BIOREDUCTION OF HEXAVALENT CHROMIUM:

FLOW-THROUGH COLUMN EXPERIMENTS AND

REACTIVE TRANSPORT MODELING

By

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Abstract

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Bioreduction of Cr(VI) is considered a novel remediation alternative for Cr(VI) contaminated soil and groundwater. Many bacterial species can use Cr(VI) as an electron acceptor and reduce soluble and toxic Cr(VI) compounds to less soluble and less toxic Cr(III) compounds. Laboratory scale column experiments were performed to investigate Cr(VI) reduction by *Shewanella oneidensis* MR-1 in a continuous flow system. The column was packed with silica sand inoculated with *S. oneidensis* MR-1 as slurry in a laminar flow hood maintaining aseptic conditions. The feed solution was composed of simulated groundwater media (SGM) - lactate as the electron donor, and fumarate as the limiting electron acceptor. Following an initial growth period, Cr(VI) in the form of chromate was added to the feed. The feed and the effluent from the column were monitored for Cr(VI), total Cr, lactate, acetate, fumarate and succinate. Results of

several experimental runs indicated that Cr(VI) in the effluent was below detection limit for several pore volumes, followed by Cr(VI) breakthrough for Cr(VI) feed concentrations equal to or greater than 1.94 mg/L. It is hypothesized that Cr(VI) breakthrough occurred due to Cr(VI) toxicity that inhibited bacterial growth. The Cr(VI) breakthrough corresponded with the breakthrough of fumarate, indicating microbial growth inhibition. A residual Cr(VI) reduction, ranging from 20% - 45% of the feed concentration, occurred after breakthrough, while no Cr(VI) or fumarate breakthrough was observed for feed Cr(VI) concentrations equal to or smaller than 1.32 mg/L. Therefore, long term biological reduction of Cr(VI) was dependent on the feed Cr(VI) concentration. Further, some soluble forms of Cr(III) complexes that may have been microbially produced were found in the column effluent. The column data, together with the batch kinetic information, was used to calibrate a mathematical model that describes reaction and transport of Cr(VI) in a column environment. The model was developed using RT3D, Reactive Transport in 3 Dimensions, with Cr(VI) biotransformation kinetics based on a recently developed dual enzyme Cr(VI) reduction kinetic model. The calibrated model was able to predict fate and transport of Cr(VI) in our laboratory scale sand columns. The model may be very useful for engineering design of *in situ* bioremediation.

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Attribution

The author of this dissertation is the first author of the two manuscripts contained herein. He was responsible for all laboratory work including all data acquisition and analysis, computer simulation, and interpretation of results. Contributions of coauthors are recognized in the Acknowledgments section.

Dedication

This dissertation is dedicated to my mother and father

CHAPTER ONE

INTRODUCTION

Overview

This dissertation describes research performed to study biological Cr(VI) reduction in flow-through systems. The organization of the dissertation is based on the Washington State University manuscript format. The introductory chapter is followed by two manuscripts (chapter Two and chapter Three) that have been submitted for publication in peer-reviewed journals, future work (chapter Four), and an appendix of supporting data and computer code. Chapter Two describes the experimental investigations of Cr(VI) bioreduction in a continuous flow silica sand column. Chapter Three contains the development, calibration, and validation of a reactive transport model that describes fate and transport of chromium in a saturated subsurface environment.

Background

Chromium (Cr) is found in air, soil, and water in small quantities. As a transition metal, Cr can occur under several oxidation states from 0 to +6. In aqueous environments, however, Cr has two stable forms: hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)]. Cr(VI) is usually associated with anthropogenic contamination, primarily from industrial and commercial processes, including

electroplating, leather tanning, ore and petroleum refining, textile manufacturing, wood

preservation, inorganic chemicals and pulp production and many metal finishing

industries (Langard, 1980; James, 1996). Of these two valence states, Cr(VI) is toxic and

carcinogenic (Roe and Carter, 1969; Enterline, 1974; Mertz, 1974). The Maximum

Contaminant Level (MCL) for chromium in drinking water has been established by the

U.S. Environmental Protection Agency at 0.1 mg/L total chromium (U.S. EPA, 1995).

The oxyanions of Cr(VI) (chromate, CrO_4^{2-}, and dichromate, $Cr_2O_7^{2-}$) are soluble and

mobile in groundwater (Dragun, 1988). Cr(VI) contamination is a major environmental

problem in many U.S. Department of Energy (DOE) sites, and exists in 13 of the 18

DOE installations studied by Riley et al. (1992). They reported that Cr(VI) constitutes

more than 90% of the total chromium present at these facilities. Cr(III), the other stable

form of Cr, is naturally occurring. It is less toxic and less mobile (Fendorf and Sparks,

1994). It adsorbs on most soils and has a relatively high soil partition coefficient

(Barnhart, 1997). Consequently, research has been focused on the reduction of Cr(VI) to

Cr(III) for remediation of Cr(VI)-contaminated soil and groundwater.

The existing treatment methods for Cr(VI) contaminated soil or groundwater

involve physical and chemical methods, e.g., excavation and off-site disposal or

pumping and subsequent chemical or electrochemical reduction and precipitation or ion

exchange (Patterson, 1985; Nyer, 1992). However, these methods are relatively

expensive and sometimes generate secondary wastes that require subsequent disposal.

Alternatively, *in-situ* bioremediation technology can be applied to circumvent the

limitations of physical and/or chemical methods. Direct metabolic reduction of Cr(VI) by bacteria has been documented by several researchers (Romanenko and Koren'kov, 1977; Horitsu, 1987; Bopp and Erlich, 1988; Wang et al., 1989; Fujie et al., 1994; Shen and Wang, 1994; Shen and Wang, 1995; Wang and Shen, 1995; Chen and Hao, 1996; Fujie et al., 1996; Turick et al., 1996; Garbisu et al., 1998; Schmieman et al., 1998; Tebo and Obraztsova, 1998; Guha et al, 2001). Bioreduction of Cr(VI) appears to be ubiquitous since Cr(VI) reducing consortia were isolated from Cr(VI) contaminated sites as well as uncontaminated sites (Turick et al., 1996; Chen and Hao, 1998; Schmieman et al., 1998; Sani et al., 2002; Camargo et al., 2003). Following microbial reduction, it is commonly assumed that Cr(VI) species are transformed to insoluble and immobile chromium hydroxide. Hence, this technology has potential to be applied at field sites to immobilize Cr in the subsurface.

Scope of Sand Column Experiment

Most of the studies on microbial Cr(VI) reduction have been conducted in batch experiments (Chen and Hao, 1998) and these studies often cannot be directly applied to *in situ* biotransformation of Cr(VI). Obviously, Cr(VI) transport through saturated porous media is a highly dynamic process that cannot be fully defined through batch reactor experiments. Cr(VI) reduction kinetics obtained from batch studies must be tested with continuous flow soil column experiments to elucidate the efficacy of this technology and to define field application design and operational parameters. Currently, there is a lack of understanding with regard to the interaction of microbial Cr(VI)

reduction processes, Cr(VI) toxicity and subsurface Cr transport and speciation. Sand column experimental data can be very helpful to address these issues.

Soil column studies focused on biotransformation of chromate are rarely found in literature. Oliver et al. (2003) performed column experiments to assess Cr(VI) reduction under unsaturated flow conditions using a native microbial community. The findings of this study can not be applied to many Cr(VI) contaminated sites, where Cr(VI) is moving along the saturated groundwater. Tokunaga et al. (2003) studied acceleration of microbial Cr(VI) reduction in soil columns where transport in diffusion-limited. Another study by Guha et al. (2003) focused on Cr(VI) reduction in presence of pyrolusite-coated sand and uncoated quartz sand by *Shewanella alga* simidu ATCC 55627 in laboratory column. The findings from this study emphasized the need for a more detailed understanding of integrated hydrobiogeochemical processes. As an initial step in developing a more complete understanding of the complex processes associated with subsurface Cr(VI) reduction, continuous flow experiments using pure quartz sand were performed.

