one observed in the inoculated column reactor without carbon source. This indicates that the rate at which the contaminant disperses into the cell layer attached to the aquifer soil particles influence the removal of Cr(VI) by biomass. Therefore higher rates of Cr(VI) reduction in the inoculated carbon source reactor can be attributed to higher dispersion rate in the column. It is also shown in Table (5-3) that the cell death rate is relatively faster in the non-carbon source reactor than in the carbon source reactor; this indicate that the organic carbon source in the reactor enhance the cell activity.

Results on Cr(VI) effluent simulation in a sterile control reactor, inoculated carbon source, and non-carbon source reactor at various initial Cr(VI) concentrations are demonstrated in Figure (5-10 and 5-11). It is observed in Figure (5-10 and 5-11) that in the order of 5-10

hours of operation, adsorption sites on the aquifer media particles is saturated. This implies that the adsorptive process reaches the equilibrium state in the column, thus adsorption coefficient ($q_c$) approaches zero. Therefore the mechanisms which are responsible for Cr(VI) removal in a long run are limited to reduction by kinetics adapted from the anaerobic batch reaction, advection, and mass transport.

### 5.5.5 Summary of Parameters

The model for saturated soil column with dispersion was adapted from AQUSIM 2.0 for simulation of soil columns. The kinetic parameters for reduction rate process were obtained and optimised in a batch reactor system and were directly applied in continuous flow process. However minor adjustments were applied in continuous-flow process as a result of low levels of biomass in the continuous flow reactor system as compared to the batch reactor system.

Cultures grown under carbon source showed higher Cr(VI) reduction capacity than the cultures grown on inorganic carbon source from the soil. Most physical parameters were determined from known literature values of similar systems. Mass transport and adsorption parameters were estimated from continuous-flow reactor data. The breakthrough characteristics of the saturated soil column were observed to be typically of packed-bed reactor systems with moderate dispersion depicting an exponential rise up to maximum point and then followed by reduction in effluent as Cr(VI) reducing culture become more established. The model for the saturated soil columns with dispersion adapted from AQUASIM 2.0 successfully simulated the operation of microcosm used in this study.

Table5- 2: Optimum kinetic parameter values obtained for the biofilm in a carbon source reactor

| **Parameter Symbol** | **Definition** | **Constrains [ lower, upper]** | **Optimum value** |
|---|---|---|---|
| Biological parameters | | | |
| $C$ (mg/L) | State variable | -- | $1\times10^{-6}$ |
| $C_{in}$ (mg/L) | Influent Cr(VI) concentration | -- | 20-40 |
| $K_c$ (mg/L) | Half velocity concentration | [0, 15] | 11.3 |
| $k_m$ ($h^{-1}$) | Specific reduction rate | [0, 0.02] | 0.0051 |
| $K_d$ ($h^{-1}$) | Cell death rate coefficient | [0, 1000] | 0.0025 |
| $\mu$ ($h^{-1}$) | Biomass growth rate | [0, 1000] | 0.023 |
| $R_c$ (mg/mg) | Cr(VI) reduction capacity | [0, 0.5] | 0.283 |
| $C_r$ (mg/L) | Cr(VI) toxicity threshold | -- | 50 |
| Physical parameters | | | |
| $\theta$ (%) | porosity | -- | 0.4 |
| $\alpha$ | alpha | -- | 0.5 |
| $rho\_s$ ($kg/m^3$) | Soil particle density | -- | 2300 |
| $Q_{in}$ (L/h) | Influent flow rate | -- | 0.015 |
| $D$ ($m^2/s$) | Dispersion coefficient | [0, 100] | 6.02-95.4 |
| $A$ ($m^2$) | Cross sectional area | -- | 0.00196 |
| $A_f$ ($m^2$) | Biofilm surface area | -- | 0.000785 |

-- Constant values

Table5- 3: Optimum kinetic parameter values obtained for the biofilm in a non carbon source reactor

| **Parameter Symbol** | **Definition** | **Constrains [ lower, upper]** | **Optimum value** |
|---|---|---|---|
| Biological parameters | | | |
| $C$ (mg/L) | State variable | -- | $1\times10^{-6}$ |
| $C_{in}$ (mg/L) | Influent Cr(VI) concentration | -- | 20-30 |
| $K_c$ (mg/L) | Half velocity concentration | [0, 15] | 12.51 |
| $k_m$ ($h^{-1}$) | Specific reduction rate | [0, 0.02] | 0.0010 |
| $K_d$ ($h^{-1}$) | Cell death rate coefficient | [0, 1000] | 0.0031 |
| $\mu$ ($h^{-1}$) | Biomass growth rate | [0, 1000] | 0.018 |
| $R_c$ (mg/mg) | Cr(VI) reduction capacity | [0, 0.5] | 0.099 |
| $C_r$ (mg/L) | Cr(VI) toxicity threshold | -- | 50 |
| Physical parameters | | | |
| $\theta$ (%) | porosity | -- | 0.4 |
| $\alpha$ | alpha | -- | 0.5 |
| $rho\_s$ ($kg/m^3$) | Soil particle density | -- | 2300 |
| $Q_{in}$ (L/h) | Influent flow rate | -- | 0.015 |
| $D$ ($m^2/s$) | Dispersion coefficient | [0, 100] | 1.52-11.7 |
| $A$ ($m^2$) | Cross sectional area | -- | 0.00196 |
| $A_f$ ($m^2$) | Biofilm surface area | -- | 0.000785 |

-- Constant values

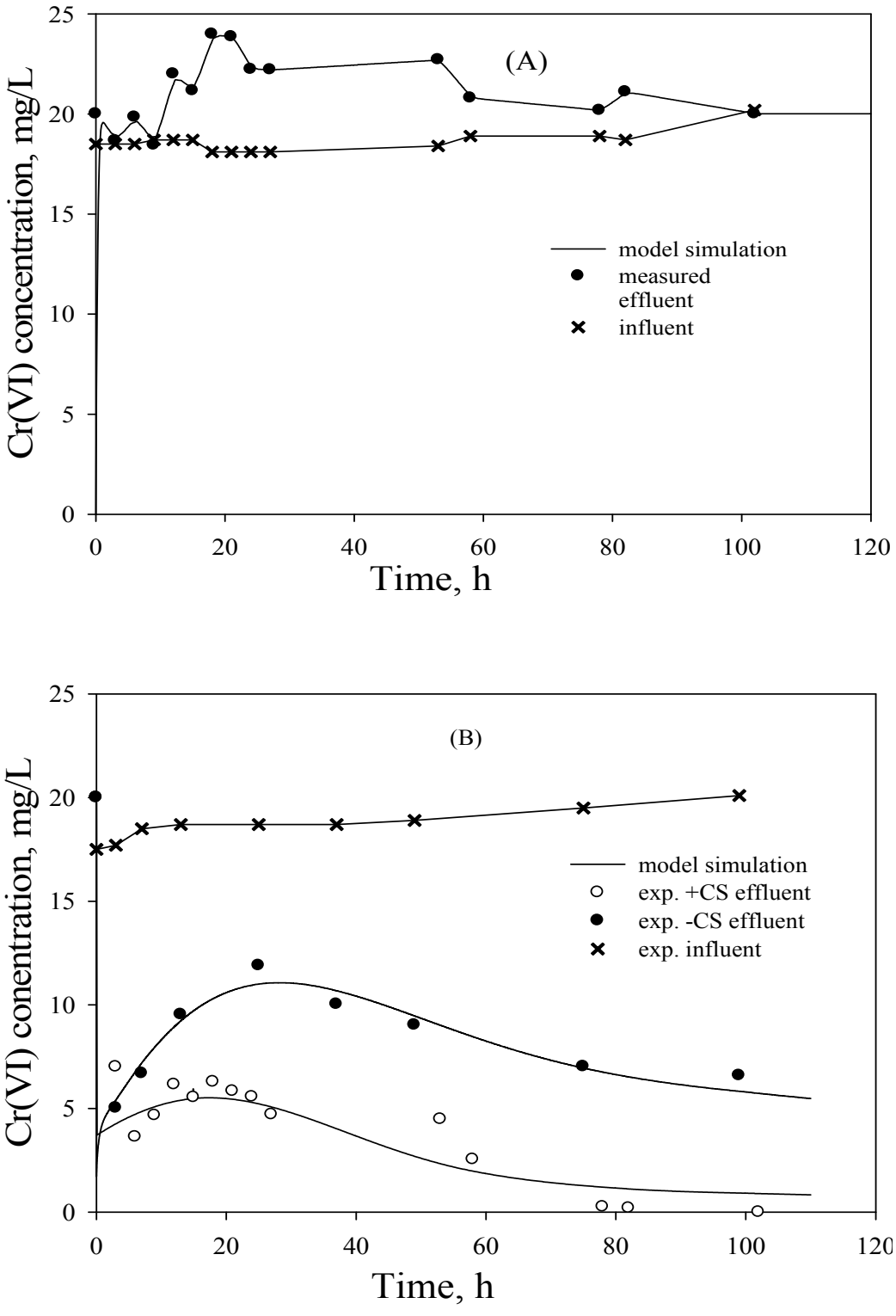


Figure5- 10: Simulation of Cr(VI) effluent at 20 mg/L in a (A) sterile control column, (B) carbon source and non-carbon source reactor