Cr(VI) Reduction by *Shewanella oneidensis* MR-1

Shewanella oneidensis MR-1 (MR-1), formerly known as *Shewanella putrefaciens* MR-1, is a facutively aerobic Gram-negative bacterium that was originally isolated from anaerobic sediments in Oneida Lake, NY (Myers and Nealson, 1988). As in other bacteria during aerobic respiration, *S. oneidensis* MR-1 utilizes oxygen as the

terminal electron acceptor, but under anaerobic conditions MR-1 can use a variety of alternative electron acceptors, including oxidized metals such as Mn(IV), Fe(III), Co(III), U(VI), Tc(VII), and Cr(VI). Previous research in our laboratory showed that MR-1 reduced Cr(VI) at a relatively high rate in batch reactors under a variety of growth conditions (Viamajala et al., 2002). Recently, the genome of MR-1 has been sequenced and it has been proposed for bioremediation of polluted sites under anoxic conditions (Heidelberg et al., 2002). Considering the bioremediation potential of MR-1, laboratory scale sand column experiments were performed to investigate the bioreduction of Cr(VI) in a continuous flow environment. The details of the experimental system and results can be found in Chapter Two of this dissertation.

Necessity of Mathematical Modeling

A well developed mathematical model can assist in describing the interrelated processes involved in microbial Cr(VI) reduction. It has been generally accepted that microbial growth and transport with biodegradation can be described by the advection-dispersion equation with modifications to account for growth, decay, attachment and detachment (Corapcioglu and Haridas, 1984, 1985; Taylor and jaffe, 1990; Harvey and Garabedian, 1991; Hornberger et al., 1992; Tan et al. 1992, 1994; Clement et al., 1997). Microbial transport, controlled by attachment and detachment, is very complex and arguably the least understood process (Tan et al. 1994). A properly developed model needs to be both calibrated and validated. The model should then be able to predict field scale systems and can aid in the design and operation of site cleanup.

There are a number of mass transport models, such as, MT3D (Zheng, 1990), RT3D (Clement, 1997), and CTRAN/W (Geo-Slope International, 2004) that can simulate subsurface fate and transport of contaminants. Among these models, RT3D is well suited for simulating contaminant fate and transport that includes biodegradation and the model can accommodate transport involving multiple species and reaction kinetics. A distinct advantage of RT3D is that user-defined reaction kinetics can be incorporated into the model. As with any model, RT3D must be calibrated and validated prior to its application in the field. Following bench scale calibration and validation, a meso-scale validation should be performed prior to full scale application. Chapter Three of this dissertation presents the formulation, calibration, and validation of a mathematical model based on RT3D.

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CHAPTER TWO

BIOREDUCTION OF HEXAVALENT CHROMIUM IN FLOW-THROUGH SAND COLUMNS

This chapter is presented as a paper that was submitted to Journal of Environmental Engineering on July 14, 2004.

BIOREDUCTION OF HEXAVALENT CHROMIUM IN FLOW-THROUGH SAND COLUMNS

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ABSTRACT

Chromium(VI) (Cr(VI)) contamination of soil and groundwater is a major environmental concern for some industrial sites and most of the US DOE's sites. Cr(VI) is toxic and fairly mobile in groundwater. Bioreduction of Cr(VI) to less toxic and less mobile Cr(III) is considered to be a feasible option. Among the Cr(VI) reducing bacteria *Shewanella oneidensis* MR-1 (MR-1) is the most widely studied and relatively effective under anaerobic conditions. Data on bioreduction of Cr(VI) by MR-1 accounting for dynamics of groundwater flow is not available. The objective of this research project was to study the bioreduction of Cr(VI) by MR-1 in a continuous flow silica sand column system. MR-1 was found to be very effective in reducing Cr(VI) to Cr(III) for

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concentrations ranging from 0.006 mM (0.31 mg/L) to 0.055 mM (2.85 mg/L). The mass of Cr(VI) reduced prior to its breakthrough was found to be inversely proportional to its concentration in the feed due to increased inhibition of microbial activity. Following breakthrough a residual reduction of 20 – 45% of Cr(VI) was observed.

Key words: Bioreduction, Cr(VI), sand column, Shewanella oneidensis MR-1.

INTRODUCTION

Chromium (Cr) compounds are widely used in many industrial and commercial processes, including electroplating, leather tanning, ore and petroleum refining, textile manufacturing, wood preservation, inorganic chemicals and pulp production and many other metal finishing industries (NRC 1974; Wang and Xiao 1995). In addition, Cr compounds are added to cooling water to inhibit corrosion in power plants (Patterson 1985). The widespread use of this heavy metal has resulted in chromium contaminated soil and groundwater in many areas including United States Department of Energy (DOE) facilities (Riley et al. 1992). As a redox-sensitive transition metal, Cr can occur in several oxidation states from -2 to +6 (Fendorf 1995). In natural environments, however, Cr has two stable forms: hexavalent chromium, Cr(VI) - present almost exclusively as a result of anthropogenic activities, and trivalent chromium, Cr(III) (NRC 1974; Fendorf 1995). Cr(VI) constitutes more than 90% of the total chromium present at the Cr-contaminated DOE sites (Riley et al. 1992). It is very toxic and is known to have carcinogenic and mutagenic effects (Enterline 1974; ATSDR 2000). It was ranked 17th

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among the top 20 hazardous substances in the USA in 2003 (ATSDR 2003). Cr(VI) species, predominantly chromate and dichromate, are soluble in aqueous systems and are readily transported in groundwater (Dragun 1988). Conversely, most of the Cr(III) species are relatively insoluble, more stable, and much less toxic (Ross et al. 1981). In fact, it is needed in trace amounts for humans, contributing to the glucose tolerance factor necessary for insulin-regulated metabolism (NRC 1974; ATSDR 2000). Considering the toxicity and mobility of Cr(VI) in the environment, its removal or transformation to less mobile and less toxic Cr(III) is important.

Some of the existing methods for the treatment of Cr(VI) contaminated soil and groundwater include excavation and off-site disposal or pumping and subsequent chemical or electrochemical reduction and precipitation or ion exchange (Patterson 1985). These physico-chemical methods are relatively expensive because they are either energy intensive or require large quantities of chemical reagents and sometimes generate secondary wastes that require subsequent disposal (Chen and Hao 1997). Alternatively, on-site remediation of Cr(VI) contaminated soils may include heap leaching followed by leachate collection and treatment (Hanson 1993); reduction and sorption onto soil and microorganisms (Cifuentes et al. 1996); and permeable Fe^0 reactive walls (Blowes et al. 1997). Due to operational difficulties, low efficiency, high cost, and generation of secondary wastes, feasibility of these methods is yet to be established.

Recently, bioremediation of Cr(VI) has gained considerable attention (Romanenko and Koren'kov 1977; Horitsu et al. 1987; Bopp and Erlich 1988; Wang et al. 1989; Yamamoto et al. 1993; Garbisu et al. 1998). Some microbial species can utilize Cr(VI) as a terminal electron acceptor in their respiratory process and transform Cr(VI) to less toxic Cr(III) compounds (Lovely and Phillips 1994; Shen et al. 1996; Wang and Shen 1995). In addition, Cr(III) compounds are reported as having significantly greater soil partition coefficients and tend to precipitate from solution (Barnhart 1997). *In situ* biotransformation technology is, therefore, being considered as a novel remediation alternative to the current practice of employing physico-chemical treatments because it does not produce secondary wastes and may be less expensive.

Cr(VI) reducing bacteria appear to be ubiquitous since they have been isolated from Cr(VI) contaminated sites as well as uncontaminated sites (Myers and Nealson 1988; Turick et al. 1996; Schmieman et al. 1998; Sani et al. 2002; Cheung and Gu 2003). However, most of the studies on microbial reduction of Cr(VI) have been conducted by employing batch reactors (Chen and Hao 1998). Batch reactors are very useful tools to evaluate the feasibility of bioreduction of Cr(VI). However, results obtained from batch studies may not reflect the effect of subsurface flow dynamics. Obviously, Cr(VI) bioreduction and transport through the subsurface porous media is a highly dynamic process which cannot be fully defined through batch reactor experiments that employ a closed system with no solid phase material. Therefore, effect of flow dynamics and bacterial interaction with the solid matrix of the subsurface on Cr(VI)

reduction must be studied by performing continuous flow soil column experiments to elucidate the efficacy of this technology and to develop design and operational parameters for field application. Soil column studies focused on bioreduction of chromate have not been reported in literature except for a study that focused on Cr(VI) reduction by *Shewanella alga* Simidu (BrY-MT) in the presence of pyrolusite (β-MnO_2) coated sand (Guha et al. 2003). Therefore, there is a need for continuous flow soil column experiments to enhance our current understanding of *in situ* biotransformation of Cr(VI) to Cr(III) in the subsurface.

Sand column experiments were carried out in our laboratory to observe chromate reduction under continuous flow with *Shewanella oneidensis* MR-1 (MR-1). MR-1 is a Gram-negative, nonfermentative, facultatively anaerobic bacterium originally isolated from anaerobic sediments in Oneida Lake, New York (Myers and Nealson 1988). It can reduce a number of metals including manganese(III/IV), iron(III), uranium(VI), technetium(VII), cobalt(III) and chromium(VI) and, therefore, is commonly used as a model microorganism to gain a fundamental understanding of anaerobic microbial metal reduction processes (Myers and Nealson 1988; Myers and Nealson 1990; Myers et al. 2000; Liu et al. 2002). Previous research in our laboratory has shown that MR-1 is able to reduce Cr(VI) at a rapid rate in a batch reactor under fumarate reducing condition whereas Cr(VI) reduction was inhibited under denitrifying condition (Viamajala et al. 2002). Therefore, the experiments were conducted under fumarate reducing condition

for a number of feed concentrations to evaluate the effect of feed concentrations on bioreduction of Cr(VI) by MR-1.

In summary, the objective of this research work was to study the bioreduction of Cr(VI) in continuous flow environment under fumarate reducing condition, in general, and to study short and long term reduction of Cr(VI), fate and distribution of Cr, and the biomass profile after the bioreduction, in particular.

MATERIALS AND METHODS

Continuous Flow Sand Column System

A schematic of the sand column system is shown in Figure 1. The columns were constructed using 316 stainless steel High Performance Liquid Chromatography (HPLC) columns (Alltech Associates Inc., Deerfield, IL) of 2.1 cm internal diameter and 15 cm length. Columns were equipped with a flow distributor and a stainless steel frit at the entrance and exit to minimize channeling. Flow to the columns was delivered through a multi-channel syringe pump (Cole-Parmer Instrument Co., Vernon Hills, IL). The 60 mL syringe contained sterile simulated groundwater medium (SGM) (Petersen et al. 1994) amended with lactate as the electron donor and fumarate as the terminal electron acceptor for bacterial growth. A sterile 0.2 μm filter (Pall Life Sciences, Ann Arbor, MI) at the end of the syringe prevented bacterial movement from the reactor to the syringe feed solution. Stainless steel tubing of 1.02 mm inside diameter was used to minimize surface area and volume for biomass growth upstream of the column reactor.

The column was packed with uniform quartz sand with an average particle size of 840 μm. Details of the column and packing material properties are summarized in Table 1.

Column Startup

The quartz sand was washed with 5% HNO_3 and combusted at 550^oC for 30 min to sterilize and remove any residual metal and organic matter. The empty column, tubing, and accessories were autoclaved at 121^oC for 15 min. For column inoculation, 1.8 mL pure frozen culture of MR-1 stored inside a cryovial in 20% glycerol at -85^oC, was thawed and grown aerobically in a 165 mL serum bottle containing 50 mL of the growth medium. The growth medium was prepared with SGM amended with 15 mM (1335 mg/L) lactate as the electron donor and 12 mM (1368 mg/L) fumarate as the electron acceptor. It also contained the following micro-nutrients: 100 mg/L casamino acid, 20 mg/L of L-serine, L-arginine, and L-glutamic acid to stimulate growth (Myers and Nealson 1990). After 24 hours of incubation at 30^oC, 1 mL of the grown culture was transferred to 50 mL fresh medium to facilitate the removal of residual glycerol. The strain was grown for another 24 hours that marked the end of log growth phase. Approximately 100 g of pretreated sterile sand was then inoculated with 50 mL of MR-1 culture and mixed thoroughly in a 250 mL sterile Erlenmeyer flask. The inoculated sand was aseptically poured into the sterile column as a slurry in a laminar flow hood (Edge Gard Hood, Baker Co., Sanford, MA). The column was gently vortexed at several stages to ensure uniform packing. The inoculated column was then capped and connected to the sterile tubing and syringes of the flow through system. A syringe pump

was then attached to the assembly, allowing flow to be introduced into the column. At

the beginning of each experiment, the column was flushed with two pore volumes of

SGM containing no lactate or fumarate to remove unattached biomass from inter-particle

pore spaces. The SGM amended with the nutrients necessary to support bacterial growth

was then introduced into the column. The time when this flow was initiated was

considered "zero" time for all experiments. The initial feed solution contained SGM

amended with 3 mM (267 mg/L) lactate as the electron donor, and 2 mM (228 mg/L)

fumarate as the electron acceptor as the limiting nutrient. The initial feed did not contain

any Cr(VI) since it was observed in batch tests that MR-1 is highly susceptible to growth

inhibition by Cr(VI) toxicity, even at low concentrations (0.015 mM Cr(VI)) (Viamajala

et al. 2004). After a growth period of 8 days, Cr(VI) was added to the feed as chromate

at a predetermined concentration. Six experiments were performed at different Cr(VI)

concentrations varying from 0.006 mM (0.31 mg/L) to 0.055 mM (2.84 mg/L).

Additional details of these experiments can be found in Table 2. A sterile column

without MR-1 served as an un-inoculated control.

Sampling

Column effluent samples were collected in vials maintained in an ice bath to

minimize growth and degradation during sampling. The samples were filtered by

employing 0.2 μm syringe filters (Millipore, Bedford, MA) to remove suspended cells

and filterable precipitates. The column influent and the filtered effluent samples were

refrigerated at 4°C for subsequent analysis for Cr(VI), *total soluble Cr* assumed to be

soluble Cr(III) plus Cr(VI), lactate, and fumarate, and their by-products acetate and succinate. A small portion of the unfiltered effluent sample was used for quantification of colony forming units (CFU's) by spread plate technique (Gerhardt 1981) and measurement of unfiltered total Cr. After each experiment, the sand column was quickly frozen and later extruded in 1 cm or 2 cm segments. The sand samples were then analyzed for attached phase protein concentration and total Cr.

Tracer Test

A chloride tracer experiment was conducted after 8 days of microbial growth to measure longitudinal dispersion and column residence time. A separate tracer test with Cr(VI) and fumarate as the tracers was performed in an un-inoculated packed column to assess their sorption to the packing material.