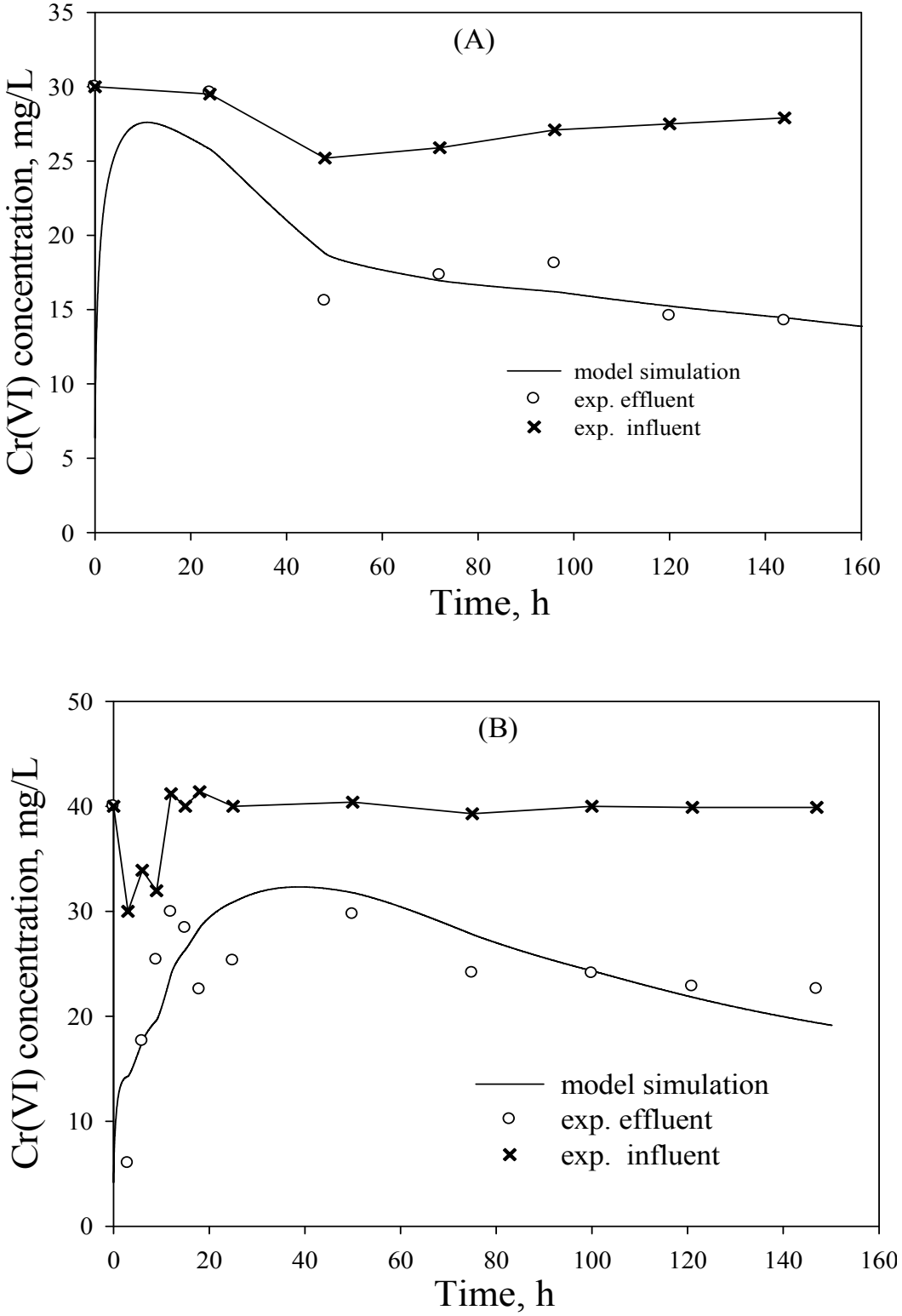


Figure5- 11: Simulation of Cr(VI) effluent at (A) 30 mg/L in a non-carbon source reactor, (B) 40 mg/L in a carbon source reactor

## 5.6 Steady State Performance Model

### 5.6.1Model Formulation

Data collected from equally space longitudinal sampling ports were used to facilitated the spatial modelling of Cr(VI) concentration profiles across the reactor under different loading conditions. Steady-state in a packed-bed system is mainly, among many other reasons associated to the enlargement of the mass transfer boundary layer thickness at low velocity which results into limitation of reaction by diffusion. The actual behaviour of Cr(VI) along the reactor under quasi-steady state conditions was modelled as a plug flow reactor using the finite difference model.

The generalized mole balance continuous equation on species (C) over a catalyst weight (W) at a steady-state can represented as follows:

$$F_c\,(W) - F_c\,(W+\Delta W) + \gamma_c\,(\Delta W) = 0 \qquad (5\text{-}7)$$

where: $F_c$ = molar flow rate of Cr(VI) $(MT^{-1})$; $W$ = mass of aquifer soil particles $(M)$; and $\gamma_c$ = reaction rate $(ML^{-3}T^{-1})$.

Dividing equation (5-7) by (ΔW) yield the following equation:

$$\frac{F_c(W) - F_c(W+\Delta W)}{\Delta W} = \gamma_c \qquad (5\text{-}8)$$

The basic limiting process for calculus states that: for any quantity $Q$ which is a smooth continuous function of $L$:

$$\lim_{L_2 \to L_1} \frac{Q_2 - Q_1}{L_2 - L_1} = \lim_{\Delta L \to 0} \frac{\Delta Q}{\Delta L} = \frac{dQ}{dL}$$

Therefore taking the limit as ΔW→0, in equation (5-8) we arrive at the differential form of the mole balance for a plug flow reactor:

$$\frac{dF_c}{dW} = \gamma_c \qquad (5\text{-}9)$$

Expressing ($F_c$) in terms of concentration yield the following equation:

$$F_c\ (MT^{-1}) = Q_{in}\,C \tag{5-10}$$

where: $Q_{in}$ = inflow rate ($L^3T^{-1}$); and $C$ = Cr(VI) concentration any time ($ML^{-3}$). On the other hand W (mass of aquifer soil particles) which is more important to the rate of reaction can be represented as:

$$W\,(M) = \rho_c A_f L \tag{5-11}$$

Levenspiel (1999) has shown that the reaction rate of the microbial reactions that are subjected to reactant toxicity can be represented as:

$$-\gamma_c = kC\left(1-\frac{C}{Cr}\right)^n \tag{5-12}$$

where: $\gamma_c$ = reaction rate ($ML^{-3}T^{-1}$); $k$ = reaction rate coefficient ($L.M^{-3}.T^{-1}$); $C$ = effluent Cr(VI) concentration at any time ($ML^{-3}$); $C_r$ = Cr(VI) toxicity concentration, ($ML^{-3}$); $n$ = empirical dimensionless variable ($M^{-1}M^{-1}$).

Therefore equation (5-9) can be represented as second order ordinary differential equation (ODE) representing both the flow characteristics and the predominant removal mechanism as follows:

$$-\frac{dC}{dL} = kC\left(\frac{\rho_c A_f}{Q_{in}}\right)\left(1-\frac{C}{C_r}\right)^n \tag{5-13}$$

where: $C$ = effluent Cr(VI) concentration at any time ($ML^{-3}$), $L$ = height of a reactor ($L$), $k$ = reaction rate coefficient ($L.M^{-3}.T^{-1}$), $\rho_c$ = density of aquifer soil particles ($ML^{-3}$), $A_f$ = biofilm surface area ($L^2$), $Q_{in}$ = inflow rate ($L^3T^{-1}$), $C_r$ = Cr(VI) toxicity concentration, ($ML^{-3}$), $n$ = empirical dimensionless variable ($M^{-1}M^{-1}$). N.B: $n$ varies with the reactor environment.

Assumptions Governing the Model:

- There is no mixing in the axial direction, this implies that molecular and/or turbulent mass dispersion is negligible in flow direction
- Uniform properties in the direction perpendicular to the flow (flow is one dimensional)
- The net growth of bacteria is zero at this state, i.e., Cr(VI) reduction is by resting cells
- The system is a homogenous catalytic system

### 5.6.2 Steady State Spatial Simulation

The slope of Cr(VI) concentration profiles across the reactor was defined by a second order ODE (Equation 5-13). The plug flow model was initially tested with control column to simulate length series data under various loading conditions in the reactor using the Computer Program for Solving Numerical Problems (Octave 3.0) as shown in Appendix B.

**Performance under Carbon Source**

The rate of Cr(VI) removal along an inoculated carbon source reactor was evaluated using the plug flow model in Equation (5-13). The model was initially tested with Cr(VI) experimental run of 50 mg/L which is considered as control due to insignificant Cr(VI) removal observed at 50mg/L in an inoculated carbon source reactor. The optimum kinetic parameters obtained at 50 mg/L which are $k$ (reaction rate coefficient) and $n$ (empirical variable) were then used to simulate Cr(VI) effluent under different loading conditions.

The experimental run at 20 mg/L in a carbon source reactor gives a clear representative picture of bioreduction Figure (5-12). It also observed in Figure (5-12 B) that at the initial Cr(VI) concentration of 40 mg/L, inhibition of Cr(VI) in Cr(VI) reducing culture occurred in the reactor. The inhibition of Cr(VI) in the reactor may be associated to Cr(VI) toxicity within the cells and the loss of Cr(VI) reduction capacity due to blockage of the media pores with Cr(III) precipitate. The experimental data in Figure 5-12 (A-C) is the average of the last three sampling times when the quasi-steady state was reached in the column. The Cr(VI)

toxicity threshold concentration was assumed to be 50 mg/L as it is observed in Figure 5-12 (C) that at the initial Cr(VI) concentration of 50 mg/L the reduction of Cr(VI) was insignificant over time across the reactor. The optimum reaction rate coefficient, $k$, was determined to be $5.2\times10^{-8}$ (L/mg/h) and the empirical variable, $n$ =2. The model depicted well the trends of Cr(VI) concentration profiles under quasi-steady state conditions for different loading conditions in a carbon source reactor with the R-squared value of 95%. The experimental data points outside the model trend line were considered as outliers.

**Performance under non Carbon Source**

The plug flow model developed in this study under quasi-steady state condition for different loading conditions Equation (5-13) was used to simulate Cr(VI) effluent concentration in an inoculated non-carbon source reactor. Cr(VI) experimental run of 30 mg/L was initially tested with the model. The optimum kinetic parameters obtained from the Cr(VI) feed concentration of 30 mg/L were then used to simulate Cr(VI) effluent concentration at 20 mg/L. Figure (5-13) gives a clear representative picture that the inhibitory effects of Cr(VI) in a non-carbon source reactor occurs at a relatively lower initial Cr(VI) feed concentration compared to carbon source reactor. This is indicates that the carbon source in the reactor greatly enhance the removal of Cr(VI) in the aquifer medium.

Compared to the kinetic parameters obtained in a carbon source reactor under quasi-steady state conditions for different loading conditions the kinetic parameters in a non-carbon source reactor were adjusted, thus $k = 9.9\times10^{-9}$ L/mg/h and $n = 1$. The model successfully captured the trends of Cr(VI) response profiles under quasi-steady state conditions for different loading conditions in the inoculated non-carbon source reactor with the R-squared value of 94.7% . The experimental data points outside the model trend line were considered as outliers.

Table5- 4: Optimum kinetic parameter values for the biofilm at steady-state in a carbon source and a non carbon source reactor

| **Kinetic parameter** | **Description** | **Carbon source reactor** | **Non Carbon source reactor** |
|---|---|---|---|
| $k$ (L/mg/h) | Reaction rate coefficient | $5.2\times10^{-8}$ | $9.9\times10^{-9}$ |
| $n$ (mg/mg) | Empirical variable | 2 | 1 |

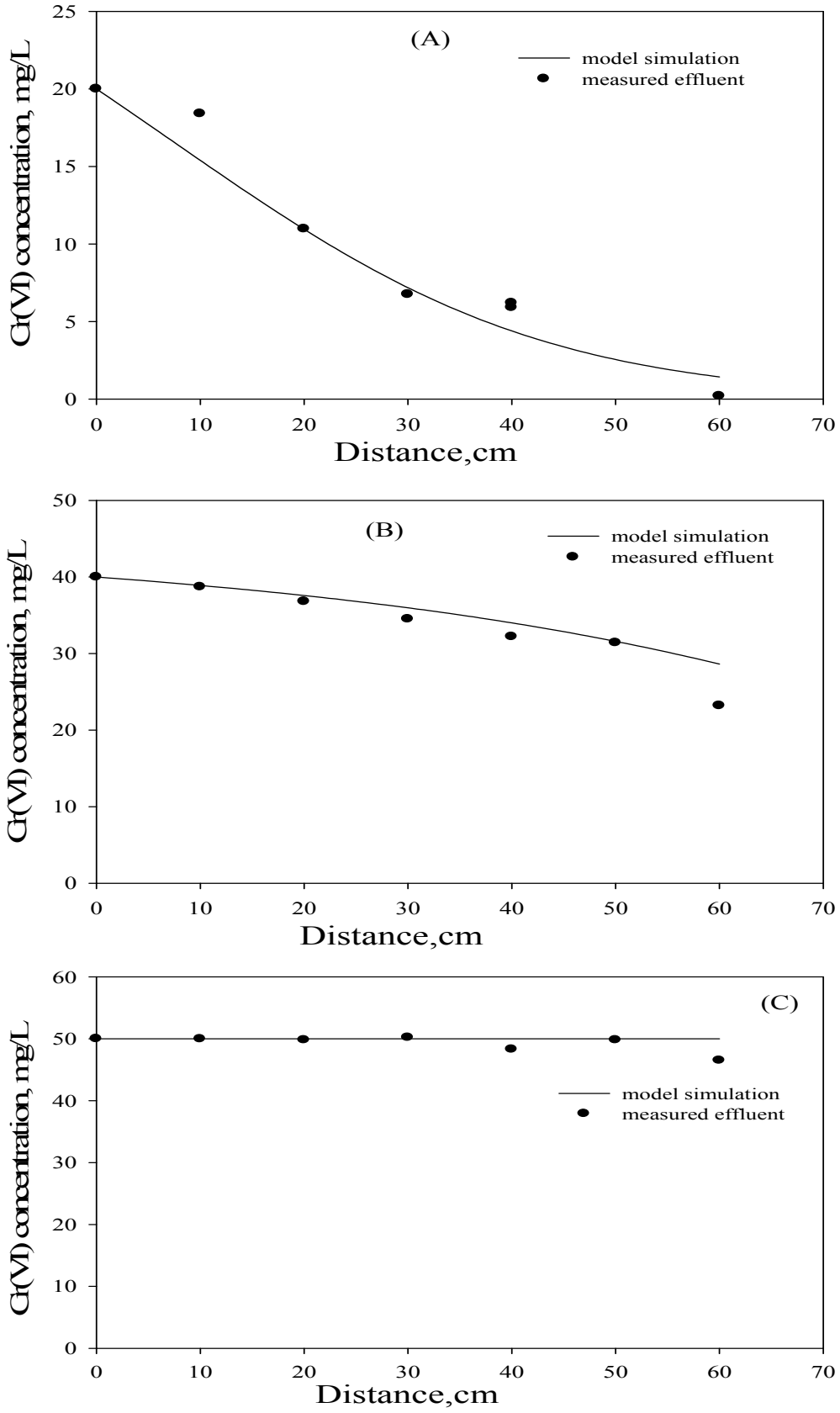


Figure5- 12: Simulation of Cr(VI) effluent in a carbon source reactor at various lengths

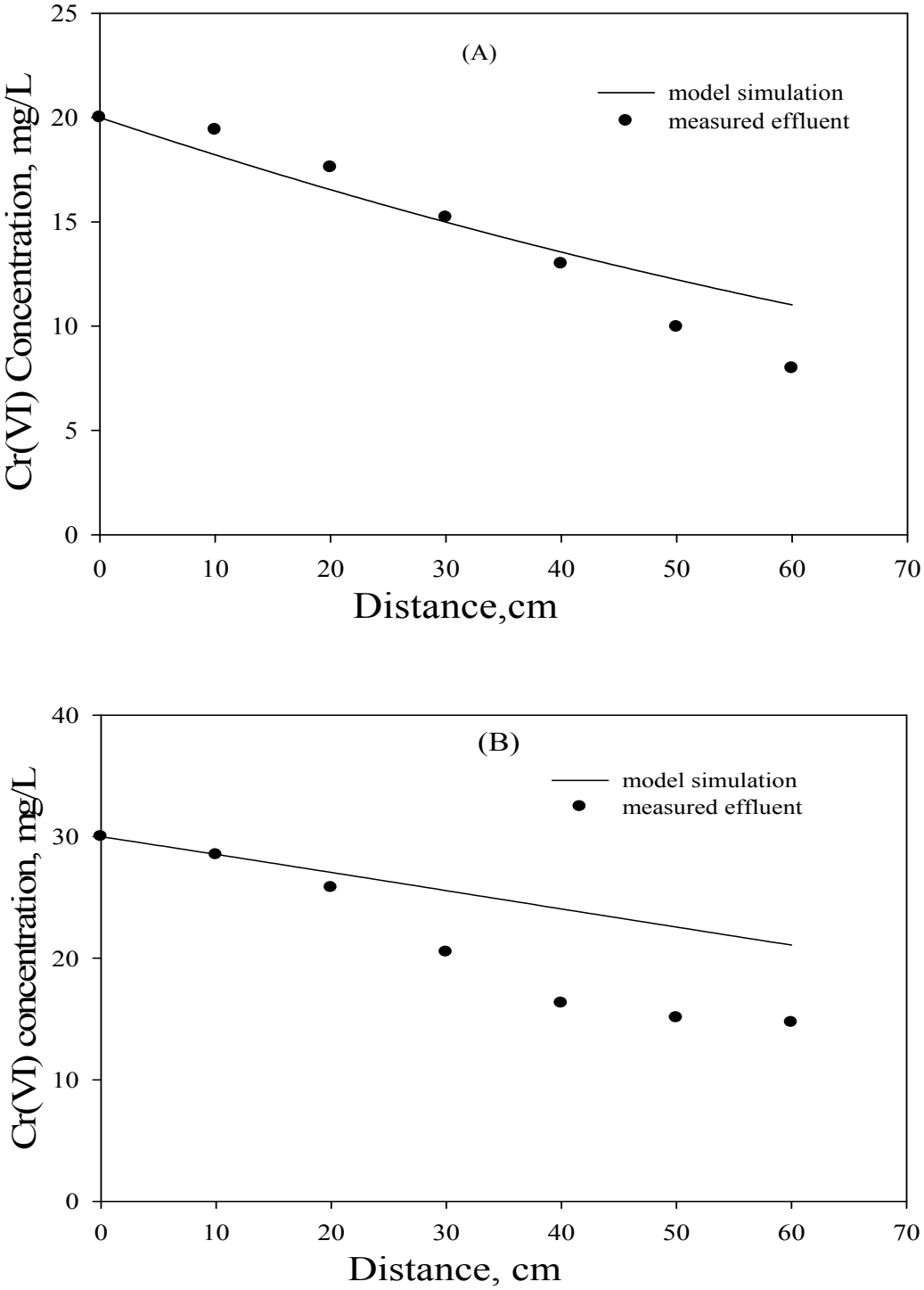


Figure5- 13: Simulation of Cr(VI) effluent in a non-carbon source reactor over length. The experimental data is the average values of the last three sampling times where the quasi-steady state was achieved in the column.

### 5.6.3 Summary of Steady State Kinetic Parameters

The model for saturated soil column at the steady state was determined by an ordinary differential equation as shown in Equation (5-13). The parameter, *n*, which is defined as the empirical dimensionless variable in this model various with the environmental conditions as it is associated to the rate of Cr(VI) toxicity in each column experimental condition. The toxicity term in Equation (5-13), $\left(1-\frac{C}{C_r}\right)^n \rightarrow 0$ as $n \rightarrow \infty$. Table (5-5) shows that the value of, *n*, in an inoculated carbon source reactor is greater than the value of, *n*, in an inoculated non carbon source reactor. This is indicates that the inhibitory effects of Cr(VI) on Cr(VI) reducing cultures is approached at a faster rate in the non carbon-source reactor than in the carbon source reactor. This implies that the higher the value of, *n*, the slower the rate of Cr(VI) toxicity within the cells and thus also imply that the rate of cell inactivity within the reactor will be slow. It is also observed in Table (5-5) that the reaction rate coefficient, *k*, also varies with the reactor experimental conditions. Minor adjustments on, *k,* in the non-carbon source reactor were determined in the model. The results obtained in a carbon source and non-carbon source reactor indicates that the presence of the carbon source may greatly influence the rate of Cr(VI) reduction in the reactor by improving the rate of cell activity.

## 5.7 Summary

Microcosm studies showed that the isolated bacterial species from the dried sludge can effectively reduce Cr(VI) from the contaminated aquifer systems. The reduction of Cr(VI) in the inoculated reactors was observed to be both time and length dependent. A one dimensional dispersion-reaction model evaluated in this study was able to simulate contaminant movement in the aquifer system for a broader range of Cr(VI) concentrations at various time intervals. At a steady-state the removal of Cr(VI) along the reactor was estimated using the plug flow model expressed as function of spatial variable, *L*, which was derived based on component/mole balance continuity equation in Equation (5-13).