ANALYTICAL METHODS

Hexavalent Chromium

Cr(VI) concentrations were determined by the diphenylcarbazide method using Hach Chroma Ver 3 reagent (Hach Company, Loveland, CO) adapted for use in a Costar 48-well tissue culture plate (Corning Inc., NY) (Schmieman et al. 1998). A pink color was developed when Cr(VI) was present, and the absorbance was measured on a 48-well bioassay reader (HTS7000 Bio Assay Reader, Perkin Elmer, Norwalk, CT) at a wavelength of 540 nm. The method provided a detection limit of 0.001 mM.

Total Cr and Cr(III)

Total Cr concentration was determined by Inductively Coupled Plasma-Mass Spectrometer, ICP-MS, model 4500 (Agilent Technologies, Palo Alto, CA). All samples and Cr standards were treated with 3% HNO_3 and then filtered with 0.2 μm filter before analysis. The core sand samples from the column were digested with 10% HNO_3 and similarly filtered. Since Cr(VI) and Cr(III) are the predominant forms of Cr (Fendorf 1995), Cr(III) was determined by subtracting Cr(VI) from total Cr. The detection limit for total Cr was 0.02 μM.

Anions

All anion (chloride, lactate, acetate, fumarate and succinate) concentrations were measured by ion chromatography, IC, (Model DX 500, Dionex Corporation, Sunnyvale, CA). The IC had a 4-mm AS11 HC column, a 4-mm AG11 HC guard column, a CD 20 conductivity detector, and an AS3500 autosampler. Peaknet software 5.1 was used to process the chromatograms. The lower detection limit for lactate and acetate was 0.1 mM and 0.05 mM for chloride, fumarate and succinate.

Viable Plate Counts

The column effluent had aqueous phase cellular protein concentrations that were below the detection limit of the Bradford method (Bradford 1976). A spread plate

technique employing Tryptic Soy Agar (TSA) was used to quantify viable cells as CFU/mL (Gerhardt 1981). This procedure also served as a visual check for contamination in the column. Colonies were counted after 48 hours of $30^{\circ}C$ incubation. A relationship between CFU and protein concentration in mg MR-1 per L was developed from batch growth test so that counted CFU's could provide an estimate of effluent cell protein.

Cellular MR-1 Protein concentration in Sand

Sand protein concentration was determined by a modification of the Bradford method (Bradford 1976) using Coomassie® Plus Protein Assay Reagent (Pierce Chemical Company, Rockford, IL). After each experimental run, the sand column was stored at $-20^{\circ}C$ until analysis. The column was partly thawed at room temperature and slowly extruded with a manual extruder. The first two samples collected were 1 cm in length and the others were extruded in 2 cm segments. Each segment was mixed thoroughly and three replicates were prepared to find an average sand protein concentration. Another sample was prepared for the acid digestion procedure to measure total Cr. Each replicate was mixed with 1 mL nanopure water and 1 mL 1N NaOH. The samples were vigorously vortexed for 30 seconds and digested at $99^{\circ}C$ for 15 minutes. Samples were allowed to cool for 15 minutes before addition of 0.2 mL 6N HCL, and were softly vortexed for 5 seconds. A 0.5 mL sub-sample was added to 0.5 mL Coomassie blue reagent at room temperature in the wells of a previously mentioned Costar 48-well tissue culture plate. A blue color was developed when protein was

present, and absorbance was read in the Bio Assay Reader at 595 nm. A calibration curve was prepared using Bovine Serum Albumin (BSA) standard. When samples were out of calibrated range, they were diluted. Following the protein assay, sand samples were placed in an oven at $105^{\circ}C$ for 24 hours to remove water. The weight of dried sand was used to calculate attached protein concentration.

RESULTS AND DISCUSSION

Abiotic Control Column Test

Results of the abiotic control tests and non-reactive chloride (Cl^{-}) tracer tests are presented in Figure 2. An examination of the figure reveals that chloride breakthrough was very sharp and occurred at one pore volume indicating that there was little longitudinal dispersion. Similar observations are evident from the Cr(VI) and fumarate breakthrough profiles. Therefore, it can be concluded that chromate and fumarate do not partition onto the sand and consequently, there is no retardation. At a flow rate of 1 mL/hr, the measured residence time was 22.5 hours. Therefore, one pore volume represents 22.5 mL and is referenced as such in this paper. This residence time corresponds to a porosity of 0.433 and the porosity of the packed column when estimated experimentally was 0.43.

Short Term Cr(VI) Reduction and Breakthrough

Short term Cr(VI) reduction is defined as the complete Cr(VI) reduction that occurs prior to its breakthrough at feed concentration greater than 0.025 mM (1.32 mg/L). Figure 3 presents results of an experiment with an average influent Cr(VI) concentration of 0.037 mM (1.94 mg/L). The non-inhibited growth, with no Cr(VI) in the feed, occurred for a period of 9 residence times or 9 pore volumes. Cr(VI) was then introduced in the feed as indicated by an arrow in the figure. Cr(VI) was completely reduced for 13 pore volumes, after which, Cr(VI) breakthrough was observed as shown in Figure 3a. The Cr(VI) breakthrough corresponded with changes in the nutrient utilization profiles as shown in Figure 3b. It can be seen that complete reduction of fumarate to succinate occurred during the uninhibited growth stage and prior to Cr(VI) breakthrough. Furthermore, fumarate breakthrough and a sharp decrease in succinate concentration were observed to coincide with Cr(VI) breakthrough. The effluent lactate concentration, which served as an electron donor and was supplied in excess of fumarate, rose to a point nearly equal to the influent lactate concentration following Cr(VI) breakthrough. At the same time, production of acetate - the oxidized product of lactate, decreased significantly indicating that MR-1 growth was inhibited by Cr(VI). The Cr(VI) breakthrough also corresponded to sharply increased detachment of MR-1 cells, temporarily resulting in a higher number of cells in the effluent as shown in Figure 3c. The increased detachment of cells may be explained by the apparent toxicity of Cr(VI).

Figure 4 presents results for an average influent Cr(VI) concentration of 0.055 mM (2.85 mg/L). An earlier breakthrough of Cr(VI) at 5 pore volumes was observed in this case. The earlier breakthrough was due to the increased inhibition at higher Cr(VI) concentration. Effluent concentration profiles for lactate, fumarate, and aqueous phase MR-1 cells were similar to trends observed for that of 0.037 mM Cr(VI). Fumarate breakthrough occurred at the same time as that of Cr(VI). Further, significantly high cell concentration was observed at the time of breakthrough. Similar results, not presented here, were also obtained for 0.049 mM (2.54 mg/L) influent Cr(VI) concentration.

The observed Cr(VI) breakthrough profiles can be explained by kinetic experiments reported in literature (Middleton et al. 2003; Viamajala et al. 2004). Batch kinetic experiments revealed that the growth of MR-1 was completely inhibited by the presence of Cr(VI). However, Cr(VI) reduction was still observed in spite of complete inhibition (Viamajala et al. 2004). Middleton et el. 2003 reported a finite reduction capacity per MR-1 cell. The finite reduction capacity was termed as the transformation capacity (TC) and was defined as the mass of Cr(VI) reduced per unit biomass present after 48 h. They reported that the rate of reduction decreased gradually for resting cells of MR-1 receiving sequential spikes of Cr(VI) ranging from 0.05 – 0.075 mM (2.6 – 3.9 mg/L). Sequential spikes of lower levels of Cr(VI), on the other hand, allowed for a larger TC over a longer duration than single spikes of higher Cr(VI) concentrations. In our continuous flow column experiments, Cr(VI) was fed continuously after an uninhibited growth period. We hypothesize that MR-1 cells were able to reduce Cr(VI)

even though their growth was inhibited by Cr(VI), and when their TC was reached, breakthrough occurred. Cr(VI) breakthrough is the result of growth inhibition due to its toxicity, which varies with its concentration in the feed. This is also supported by the data contained in Figure 5 which presents the amount of Cr(VI) reduced prior to breakthrough as a function of average feed concentration during that period. A linear correlation ($R^2 = 0.9507$) indicates that the amount of Cr(VI) reduced prior to its breakthrough, which is comparable to TC defined earlier, is inversely proportional to the feed Cr(VI) concentration. However, a more quantitative explanation of the breakthrough profile can be obtained by employing the dual enzyme kinetic model proposed by Viamajala et al. (2003) as discussed in the following section.