The results obtained in the kinetic modelling of Cr(VI) reduction at quasi-steady state demonstrated that the inhibitory effects of Cr(VI) on Cr(VI) reducing microorganisms in the inoculated reactor without carbon source are approached faster than in the inoculated reactor with carbon source. The model developed in this study under quasi-steady state condition was able to predict well the experimental data at various Cr(VI) feed concentrations. This

indicates that the developed predictive model in this study can be effective in facilitating the final scale up and operation of the microbial barrier in field.

After seven weeks of operation under oxygen stressed conditions the microbial community shift was expected. Microbial shift results showed that among all the bacterial species isolated from the dried sludge (X1 to X7) which were initially inoculated in the column reactors as a reconstituted consortium culture, *Bacillus thuringiensis* (X5 and X6) and the *Bacillus cereus* (X2), the predominant well known Cr(VI) reducers remained persistent in all inoculated reactor columns, (R2 and R4). The results obtain in this chapter correlates with the results which were obtained in the previous chapter (Chapter 4) where X5 and X6 were evaluated as potential isolates in Cr(VI) reduction process.

# CHAPTER 6

## SUMMARY AND CONCLUSION

The improper release of Cr(VI) solid and liquid waste from various industries in South Africa and around the world is a subject of paramount concern. Therefore biotransformation of hazardous Cr(VI) to less toxic Cr(III) is essential. Batch experimental studies were conducted in this study under various Cr(VI) concentration to evaluate the effectiveness of indigenous culture from the local environment in reducing Cr(VI) in the Cr(VI) contaminated environments. The rate of Cr(VI) reduction in the anaerobic batch culture was observed to be generally slower than that observed in the aerobic batch culture. Near complete Cr(VI) reduction occurred in consortium culture reconstituted from the potential pure anaerobic isolates with a lower initial Cr(VI) concentration of 100 mg/L after 65 hours of incubation.

Cr(VI) transport through saturated porous media is a highly dynamic process that can not be fully defined through batch reactor systems, therefore further experimental studies in continuous-flow system were necessary for this purpose. In order to evaluate the fundamentals of each biological process over time prior continuous-flow system operation anaerobic batch studies were initially conducted prior continuous-flow system operation as batch reactors are easy to operate and to analyse compared to continuous-flow systems.

The feasibility of using Cr(VI) reducing cultures in the aquifer environment was demonstrated by better performance of microcosm reactors inoculated with Cr(VI) reducing consortium culture from the local environment. The impact of carbon source reactor in Cr(VI) reduction was also evaluated in the study. Microcosm reactors supplemented with sawdust as carbon source outperformed the one without carbon source with Cr(VI) removal efficiency of 55% at a Cr(VI) feed concentration of 40 mg/L, which is the current highest groundwater Cr(VI) concentration at the remediation wells at the studied site. The inhibitory effects of Cr(VI) on the Cr(VI) reducing organisms in the reactor was demonstrated by a steady-state operation which was achieved after three to four days of operation. The microcosm reactor conditions favoured the well known Cr(VI) reducing species, *Bacillus cereus, Bacillus thuringiensis* after seven weeks of operation.

Batch modeling results showed that the performance of Cr(VI) reducing culture fitted well the non-competitive model associated with Cr(VI) toxicity threshold predicted under anaerobic conditions. The model used for simulation of Cr(VI) effluent within the saturated aquifer media over time in the microcosm system was adopted from AQUASIM 2.0 and used in combination with kinetic parameters obtained from the anaerobic batch cultures. The kinetic model with dispersion simulated well the soil column experimental data with a plug flow regime.

At a steady-state the plug flow model that accounts for insignificant Cr(VI) removal along the column over the last three sapling times of operation was derived in the study based on the first principle of mass conservation. The Cr(VI) mass balance model under quasi-steady state condition accounts for both flow characteristics and the predominant removal mechanism (biological transformation) in the reactor. The model simulated well the experimental data at various Cr(VI) concentration. The outcome of this study is a good basis for testing the concept in pilot scale study on site.

**Recommendations**

In order to achieve optimum application of this technology future research will be needed in the following areas:

- The experiment that accounts for Cr(III) removal in the reactor must be taken into consideration as the $Cr(OH)_3$ generated during Cr(VI) transformation process may clog in the reactor and thus will reduce the performance of the system after a long period of operation.

- The composition of sawdust must be analysed to evaluate organics which are biodegradable and non-biodegradable in the sawdust.

- Develop a method that accounts for biomass concentration profile within the column.

# APPENDIX A

AQUASIM Version 2.0

**********

**Variables**

**********

A : Description : Cross- sectional area

Type : Constant variable

Unit : $m^2$

Value : 0.00196

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: active

Parameter Estimation: active

---

Af : Description : Biofilm surface area

Type : Constant variable

Unit : $m^2$

Value : 0.000785

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: active

Parameter Estimation: active

---

$\alpha$: Description : Alpha

Type : Formula variable

Expression : 0.5

---

C : Description : Dissolved Cr(VI) concentration

Type : state variable

Unit : mg/L

Relative Accuracy: 1e-006

Absolute Accuracy: 1e-006

---

C2: Description: Cr(VI) toxicity threshold concentration

Type: Constant Variable

Unit: mg/L

Value: 50

Standard Deviation: 1

Minimum: 0

Maximum: 60

Sensitivity Analysis: active

Parameter Estimation: active

---

Calcnum: Description:

Type: Program Variable

Unit: h

Reference to: calculation number

---

Cmeas: Description: Measured Cr(VI)

Type: Real List Variable

Unit: mg/L

Argument: t

Standard Deviations: global

Rel. Stand. Deviation: 0

Abs. Stand. Deviation: 1

Minimum: 0

Maximum: 1e+009

Interpolation Method: linear interpolation

Sensitivity Analysis: inactive

Real Data Pairs (15 pairs):

| Argument | Value |
|---|---|
| 0 | 20 |
| 3 | 7 |
| 6 | 3.62 |
| 9 | 4.66 |
| 12 | 6.15 |
| 15 | 5.83 |
| 18 | 6.28 |
| 21 | 5.83 |
| 24 | 5.56 |
| 27 | 4.7 |
| 53 | 4.48 |
| 58 | 2.54 |
| 78 | 0.25 |
| 82 | 0.19 |
| 102 | 0 |

---

Co: Description: Initial Cr(VI) concentration

Type: Formula Variable

Unit: mg/L

Expression: 20

---

C_crit: Description:

Type: Formula Variable

Unit: mg/L

Expression: 0.01

---

Cin: Description: measured Cr(VI) influent

Type: Real List Variable

Unit: mg/L

Argument: t

Standard Deviations: global

Rel. Stand. Deviation: 0

Abs. Stand. Deviation: 1

Minimum: 0

Maximum: 1e+009

Interpolation Method: linear interpolation

Sensitivity Analysis: inactive

Real Data Pairs (15 pairs):

| Argument | Value |
|---|---|
| 0 | 18.5 |
| 3 | 18.5 |
| 6 | 18.5 |
| 9 | 18.7 |
| 12 | 18.7 |
| 15 | 18.7 |
| 18 | 18.1 |
| 21 | 18.8 |
| 24 | 18.9 |
| 27 | 18.1 |
| 53 | 18.4 |
| 58 | 18.9 |
| 78 | 18.9 |
| 82 | 18.8 |
| 102 | 20.2 |

---

C_in_1: Description:

Type: Real List Variable

Unit: mg/L

Argument: t

Standard Deviations: global

Rel. Stand. Deviation: 0

Abs. Stand. Deviation: 1

Minimum: 0

Maximum: 1e+009

Interpolation Method: linear interpolation

Sensitivity Analysis: inactive

Real Data Pairs (4 pairs):

| Argument | Value |
|---|---|
| 0 | 0 |
| 0.01 | 1 |
| 0.5 | 1 |
| 0.51 | 0 |

---

D: Description: Dispersion coefficient

Type: Constant Variable

Unit: $m^2/h$

Value: 95.4

Standard Deviation: 1

Minimum: 0

Maximum: 100

Sensitivity Analysis: active

Parameter Estimation: active

---

K: Description:

Type: Formula Variable

Unit: mg/L

Expression: 0.5

---

k: Description: Relaxation rate constant for sorption of B

Type: Formula Variable

Unit: 1/h

Expression: 10000

---

Kc: Description: Half velocity Cr(VI) concentration

Type: Constant Variable

Unit: mg/L

Value: 11.272

Standard Deviation: 1

Minimum: 0

Maximum: 14

Sensitivity Analysis: active

Parameter Estimation: active

---

km: Description: Maximum specific Cr(VI) reduction rate

Type: Constant Variable

Unit: 1/h

Value: 0.0051

Standard Deviation: 1

Minimum: 0

Maximum: 0.02

Sensitivity Analysis: active

Parameter Estimation: active

---

μ: Description: Specific biomass growth rate

Type: Constant Variable

Unit: 1/h

Value: 0.023

Standard Deviation: 1

Minimum: 0

Maximum: 1000

Sensitivity Analysis: inactive

Parameter Estimation: inactive

---

Kd: Description: Cell death rate

Type: Constant Variable

Unit: 1/h

Value: 0.0025

Standard Deviation: 1

Minimum: 0

Maximum: 1000

Sensitivity Analysis: active

Parameter Estimation: active

---

Kf: Description:

Type: Formula Variable

Unit:

Expression: 0.00025

---

Qin: Description: Inflow rate

Type: Formula Variable

Unit: L/h

Expression: 0.015

---

Rc: Description: Cr(VI) reduction capacity coefficient

Type: Constant Variable

Unit: mg/mg

Value: 0.283

Standard Deviation: 1

Minimum: 0

Maximum: 0.5

Sensitivity Analysis: active Parameter Estimation: active

---

rho_s: Description: Density of solid material

Type: Formula Variable

Unit: $kg/m^3$

Expression: 2300

---

S: Description: Adsorbed concentration

Type: Dynamic Surface State Variable

Unit: mg/kg

Relative Accuracy: 1e-006 Absolute Accuracy: 1e-009

---

Smax: Description:

Type: Formula Variable

Unit: mg/kg

Expression: 0.00029

---

S_eq: Description: isotherm

Type: Variable List Variable

Unit: mg/kg

Argument: calcnum

Interpolation Method: linear interpolation

List of data (1 pair):

| Argument | Value |
|---|---|
| 0 | S_eq_0 |

---

| | | |
|---|---|---|
| -S_eq_0: | Description: | Isotherm for no sorption |
| | Type: | Formula Variable |
| | Unit: | mg/kg |
| | Expression: | kd*C |

---

| | | |
|---|---|---|
| S_eq_Freundlich: | Description: | Freundlich isotherm |
| | Type: | Formula Variable |
| | Unit: | mg/kg |
| | Expression: | if C>C_crit then Kf*C^alpha else Kf*C_crit^alpha*C/C_crit endif |

---

| | | |
|---|---|---|
| S_eq_Langmiur: | Description: | Langmuir isotherm |
| | Type: | Formula Variable |
| | Unit: | mg/kg |
| | Expression: | Smax*C/(K+C) |

---

| | | |
|---|---|---|
| S_eq_lin: | Description: | Linear isotherm |
| | Type: | Formula Variable |
| | Unit: | mg/kg |
| | Expression: | Kd*C |

---

| | | |
|---|---|---|
| t: | Description: | time |
| | Type: | Program Variable |
| | Unit: | h |
| | Reference to: | Time |

---

| | | |
|---|---|---|
| theta: | Description: | Porosity |
| | Type: | Formula Variable |
| | Unit: | |
| | Expression: | 0.4 |

---

| | | |
|---|---|---|
| X: | Description: | Biomass concentration |

Type: Formula Variable

Unit: mg/L

Expression: Xo*exp-(μ-Kd)*t

---

Xo: Description: Initial biomass concentration

Type: Constant Variable

Unit: mg/L

Value: 180.8

Standard Deviation: 1

Minimum: 0

Maximum: 10000

Sensitivity Analysis: inactive

Parameter Estimation: active

**********

**Processes**

**********

Reduction: Description: Cr(VI) reduction

Type: Dynamic Process

Rate: (K^(-1*(Co-C2)/Co))*km*C*(X-(Co-C)/Rc)/(Kc+C)

Stoichiometry:

Variable: Stoichiometric Coefficient

C -1

Sorption: Description: Cr(VI) sorption

Type: Dynamic Process

Rate: k*(S_eq-S)

Stoichiometry:

Variable: Stoichiometric Coefficient

C -rho_s*(1-theta)/theta

S 1

**********

**Compartments**

**********

Column: Description: Saturated packed column

Type: Mixed Reactor Compartment

Compartment Index: 0

Start coordinate: 0

End coordinate: 1

Cross sectional area: A

Mob.Vol.Frac: theta

Dispersion: with dispersion

Number of grit points: 52 Resolution: high

Active Variables: C and S

Active Processes: reduction and sorption

Initial Conditions:

Variable(Zone) : Initial Condition

C(advection zone) : Cmeas

Input type : inlet input

Water flow: Qin

Loading variable:

C: Qin*Cin

**********

**Definitions of Calculations**

**********

Calc_0: Description:

Calculation Number: 0

Initial Time: 0

Initial State: given, made consistent

Step Size: 0.1

Num. Steps: 110

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

Active parameters: D, Kc, Kd, km, and μ

Method: simplex

Maximum number of iterations: 100

**********

**Plot Definitions**

**********

Concentration plot: Description: Cr(VI) concentration

Abscissa: Time

Title: Cr(VI) Concentration

Abscissa Label: Time [h]

Ordinate Label: Concentration [mg/L]

Curves:

Type : Variable [Calcnum, column advection zone, Time/Space]

Value : C [0,column advection zone,1]

Value : Cmeas [0,columnadvection zone,0]

Value : Cin_meas [0, column advection zone,0]

Value : Cin [0,column advection zone,0]

# APPENDIX B

Octave Version 3.0

**********

```
Lraw= [0,10, 20, 30, 40, 50, 60] ;

Craw= [20.0, 18.4, 10.97, 6.75, 6.2, 5.9, 0.19] ;

Cr= 50 ;

Af= 7.85 ;

rho_s=  2300000 ;

Qin= 0.015 ;

k = 0.000000052 ;

n=2 ;

L= linspace (0, 60, 100) ;

Co= 20 ;

dCdL= @(C, L)  (-k*C)*((Af*rho_s)/Qin)*((1-(C/Cr))^n) ;

Ci= lsode (dCdL, Co, L) ;

plot(Lraw, Craw, ‘o’, L, Ci)

data= [Ci]

data= [L’]

legend (‘experimental values’, ‘model simulation’) ;

xlabel (‘distance, cm’) ;

ylabel (‘Cr(VI) concentration, mg/L’) ;
```

# APPENDIX C

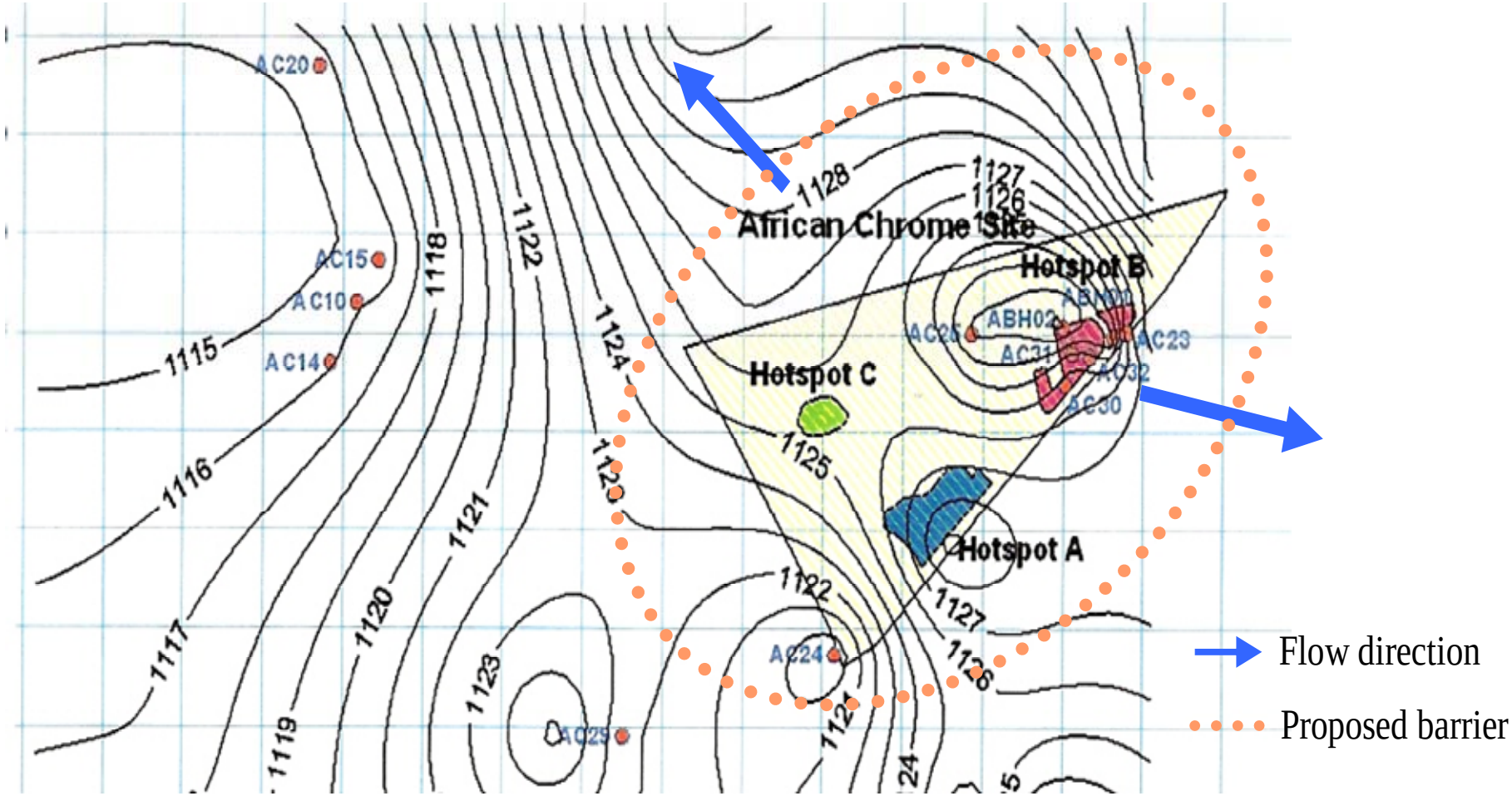


Figure C: Target Site

## REFERENCES

Ackerley, D.F., Gozalez, C.F., Keyhan, M., Blake, R., and Matin, A., 2004. Mechanism of chromate reduction by Escherichia coli protein, NfsA, and the role of different chromate reductase in minimizing oxidative stress during chromate reduction. Environ Microbiol, **6**: 851- 860.

Aeseth, J., Alexander, J., and Norseth, T., 1982. Uptake of 5Cr-chromate by human erythrocytes-a role of ghitathione. Acta Pharmacol Toxicol, **50:** 310-315.

Aguilera, S., Aguilar, M.E., Chavez, M.P., Lopez-Meza, J.E., Pedraza- Reyes, M., Campos-Garcia, J., and Cervantes, C., 2004. Essential residues in the chromate transporter ChrA of Pseudomonas aeruginosa. FEMS Microbiol Lett, **232:** 107-112.

Ahmad, W.A., Zakaria, Z.A., Khasim, A.R., Alias, M.A., and Ismail S.M.H.S., 2010. Pilot-scale removal of chromium from industrial wastewater using the ChromeBac$^{TM}$ system. Bioresource Technology, **101:** 4371-4378.

Aislabie, J., and Loutit, M.W., 1984. The effect of effluent high in chromium on marine sediment aerobic heterotrophic bacteria. Marine Environmental Research, **13:** 69-79.

Ajmal, M., Nomani, A.A., and Ahmad, A., 1984. Acute toxicity of chrome electroplating wastes to microorganisms - adsorption of chromate and chromium (VI) on a mixture of clay and sand. Water Air Soil Pollution, **23:** 119-127.