Long Term Cr(VI) Reduction

Long term Cr(VI) reduction refers to residual Cr(VI) reduction after its breakthrough and complete Cr(VI) reduction in the test which had no breakthrough. Cr(VI) breakthrough was not observed for experiments with less than, or, equal to 0.025 mM of Cr(VI) in the influent while breakthrough was observed for concentrations higher than 0.025 mM. Figure 6 presents breakthrough profiles for influent Cr(VI) concentration of 0.055, 0.049, and 0.037 mM. An examination of the figure reveals that the experimental run with the highest Cr(VI) concentration of 0.055 mM had the earliest breakthrough at 5 pore volumes after its introduction into the feed. Breakthrough for 0.049 mM and 0.037 mM were observed at 7 and 14 pore volumes, respectively. In the other three experiments with 0.006, 0.011, and 0.025 mM of Cr(VI) in the influent,

breakthrough was not observed at the termination of the experiments at 30, 60 and 38

pore volumes, respectively, as listed in Table 2. It is to be noted that Cr(VI)

breakthrough concentration did not rise up to the feed concentration and residual Cr(VI)

reduction occurred following its breakthrough. An experiment that was run with 0.037

mM influent Cr(VI) concentration showed a steady 45% residual Cr(VI) reduction

following its breakthrough as can be seen in Figure 3a. Approximately 20% residual

Cr(VI) reduction occurred for an average feed Cr(VI) concentration of 0.055 mM as

shown in Figure 4a.

The results of short and long term Cr(VI) reduction can be explained more

quantitatively, as mentioned earlier, using a dual enzyme kinetic model of Cr(VI)

reduction by MR-1 proposed by Viamajala et al. (2003). The model describes the

kinetics of Cr(VI) reduction by two parallel mechanisms: (1) a rapid Cr(VI) reduction by

an enzyme that is deactivated quickly, and (2) a slower reduction by an enzyme that has

a constant activity for longer duration. Prior to breakthrough, the quick acting enzyme is

primarily responsible for Cr(VI) reduction. At breakthrough, the quick acting enzyme

has been deactivated and Cr(VI) reduction is carried out at a significantly slower rate by

the slow acting but stable enzyme as evidenced by the steady effluent Cr(VI)

concentration that is 20% to 45% lower than the feed concentration. For the experiments

that were conducted at less than, or, equal to 0.025 mM influent Cr(VI) concentrations,

the slower mechanism persisted and no Cr(VI) breakthrough was observed for the time

period these experiments were monitored. MR-1 was able to reduce Cr(VI) completely

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for more than 50 pore volumes in the experiment with 0.011 mM (0.59 mg/L) of Cr(VI) in the influent. Therefore, it can be concluded that complete biological reduction of Cr(VI) depends upon its feed concentration.

Fate and Distribution of Cr

Fate and transport of Cr(VI) within the column was also investigated. *Total soluble Cr* was measured in the column effluent. *Total soluble Cr* is defined here as the non-filterable Cr compounds/particles that passed through a 0.2 μm filter. Cr has two stable forms in an aqueous system - Cr(VI) and Cr(III) (Fendorf 1995; Chen and Hao 1998). Therefore, soluble Cr(III) is assumed here as the difference between *total soluble Cr* and Cr(VI). Soluble Cr(III) was found to be present in the effluent as shown in Figure 7. Figure 7a and 7b present results of two experiments with influent Cr(VI) concentrations of 0.055 and 0.037 mM, respectively. It can be seen that *total soluble Cr* was present in the effluent even when Cr(VI) was completely reduced. *Total soluble Cr* and soluble Cr(III) continued to increase until Cr(VI) breakthrough but soluble Cr(III) concentrations dropped sharply following Cr(VI) breakthrough. Similar observations were made for all other experiments.

Cr(III) usually precipitates as $Cr(OH)_3$ at a pH range of 7.5 – 10 (Patterson 1985; Chen and Hao 1998). But Cr(III) can also form complexes with organic compounds (James and Bartlett 1983; Nieboer and Jusys 1988). It is possible that after Cr(VI) reduction, Cr(III) was bound with some organic ligands and produced a soluble Organo-

Cr(III) complex. This observation is in agreement with a study which found that a

bacterial flavin reductase system reduced chromate to a soluble Cr(III)-NAD$^+$ complex

(Puzon et al. 2002). Another study of Cr(VI) reduction with MR-1 (Middleton et el.

2003) reported that in a medium containing lactate as the electron donor, 13% – 14% of

the reduced Cr(III) remained soluble.

After each experiment, soil samples were collected along the length of the

column and analyzed for total Cr. Filtered effluent samples were also analyzed for total

Cr and referred to *total soluble Cr*. Percent recoveries were calculated based on total Cr

supplied as compared to the mass remaining in the column and collected in the effluent.

Figure 8 presents the percent recovery of total Cr for all the experiments. The total Cr is

comprised of total Cr in sands, and *total soluble Cr* in filtered effluent. The recovery of

total Cr is found to vary from 56% to 96%. The lower recoveries can be attributed to the

fact that Cr compounds/particles larger than 0.2 μm were trapped in the filter. However,

when unfiltered effluent total Cr was monitored, recoveries of 99% and 112% were

observed for two experiments with influent Cr(VI) concentrations of 0.037 and 0.055

mM, respectively. A greater than 100% recovery can be explained by the sensitivity of

the measurement techniques. It is also observed that the three experiments with 0.055,

0.049 and 0.037 mM influent Cr(VI) concentrations yielded higher percentage of *total

soluble Cr*, more than 50%, in the effluent. Cr(VI) breakthrough was observed for these

experiments. These results provide an estimate of how much Cr remained in the sand

column after Cr(VI) reduction and how much remained soluble at different influent

Cr(VI) concentrations. From the regulatory perspective, this observation is very important because the EPA maximum contaminant level (MCL) is measured in terms of *total soluble Cr*.

Sand Protein Profile

Figure 9a presents protein concentration, a measure of the biomass MR-1, within the column as a function of distance from the inlet for each experimental run. Figure 9b is a reproduction of 9a with the highest biomass profile omitted and with error bars representing 95% confidence interval from three replicates. The reproduction offers an easy comparison of the protein profiles. The highest protein profile was obtained for the influent Cr(VI) concentration of 0.011 mM.

The "Base Run" represents the protein profile for a column that was supplied with nutrient rich feed, without Cr(VI), for 9 pore volumes. The base run, therefore, represents conditions just prior to the addition of Cr(VI). The columns that were subjected to 0.055, 0.049 and 0.037 mM of influent Cr(VI), had less than, or, nearly equal protein concentration to that of the "Base Run". In these experiments, after the Cr(VI) breakthrough higher concentration of biomass, measured as viable cells, was observed as evidenced from Figure 3c and 4c. The higher biomass concentration in the effluent can be explained by the toxicity of Cr(VI). Higher the Cr(VI) concentration, the higher is its toxicity. Higher toxicity can result in higher detachment, a result of growth inhibition. On the other hand, experiments with 0.025, 0.011, and 0.006 mM of influent

Cr(VI), yielded higher protein concentrations than the "Base Run". Cr(VI) breakthrough was not observed for these experiments.