Alvarez, A.H., Moreno-Sanchez, R., and Cervantes, C., 1999. Chromate efflux by means of the ChrA chromate resistance protein from Pseudomonas aeruginosa. J Bacteriol, **181:** 7398-7400.

APHA, 2005. Standard methods for the examination of water and wastewater. In: Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Franson, M.A.H. (Eds.), 21st ed. American Public Health Association, American Water Works Association, Water Environment Federation, USA (centennial edition).

Asku, Z., Kutsal, T, Gun, S., Haciosmanoglu, N., and Gholminejad, M., 1991. Investigation of biosorption of Cu(II), Ni(II) and Cr(VI) ions to activated sludge bacteria. Environ Technol, **12:** 915-921.

Appenroth, K.J., Bischoff, M.B., Gabrys, H., Stoeckel, J., Swartz, H.M., Walczak, T., and Winnefeld, K., 2000. Kinetics of chromium (VI) formation and reduction in fronds of the duckweed spirodela polyrhiza-a low frequency EPR study. Journal of Inorganic Biochemistry, **78:** 235-242.

Armitage, W.K., 2002. Chromium in South Africa's minerals industry 2001/200219th: South Africa Department of Minerals and Energy December, 108-112.

Asmatullah, Qureshi, S.N., and Shakoori, A.R., 1998. Hexavalent chromium induced congenital abnormalities in chick embryos. J Appl Toxicol, **18(3):** 167-171.

Baldi, F., Vaughan, A.M., and Olson, G.J., 1990. Chromium (VI)-resistant yeast isolated from a sewage treatment plant receiving tannery wastes. Appl Environ Microbiol, **56(8):** 913-918.

Baral, A., and Engelken, R.D., 2002. Chromium-based regulations and greening in the metal finishing industries in the USA. Environ Sci Policy, **5:** 121-133.

Bartlett, R.J., 1991. Chromium cycling in soils and water: Links, gaps, and methods. Environmental Health Perspectives, **92:** 17-24.

Barnhart, J., 1997. Occurrences, Uses and Properties of chromium. Regul Toxicol Pharmacol, **26:** S3-S7.

Basu, M., Bhattacharya, S., and Paul, A.K., 1997. Isolation and characterization of chromium-resistant bacteria from tannery effluents. Bull Environ Contam Toxicol, **58:** 535-542.

Bayer, P., and Finkel, M., 2006. Life cycle assessment of active and passive groundwater remediation technologies. Journal of Contaminant Hydrology, **83:**171-199.

Beaudoin, D.L., Bryers, J.D., Cunningham, A.B., and Peretti, S.W., 1998. Mobilization of broad host range plasmid from Pseudomonas putida to establish biofilm of Bacillus azotoformans. II. Modeling. Biotechnol Bioeng, **57(3):** 280-286.

Blowes, D., 2002. Environmental chemistry. Tracking hexavalent chromium in groundwater, Science, **295:** 2024-2025.

Bopp, L.H., and Ehrlich, H.L., 1988. Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300. Arch Microbiol, **150:** 426-431.

Bondarenko, B.M., and Ctarodoobova, A.T., 1981. Morphological and cultural changes in bacteria under the effect of chromium salts. J Microbiol Epidemiol Immunobiol., USSR. **4:** 99-100.

Brock, T.D., and Madigan, M.T., 1991. Biology of Microorganisms, 6$^{th}$ Edition, Prentice-Hall, Englewood Cliffs, NJ.

Cabatingan, L.K., Agapay, R.C., Rakels, J.L.L., Ottens, M., and Van Der Wielen, L.A.M., 2001. Potential of biosorption for the recovery of chromate in industrial wastewaters. Ind Eng Chem Res, **40:** 2302-2309.

Caglieri, A., Goldoni, M., Acampa, O., Andreoli, R., Vettori, M.V., Carradi, M., Apostoli, P., and Mutti, A., 2006. The effect of inhaled chromium on different exhaled breath condasate biomarkers among chrome-plating workers. Environ Health Perspect, **114:** 542-546.

Camargo, F.A.O., Okeke, B.C., Bento, F.M., and Frankenberger, W.T., 2003. In vitro reduction of hexavalent chromium by a cell-free extract of *Bacillus* sp. ES 29 stimulated by $Cu^{2+}$. Appl Microbiol Biotechnol, **62:** 569-573.

Cervantes, C., Campos-Garcia, J., Devars, S., Gutierrez-Corona, F., Loza-Tavera, H., Torres-Guzman, J.C., and Moreno-Sanchez, R., 2001. Interactions of chromium with microorganisms and plants. FEMS Microbiology, **25(3):** 335-347.

Chang, I.S., and Kim, B.H., 2007. Effect of sulfate reduction activity on biological treatment of hexavalent chromium [Cr (VI)] contaminated electroplating wastewater under sulfate-rich condition. Chemosphere, **68(2):** 218-226.

Chen, J.M., and Hao, O.J., 1998. Microbial chromium (VI) reduction. Critical Reviewers in Environmental Science and Technology, **28(3):**219-251.

Chen, Y., and Gu, G., 2005. Preliminary studies on continuous chromium (VI) biological removal from wastewater by anaerobic-aerobic activated sludge process. Bioresource Technology, **96(15):** 1713-1721.

Chirwa, E.M.N., and Wang, Y.T., 1997. Chromium(VI) reduction by *Pseudomonas fluorescens* LB300 in fixed-film bioreactor, Journal of Environmental Engineering, **123:** 760-766.

Chirwa, E.M.N., and Wang, Y.T., 1997a. Hexavalent chromium reduction by *Bacillus sp.* in a packed-bed bioreactor. Environmental Science and Technology, **31(5):**1446-1451.

Chirwa, E.M.N., and Wang, Y.T., 1997b. Chromium(VI) reduction by *Pseudomonas fluorescence* LB300 in fixed-film bioreactor. Journal of Environmental Engineering, **123(8):**760-766.

Chirwa, E.M.N., and Wang, Y.T., 2000. Simultaneous Cr (VI) reduction and phenol degradation in an anaerobic consortium of bacteria. Water Research, **34(8):** 2376-2384.

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

Chirwa, E.M.N., and Wang, Y.T., 2004. Modeling hexavalent chromium removal in a Bacillus sp. fixed-film bioreactor. Biotechnol Bioeng, **87:** 874-883.

Coleman, R.N., and Paran, J.H., 1983. Accumulation of hexavalent chromium by selected bacteria. Environ Technol Lett, **4:** 149-156.

Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., and Thamaraiselvi, K., 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J Hazard Mater, **146:** 270-277.

Das, A., and Mishra, S., 2008. Hexavalent Chromium (VI): Health hazards & Environmental Pollutant. Journal of Environmental Research and Development, Volume 2 No. 3(S).

Dermou, E., Velissariou, A., Xenos, D., and Vayenas, D.V., 2005. Biological chromium (VI) reduction using a trickling filter. J Hazardous Materials, **B126:** 78-85.

Eid, and Zahir, 1996. Characterization and Treatment of the soil of an industrial site contaminated with Cr(VI). J Environ Sci Health, **A31(1):** 227-247.

Ehrlich, H.L., 1986. Interactions of heavy metals and microorganisms in D. Carlisle (ed.). Mineral Exploration Biological Systems and Organic Matter, Rubey Volume V, Prentice-Hall, Englewood Cliffs, New Jersey, pg 221-237.

Fendorf, S.E., 1995. Surface-Research of chromium in Soils and Waters. Geoderma, **67:** 55-71.

Ferro Orozco, A.M., Contreras, E.M., and Zaritzky, N.E., 2010. Cr(VI) reduction capacity of activated sludge as affected by nitrogen and carbon sources, microbial acclimation and cell multiplication. J Hazard Materials, **176:** 657-665.

Flores, A., and Perez, J.M., 1999. Cytotoxicity, apoptosis and in vitro DNA damage induced by potassium chromate. Toxicol Appl Pharmacol, **161:** 75-81.

Francis, C.A., Obraztsova, A.Y., and Tebo, B.M., 2000. Dissimilatory metal reduction by the facultative anaerobe Pantoea agglumerans SP1. Appl Environ Microbiol, **66:** 543-548.

Francisco, R., Alpoim, M.C., and Morais, P.V., 2002. Diversity of chromium-resistant and reducing bacteria in a chromium-contaminated activated sludge. Journal of applied Microbiology, **92(5):** 837-843.

Fujie, K., Hu, H.K., Huang, X., Tanaka, Y., Urano, K., and Ohtake, H., 1996. Optimal operation of bioreactor system developed for the treatment of chromate waste water using *Enterobacter cloacae* HO1. Water Sci Technol, **33:** 173-182.

Fulladosa, E., Desjardin, V., Murat, J.C., Gourdon, R., and Villaescusa, I., 2006. Cr(VI) reduction into Cr(III) as a mechanism to explain the low sensitivity of Vibrio fischeri bioassay to detect chromium pollution. Chemosphere, **65:** 644-650.

Ganguli, A., and Tripathi, A.K., 2002. Bioremediation of toxic chromium from electroplating effluent by chromate-reducing Pseudomonas aeruginosa A2Chr in two bioreactors, Appl Microbiol Biotechnol, **58:** 416-420.

Garbisu, C., Alkorta, I., Llama, M.J., and Serra, J.L., 1998. Aerobic chromate reduction by *Bacillus subtilis*. Biodegradation, **9:** 133-141.

Glaze, W.H., 1990. Chemical precipitation. Water Quality and Treatment Edition, Pontius, FW. McGraw-Hill, Icl., NY, pp 747-779.

Glazer, A.N., and Nikoid, H., 1995. Microbial Biotechnology: Fundamentals of Applied Microbiology. Freeman, New York.

Gonzalez, C.F., Ackerley, D.F., Park, C.H., and Matin, A., 2003. A soluble flavoprotein contributes to chromate reduction and tolerance by *Pseudomonas putida.* Acta Biotechnologica, **23:** 233-239.

Guha, H., Saiers, J.E., Brooks, S., Jardine, P., and Jayachandran, K., 2001. Chromium transport, oxidation, and adsorption in manganese-coated sand. J of Contaminant Hydrology, **49:** 311-334.

Horitsu, H.S., Futo, Y., Miyazawa, S., Ogai, S., and Kanwel, K., 1987. Enzymatic Reduction of hexavalent chromium by hexavalent tolerant *Pseudomonas ambigua* G1.Agric Biol Chem, **51:** 2417-2420.