In situ bioremediation of soils and groundwater strategies often involve nutrient injection wells to stimulate the growth of microbes. Sand protein measurement provides an estimate of biomass population available for bioreduction. It is also important to know the distribution of biomass throughout the subsurface because the success of *in situ* bioremediation depends on distributing the biomass more evenly. The sand protein data presented in this paper can be helpful in developing nutrient addition strategies for in situ bioremediation. In addition, the data can be used to model MR-1 growth and transport in a continuous flow environment.

SUMMARY AND CONCLUSIONS

Bioreduction of Cr(VI) by MR-1 was investigated in the laboratory scale flow-through sand columns. Cr(VI) was added to the feed at a range of concentrations varying from 0.006 mM to 0.055 mM after 8 days of uninhibited growth. MR-1 reduced Cr(VI) completely for several pore volumes after which a Cr(VI) breakthrough was observed in the effluent for feed concentrations greater than 0.025 mM. Amount of Cr(VI) reduced prior to its breakthrough was inversely related to the feed Cr(VI) concentration. The Cr(VI) breakthrough corresponded with the breakthrough of fumarate, the limiting electron acceptor, indicating inhibition of microbial activity due to Cr(VI) toxicity. A residual Cr(VI) reduction, ranging from 20% – 45%, occurred after

the breakthrough and no Cr(VI) or fumarate breakthrough was observed for feed Cr(VI)
concentrations less than, or, equal to 0.025 mM. Therefore, influent concentration can
have significant impact on the MR-1's ability to reduce Cr(VI).

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TABLE 1. Summary of Column and Packing Material (Quartz sand) Properties

Length of Column	15 cm
Inside Diameter	2.1 cm
Average Grain Size	0.84 mm
Dry Bulk Density	1.48 gm/cc
Porosity	0.43
Dispersivity	0.075 cm
Residence Time	22.5 hr
Flow Velocity	16 cm/day
Flow rate	1.0 mL/hr

TABLE 2. Summary of Feed Cr(VI) Concentration, Cr(VI) Breakthrough and Total Run Time of All Experiments

Test Run No.	Average Feed Cr(VI)[1] mM	Cr(VI) Feed Start Time Pore Volume	Cr(VI) Breakthrough Pore Volume	Total Run Time Pore Volume
1	0.055 (0.002)	8.92	13.90	24.27
2	0.049 (0.004)	8.62	15.99	20.33
3	0.037 (0.001)	8.92	22.65	31.48
4	0.025 (0.002)	8.76	>30.31	30.31
5	0.011 (0.001)	8.76	>60.37	60.37
6	0.006 (0.001)	8.91	>37.67	37.67

[1]values in the parenthesis indicate 95% confidence interval

FIGURE CAPTION

FIGURE 1. Schematic of the experimental setup.

FIGURE 2. Results of abiotic control column experiment showing breakthrough curves of non reactive chloride tracer, fumarate and Cr(VI).

FIGURE 3. Results for an average influent Cr(VI) concentration of 0.037 mM (1.94 mg/L). **a.** Influent and effluent Cr(VI) concentrations as a function of pore volume treated. **b.** Influent fumarate with corresponding effluent concentrations and effluent succinate concentration as a function of pore volume treated. **c.** Viable cells in the effluent as a function of pore volume treated.

FIGURE 4. Results for an average influent Cr(VI) concentration of 0.055 mM (2.84 mg/L). **a.** Influent and effluent Cr(VI) concentrations as a function of pore volume treated. **b.** Influent fumarate with corresponding effluent concentrations and effluent succinate concentration as a function of pore volume treated. **c.** Viable cells in the effluent as a function of pore volume treated.

FIGURE 5. Cr(VI) reduction prior to its breakthrough as a function of influent concentrations.

FIGURE 6. Breakthrough profiles for Cr(VI) for different influent concentrations.

FIGURE 7. a. Breakthrough profiles for influent and effluent *total soluble Cr*, effluent soluble Cr(VI), and effluent soluble Cr(III) for an influent Cr(VI) of 0.055 mM (2.84 mg/L) **b.** Breakthrough profiles for influent and effluent *total soluble Cr*, effluent

soluble Cr(VI), and effluent soluble Cr(III) for an influent Cr(VI) of 0.037 mM (1.94 mg/L).

FIGURE 8. Recovery of total Cr from the column for different influent Cr(VI) concentrations. Total Cr recovered from sand after the column extrusion is shown as black filled box and *total soluble Cr* collected in the column effluent throughout the experiment is shown as white hollow box.

FIGURE 9. a. Distribution of sand protein, a measure of biomass, within the column for different influent Cr(VI) concentrations after each experimental run. **b.** Reproduction of (a) by omitting the highest biomass profile. Error bars indicate 95% confidence level.