Horton, R.N., Apel, W.A., Thompson, V.S., and Sheridan, P.P., 2006. Low temperature reduction of hexavalent chromium by microbial enrichment consortium and novel strains of *Arthrobacter aeurescens*. BMC Microbil , **6:** 5.

Ishibashi, Y., Cervantes, C., and Silver, S., 1990. Chromium reduction in *Pseudomonas putida*. Applied Environmental Microbiology, **56:** 2268-2270.

James, B.R., 1996. The challenge of remediating chromium-contaminated soil. Environ Sci Technol, **30:** 248-251.

James, B.R., and Bartlett, R.J., 1983b. Behaviour of chromium in soils:V. Fate of organically complexed Cr(III) added to soil. J Environ Quality, **12:** 169-173.

Jain, A.K., Gupta, V.K., Singh, L.P., Srivastava, P., and Raisoni, J.R., 2005. Anion Recognition Through Novel C-thiophenecalix [4] resorcinarene : PVC Based Sensor for Chromate Ions. Talanta. **65:** 716-721.

Juwarkar, A.A., and Jambhulkar, H.P., 2008. Phytoremediation of coal mine spoil dump through intergraded biotechnological approach. Bioresource Technol, **99:** 4732-4741.

Kadiiska, M.B., Xiang, Q.H., and Mason, P.R., 1994. In vivo free radical generation by Cr(VI): an electron resonance spin-trapping investigation. Chem Res Toxicol, **7:** 800-805.

Kamaludeen, S.P., Arunkumar, K.R., Avudainayagam, S., and Ramasamy, K., 2003. Bioremediation of chromium contaminated environments. Indian J Exp Biol, **41:** 972-985.

Kiilunen, M., 1994. Occupational exposure to chromium and nickel in Finland – analysis of registries of hygienic measurements and biological monitoring. Annals of Occupational Hygiene, **38(2):** 171-187.

Kobya, M., 2004. Removal of Cr(VI) from the aqueous solutions by adsorption onto hazelnut shell activated carbon: Kinetic and equilibrium studies. Bioresource Technology, **91(3):** 317-321.

Komori, K.A., Rivas, A., Toda, K., and Ohtake, H., 1990. A method for removal of toxic chromium using dialysis-sac cultures of chromate- reducing strain *Enterobacter cloacae* strain . Appl Microbiol Biotechnol, **33:** 117-119.

Kotas, J., and Stasicka, Z., 2000. Chromium occurrence in the environment and methods of its specification. Environ Pollution, **107:** 263-283.

Krishna, K.R., and Philip, L., 2005. Bioremediation of Cr(VI) in contaminated soils. Journal of Hazardous Materials, **B121:** 109-117.

Kurniawan, T.A., Chan, G.Y.S, Lo, W.H., and Babel, S, 2006. Physicochemical treatment technique for wastewater laden with heavy metals. Chem Eng J, **118:** 83-98.

Levenspiel, O., 1999. Chemical Reaction Engineering, 2nd Ed. Wiley Eastern Ltd, 41-92.

Lin, S.H., and Kiang, C.D., 2003. Chromic acid recovery from waste acid solution by an ion exchange process: equilibrium and column ion exchange modeling. Chem Eng J, **92(1-3):** 193-199.

Llovera, S., Bonet, R., Simon-Pujol, M.D., and Congregado, F., 1993. Effects of culture medium ions on chromate reduction by resting cells of Agrobacterium radiobacter. Appl Microbiol Biotechnol, **39:** 424–426.

Losi, M.E., Amrhein, C., and Frankenberger, W.T., 1994b. Environmental biochemistry of chromium. Rev Environ Contamination Toxic, **136:** 91.

Lovely, D.R., and Philip, E.J., 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its C3 cytochrome. Appl and Env Microb, **60:**726-728.

Liu, S.J., Jiang, B., Huang, G.Q., and Lia, 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, K.J., Jiang, J., Shi, X., Gabrys, H., Walczak, T., and Swartz, M., 1995. Low frequency EPR study of Cr(V) formation from Cr(VI) in living plants. Biochem Biophys Res Commun, **206:** 829-834.

Lu, Y.L., and Yang, J.L., 1995. Long –term exposure to chromium (VI) oxide leads to defect is sulfate transport system in *Chinese hamster ovary cells*. J Cell Biochem, **57:** 655-665.

Makino, T., Kamewada, K., Hatta, T., Takahashi, Y., and Sakurai, Y., 1998. Determination of optimal chromium oxidation conditions and evaluation of soil oxidative activity in soils. J. Geochem Explor, **64:** 435-441.

Mazierski, J., 1994. Effect of chromium Cr(VI) on the growth rate of denitrifying bacteria. Water Research, **28(9):**1981-1985.

McBride, M.B., 1994. Environmental chemistry of soils. New York, Oxford University Press, 406. ISBN 0-19-507011-9.

McGrath, S. P., and Smith, S., 1990. 'Chromium and Nickel', in B. J. Alloway (ed.), Heavy Metals in Soils, John Wiley & Sons, New York, 125-147.

McLean J., and Beveridge T.J., 2001. Chromate reduction by a pseudomonad isolated from a site contaminated with chromated copper arsenate**.** Applied and environmental microbiology, **67(3):** 1076-1084.

Megharaj, M., Avudainayagam, S., and Naidu, R., 2003. Toxicity of hexavalent chromium and its reduction by bacteria isolated from soil contaminated with tannery waste. Current Microbiol, **47:** 51-54.

Merian, E., 1984. Introduction on environmental chemistry and global cycles of Arsenic, Beryllium, Cadmium, Chromium, Cobalt, Nickel, Selenium, and their derivatives. Toxicological and Environmental Chemistry, **8:** 9-38.

Middleton , S.S., Latimani, R.B., Mackey, M.R., Ellisman, M.H., Tebo, B.M., and Criddle, C.S., 2003. Cometabolism of Cr(VI) by *Shawella oneidensis* MR-1 produces cell-associated reduced chromium and inhibits growth. Biotechnol Bioeng, **83:** 627-637.

Michel, C., Brugna, M., Aubert, C., Bernadac, A., and Bruschi, M., 2001. Enzymatic reduction of chromate: comparative studies using sulfate-reducing bacteria. Key role of polyheme cytochrome *c* and hydrogenases. Appl Microbiol Biotechnol, **55:** 95-100.

Mintek, 2004. Mining and Metallurgy in South Africa, a Pictorial History. Mintek,in association with Phase 4, pg. 50.

Molokwane, P.E., Meli, C.K., and Chirwa, E.M.N., 2008. Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading. Water Science and Technology, **58(2):** 399-405.

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

Molokwane, P.E., (Ph.D., 2010). Simulation of in situ bioremediation of Cr(VI) in groundwater. University of Pretoria, Pretoria, South Africa. http://www.google.co.za/search?hl=en&biw=779&bih=409&q=pulane+molokwane. Last access date: 2011-07-20.

Moncur, M.C., Ptacek, C.J., Blowes, D.W., and Jambor, J.L., 2005. Release, transport, and attenuation of metals from an old tailings impoundment. Applied Geochemistry, **20:** 639-659.

Nelson, Y.M., Lion, L.W., Schuler, M.L., and Ghiorse, W.C., 1996. Modeling oligotrophic biofilm formation and lead adsorption to biofilm components. Environ Sci and Technology, **30(6):** 2027-2035.

Nyer, E.K., 1992. Treatment methods for inorganic compounds. Groundwater technology, Van Norstrand Reinhold, New York, 218.

Ohtake, H., Fuji, E., and Toda, K., 1990. Reduction of toxic chromate in an industrial effluent by use of a chromate-reducing strain Enterobacter cloacae. Eviron Technol, **11:** 663-338.

Opperman, D.J., and van Heerden, E., 2007. Aerobic Cr(VI) reduction by Thermus scotoductus strain SA-01. J Appl Microbiol, **103:** 1907-1913.

Pacyna, J.M., and Nriagu, J.O., 1988. Atmospheric emissions of chromium from natural and anthropogenic sources. In: Nriagu, J.O. & Nieboer, E. (eds). 1988. Chromium in natural and human environments. Wiley Interscience, New York. 105-125.

Palmer, C.D., Wittbrodt, P.R., 1991. Process affecting the remediation of chromium-contaminated sites. Environ Health Perspect, **92:** 25-40.

Papp, J. F., 1999. U.S. Geological Survey. Minerals Yearbook, **17:** 1-17.8

Pattanapipitpaisal, P., Mabbett, A.N., Finalay, J.A., Beswick,A.J., Paterson-Beedle, M., and Essa, 2002. Reduction of Cr(VI) and bioaccumulation of chromium by gram positive and gram negative microorganisms not previously exposed to Cr stress. Environ Tech, **23:** 731-745.

Patterson, R.R., Fendorf, S., and Fendorf, M., 1997. Reduction of hexavalent chromium by amorphous iron sulfide. Environ Sci Technol., **31:** 2039-2044.

Pal, A., Sumuna Duutta, S., and Paul, A.K., 2005. Reduction of hexavalent chromium by cell-free extracts of *Bacillus Sphaericus* and 303 isolated from serpentine soil. Current Microbiology, **51(5):**327-330.

Park, C.H., Keyhan, M., Wielinga, B., Fendorf, S., and Matin, A., 2000. Purification to homogeneity and characterization of a novel Pseudomonas putida chromate reductase. Appl Environ Microbiol, **66:** 1788-1795.

Pettine, M., Millero, F.J., and Passino, R., 1994. Reduction of chromium (VI) with hydrogen sulfide in NaCl media. Mar Chem, **46:** 335-344.

Pettine, M., Barra, I., Campanella, L., and Millero, F.J., 1998. Effects of metals on the reduction of chromium (VI) with hydrogen sulfide. Water Research, **32:** 2807-2813.

Petrilli, F.L., and DeFlora, S., 1977. Toxicity and mutagenicity of hexavalent chromium on *Salmonella typhimurium*. Appl Environ Microbiol, **33(4):** 805-809.

Petrilli, F.L., and DeFlora, S., 1978. Metabolic deactivation of hexavalent chromium mutagenicity. Mutat Res, **54:**139-147.