FIGURE 1. Schematic of the experimental setup

Column

Effluent jug

Syringe feed

Filter

Syringe pump
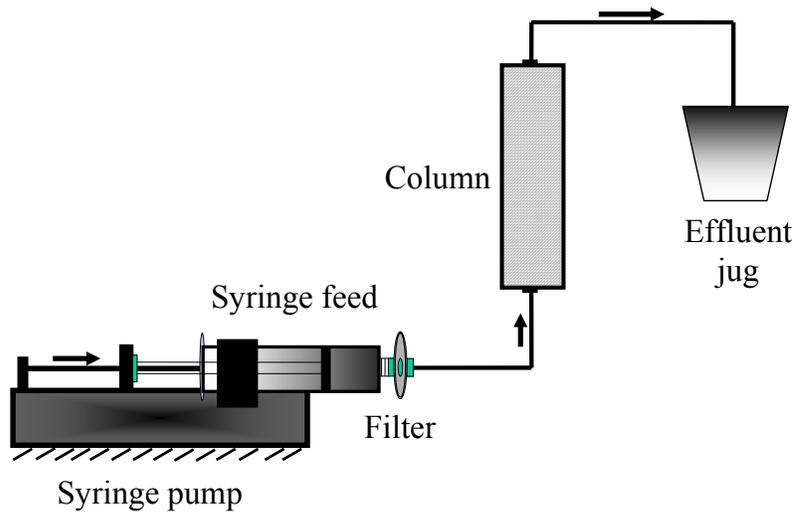
FIGURE 2. Results of abiotic control column experiment
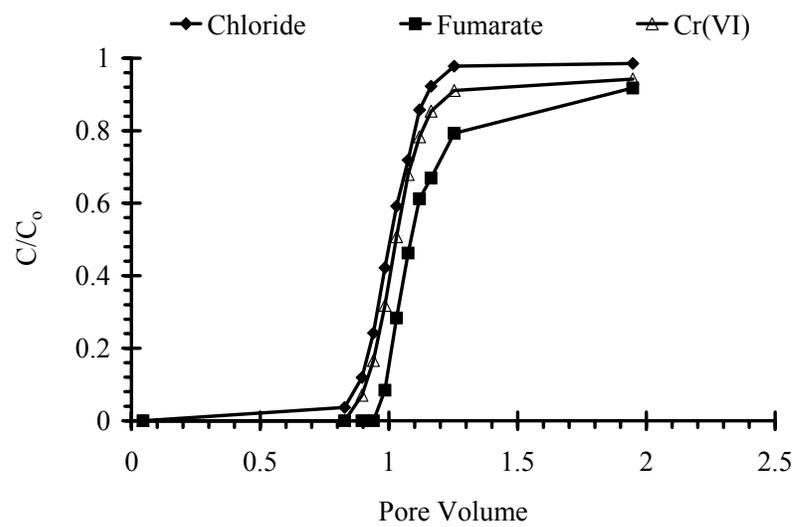
FIGURE 3. Results for an average influent Cr(VI) concentration of 0.037 mM
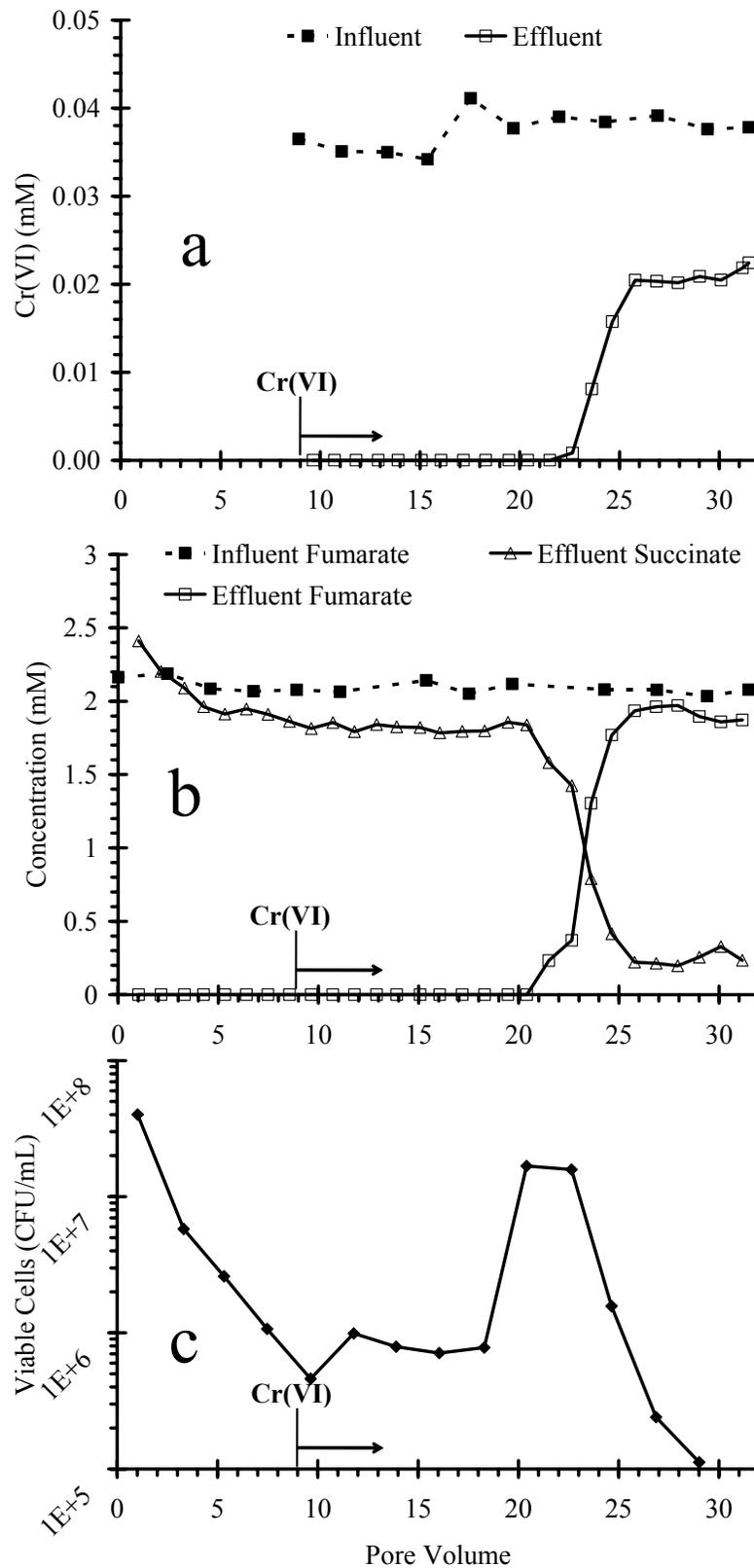
FIGURE 4. Results for an average influent Cr(VI) concentration of 0.055 mM
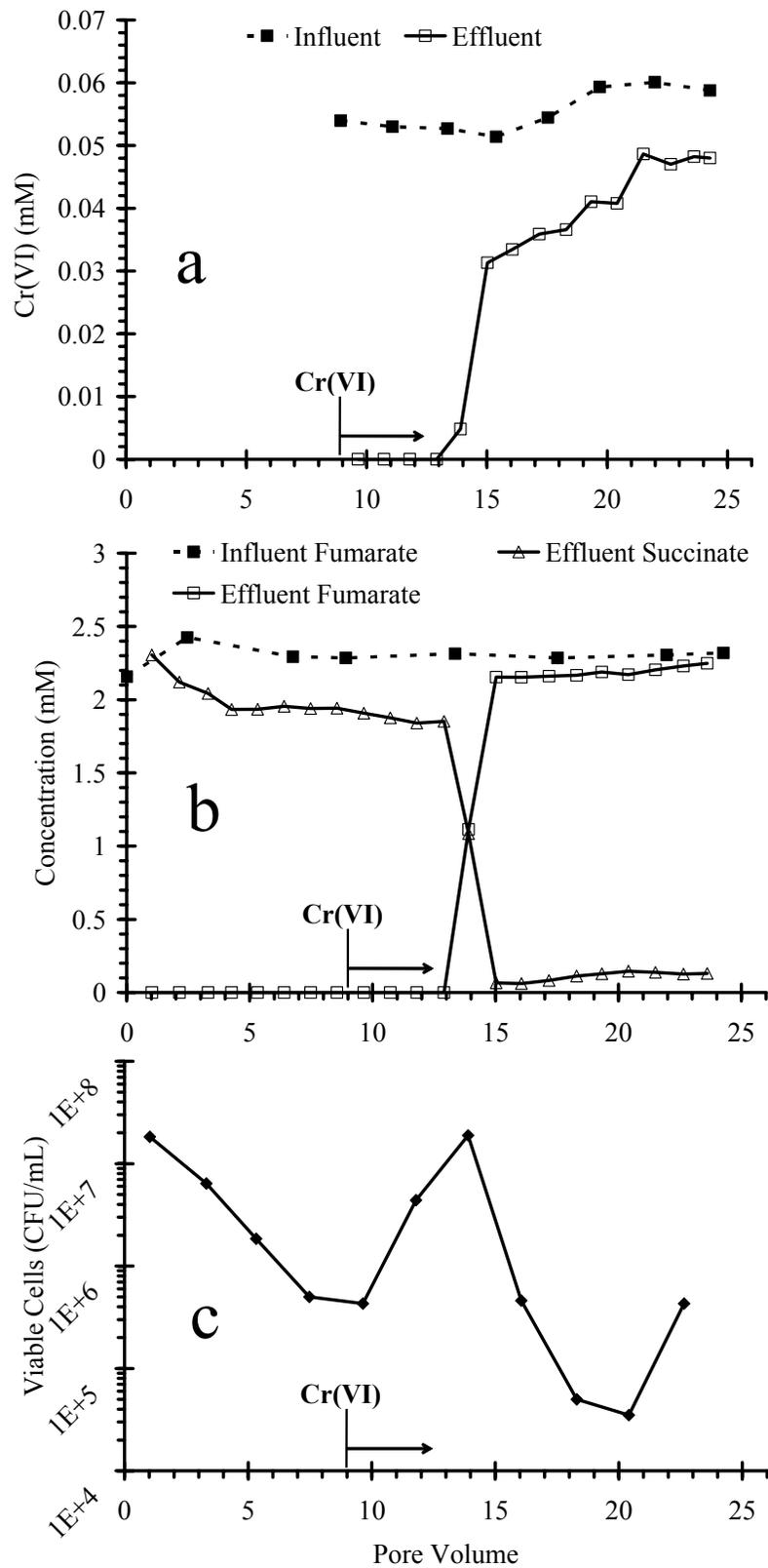
FIGURE 5. Cr(VI) reduction prior to its breakthrough
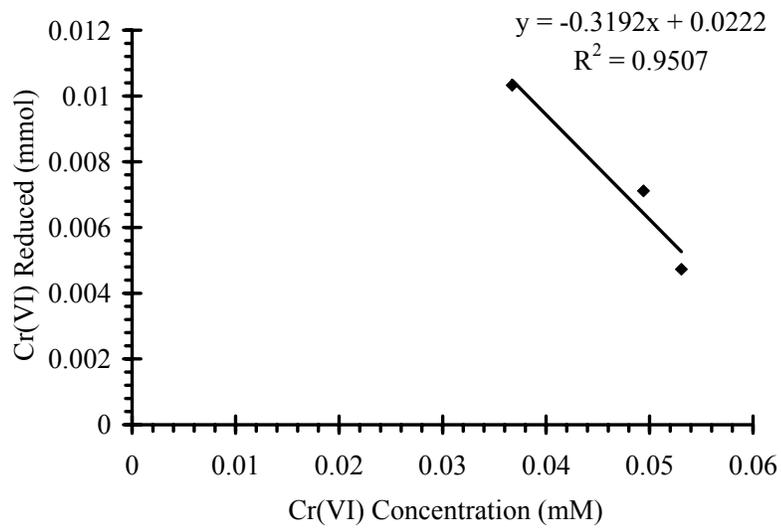
FIGURE 6. Breakthrough profiles for Cr(VI) for different influent concentrations
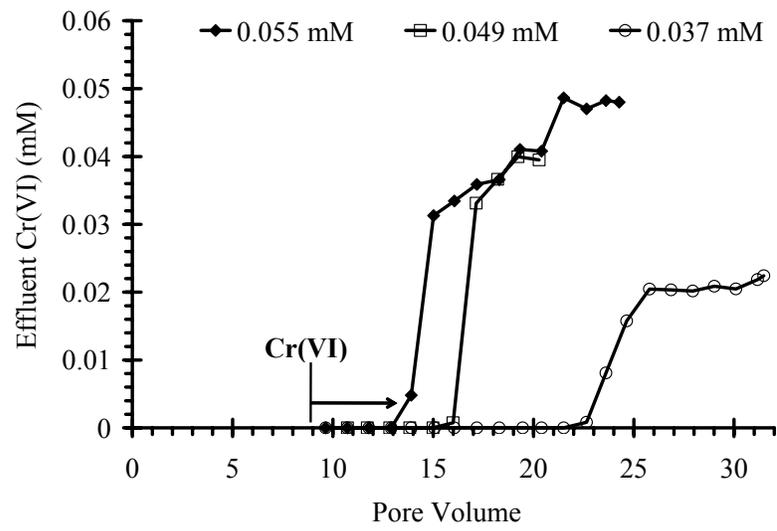
FIGURE 7. Breakthrough profiles for influent and effluent *total soluble Cr*, effluent