Polti, M.A., Amoroso, M.J., and Abate, C.M., 2007. Chromium (VI) resistance and removal by actinomycete strains isolated from sediments. Chemisphere, **67:** 660-667.

Reichart, P., 1998. Swiss Federal Institute for Environmental Science and Technology (EAWAG) CH Dubendorf, Switzerland, ISBN: 3906484-16-5.

Ross, D.S., Sjogren, R.E., and Bartlett, R.J., 1981. Behaviour of chromium (VI) toxicity to microorganisms in soils: IV. J Environ Qual, **2:** 145-168.

Ryan, M.P., Williams, D.E., Chater, R.J., Hutton, B.M., and McPhail, D.S., 2002. Why stainless steel corrodes? Nature, **415:** 770-774.

Sani, R.K., Peyton, B.M., Smith W.A., Apel, W.A., and Peterson, J.N., 2002. Dissimilatory reduction of Cr(VI), Fe(III), and U(VI) by cellulose isolates. Appl Microbiol, **60:** 192-199.

Schmieman, E.A., Yonge, D.R., Rege, M.A., Petersen, J.N., Turick, C.E., Johnstone, D.L.,, and Apel, W.A., 1998. Comparative kinetics of bacterial reduction of chromium. J Environ Eng, **124:** 449-455.

Sedlak, D.L., and Chan, P.G., 1997. Reduction of hexavalent chromium by ferrous iron. Geochim Cosmochim Acta, **61:** 2185-2192.

Seigneur, C. H., and Constantinou, E., 1995. Chemical kinetics mechanism for atmospheric chromium. Environ Sci Technol, **29:** 222-231.

Shakoori, A.R., Makhdoom, M., and Haq, R.U., 2000. Hexavalent chromium reduction by a dichromate-resistant Gram-positive bacterium isolated from effluents of tanneries. Appl Microbiol Biotechnol, **53:** 348-351.

Sharma, S., Dastidar, M.G., and Sreekrishnan, T.R., 2002. Zinc uptake by a fungal biomass isolated from industrial wastewater. ASCE: Practise Period Toxic Hazard Radioact Waste Manage, **64:** 256-261.

Shashidhar, T., Bhallamudi, S.M., and Philip, L., 2007. Development and validation of a model of bio-barriers for remediation of Cr(VI) contaminated aquifers using laboratory column experiments. J Hazard Mater, **145:** 437-452.

Shen, H., and Wang, Y.T., 1993. Characterization of enzymatic reduction of hexavalent chromium by *Eschericia coli* ATCC 33456. Appl Environ Microbiol, **59(11):**3771.

Shen, H., and Wang, Y.T., 1994a. Biological reduction of chromium by *E. Coli*. J Environ Eng Am Soc Civil Eng, **120(3):** 560-572.

Shen H. and Wang Y.T. 1994b Biological reduction of chromium by *E. coli*. J Environ Engng, **120:** 560-572.

Shen, H., and Wang, Y.T., 1995. Modeling simultaneous hexavalent chromium reduction and phenol degradation by a defined coculture of bacteria. Biotechnol Bioeng, **48:** 606-613.

Shi, W.Y., Shao H.B., Li, H., Shao, M., and Du, S., 2009. Progress in the remediation of hazardous heavy metal-polluted soils by natural zeolite. J Hazard Mater, **170:**1-6.

Smith, W.A., Apel, W.A., Petersen, J.N., and Peyton, B.M., 2002. Effect of carbon and energy source on bacterial chromate reduction. Bioremediation Journal, **6:** 205-215.

Smith, W.L., and Gadd, G.M., 2000. Reduction and Precipitation of chromate by mixed culture sulphate reducing bacterial biofilm. J Appl Microbiol, **88(6):** 983-991.

Stasinakis, A.S., Thomaidis, N.S., Mammais, D., and Lekkas, T.D., 2004. Investigation of Cr (VI) reduction in continuous-flow activated sludge systems. Chemosphere, **57(9):**1069–1077.

Srinath, T., Verma, T., Ramteke, P.W., and Garg, S.K., 2002. Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere, **48:** 427–435.

Sullivan, R.J., 1969. Air pollution aspects of chromium and its compounds, Raleigh, North Carolina, US Department of Health, Education and Welfare (National Air Pollution Control Administration Publication No. APTD 69-34), pg 75.

Suzuki, T., Miyata, N., and Horitsu, H., 1992. NAD(P)H-dependent chromium (VI) reductase of *Pseudomonas ambigua* G-1: a Cr(V) intermediate is formed during the reduction of Cr(VI) to Cr(III). J Bacteriol, **174(16):** 5340.

Tebo, B.M., and Obraztsova, A.Y., 1998. Sulfate-reducing bacterium grows with Cr (VI), U(VI), Mn(IV) and Fe(III) as electron acceptors. FEMS Microbiol Lett, **162:** 193– 198.

Tekerlekopoulou, A.G., Papazafiris, P.G.D., and Vayenas, D.V., 2010. A full-scale trickling filter for simultaneous removal of ammonium, iron and manganese from portable water. Journal of Chemical Technology and Biotechnology, **85(7):** 1023-1026.

Turick, C.E., Appel, W.A., and Carmiol, N.S., 1996. Isolation of hexavalent chromium-reducing anaerobes from hexavalent-chromium-contaminated and non contaminated environments. Appl Microbiol Biotechnol, **44:** 683-688.

U.S. (NAS), (1974). Geochemistry and the environment. Washington DC. U.S. Government Printing Office.

Uyguner, C.S., and Bekbolet, M., 2003. Evaluation of humic acid, chromium (VI), and TiO2 ternary system in relation to adsorptive interactions. Appl Catal. B: Environ, **49:** 267-275.

Velasquez, L., and Dussan, J., 2009. Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus Sphaericus.* J Hazard Material, **167:** 713-716.

Viamajala, S., Peyton, B.M., and Petersen, J.N., 2003. Modeling chromate reduction in *Shewanella oneidensis* MR- 1: Development of a novel dual-enzyme kinetic model. Biotech Bioeng**, 83:** 790-797.

Viamajala, S., Peyton, B.M., Sani, R.K., Apel, W.A., and Petersen, J.N., 2004. Toxic effects of chromium (VI) on anaerobic and aerobic growth of Shewanella oneidensis MR-1, Biotechnology Progress, **20(1):** 87-95.

Vadali, M., 2001. Bioremediation: An overview. Pure Appl Chem, **73:** 1163-1172.

Venitt, S. and Levy, L. S., 1974. Mutagenicity of chromate in bacteria and its relevance to chromate carcinogenesis. Nature, **250(4):** 493.

Viti, C., and Giovannetti, L., 2001. The impact of chromium contamination on soil heterotrophic and photosynthetic microorganisms. Ann Microbiol, **51:** 201-213.

Viti, C., and Giovannetti, L., 2005. Characterization of cultivable heterotrophic bacterial communities in Cr-polluted and unpolluted soils using Biology and ARDRA approaches. Applied Soil Ecology, **28:** 101-112.

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

Wang, Y.T., and Shen, H., 1995. Bacterial reduction of hexavalent chromium. J Ind Microbiol, **14:** 159-163.

Wang, P-C., Mori, T., Komori, K., Sasatsu, M., Toda, K., and Ohtake, H., 1989. Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. Appl Environ Microbiol, **55(7):**1665.

Wang, Y-T., Chirwa, E.N., and Shen, H., 2000. Cr(VI) reduction in a continuous-flow coculture reactor. Journal of Environmental Engineering, **126(4):** 300-306.

Wanner, O., 1995. Modeling of biofilms. Abstracts 9th International Congress on Marine Corrosion and Fouling, Portsmouth, UK, 15-21 July.

Watts, R.J., 1998. *Hazardous wastes: sources, pathways, receptors*. John Wiley & Sons, Inc.,New York,123.

Wu, J., Zhang, H., Yao, Q., and Shao, L.M., 2010. Cr(VI) removal from aqueous solution by dried activated sludge biomass. J Hazard Material, **176:** 697-703.

Yang, Q., Li, C., Li, H., Li , Y., and Yu, N., 2009. Degradation of synthetic reactive azo dyes and treatment of textile wastewater by a fungi consortium reactor, Biochemical Engineering Journal, **43:** 225-230.

Yassi, A., and Nieboer, E., 1988. Carcinogenicity of chromium compounds. In J. O. Nriagu and E. Nieboer (eds.), Chromium in natural and human environments. John Wiley and Sons, New York, 443-496.

Ye, J., Yin, H., Mai, B., Peng, H., Quin, H., He, B., and Zhang, N., 2010. Bisorption of chromium from aqueous solution and electroplating wastewater using mixture of Candida lipolytica and dewatered sewage sludge. Bio- resource Technology, **101:** 3893-3902.

Zakaria, Z.A., Zakaria, Z., Surif, S., and Ahmad, W.A., 2007. Biological detoxification of Cr (VI) using wood-husk immobilized Acinetobacter haemolyticus. Journal of Hazardous Materials, **148 (1–2):**164-171.

Zahoor, A., and Rehman, A., 2009. Isolation of Cr(VI) reduction bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater. J Environ Sci, **21:** 814-820.

Zayed, A.M., and Terry, N., 2003. Chromium in the environment: Factors affecting biological remediation. Plant soil, **249:** 139-156.

Zhighou, H., Fengling, G., Tao, S.,Yuehua, H., and Chao, H., 2009. Isolation and characterization of Cr(VI)-reducing Ochrobactrum sp. Strain CSCr-3 from chromium landfill. Journal of Hazardous Materials, **163:** 869-873.

Zhitkovich, A., Voikun, V., and 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. Biochem, **35:** 7275-7282.

Zhu, W., Chai, L., Ma, Z., Wang, Y., Xiao, H., and Zhao, K., 2008. Anaerobic reduction of hexavalent chromium by bacterial cells of Achromobacter sp. Strain Ch1. Microbilogical Research, **63:** 616-623.

Zibilske, L.M., and Wagner, G.H., 1982. Bacterial growth and fungal genera distribution in soil amended with sewage sludge containing cadmium, chromium and copper. Soil Sci, **134(6):** 364.

Zouboulis, A.I., Loukidou, M.X., and Matis, K.A., 2004. Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. Process Biochem, **39:** 909-916.