soluble Cr(VI), and effluent soluble Cr(III)
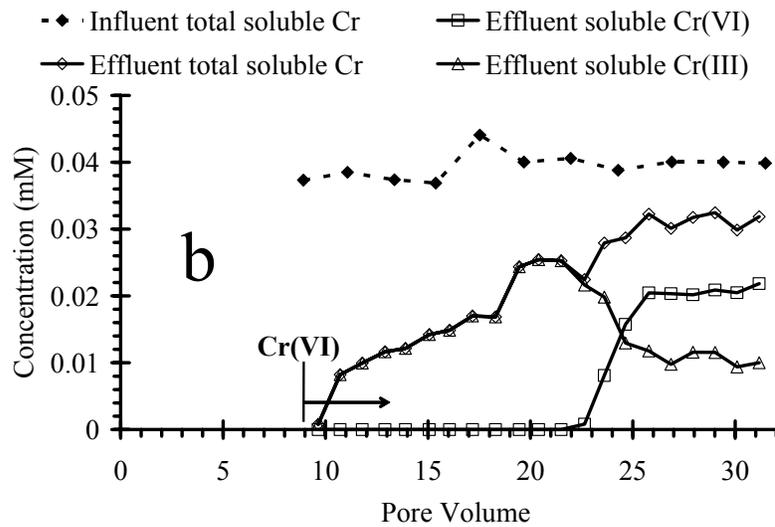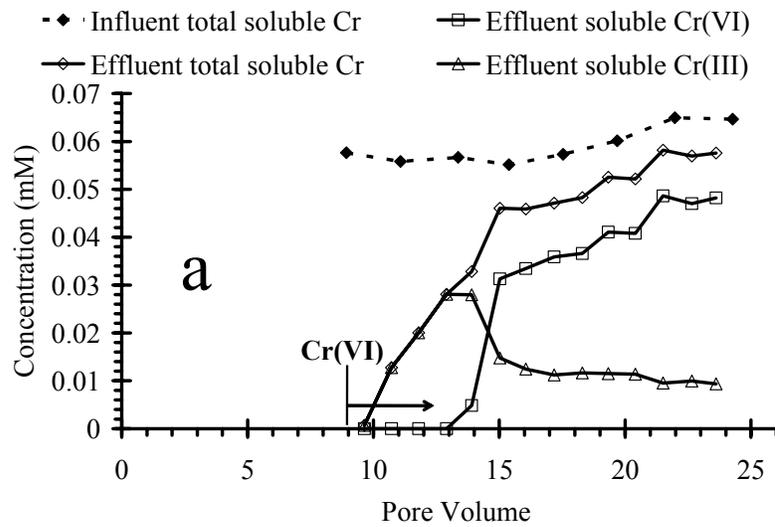
FIGURE 8. Recovery of total Cr from the column
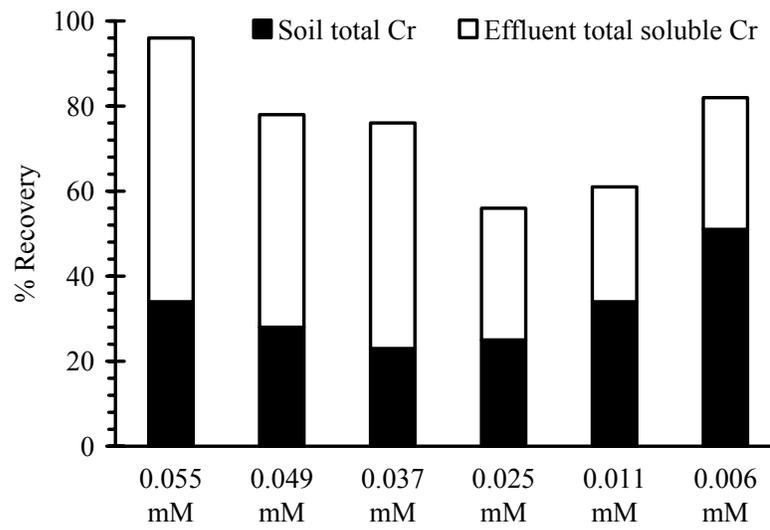
FIGURE 9. Distribution of sand protein
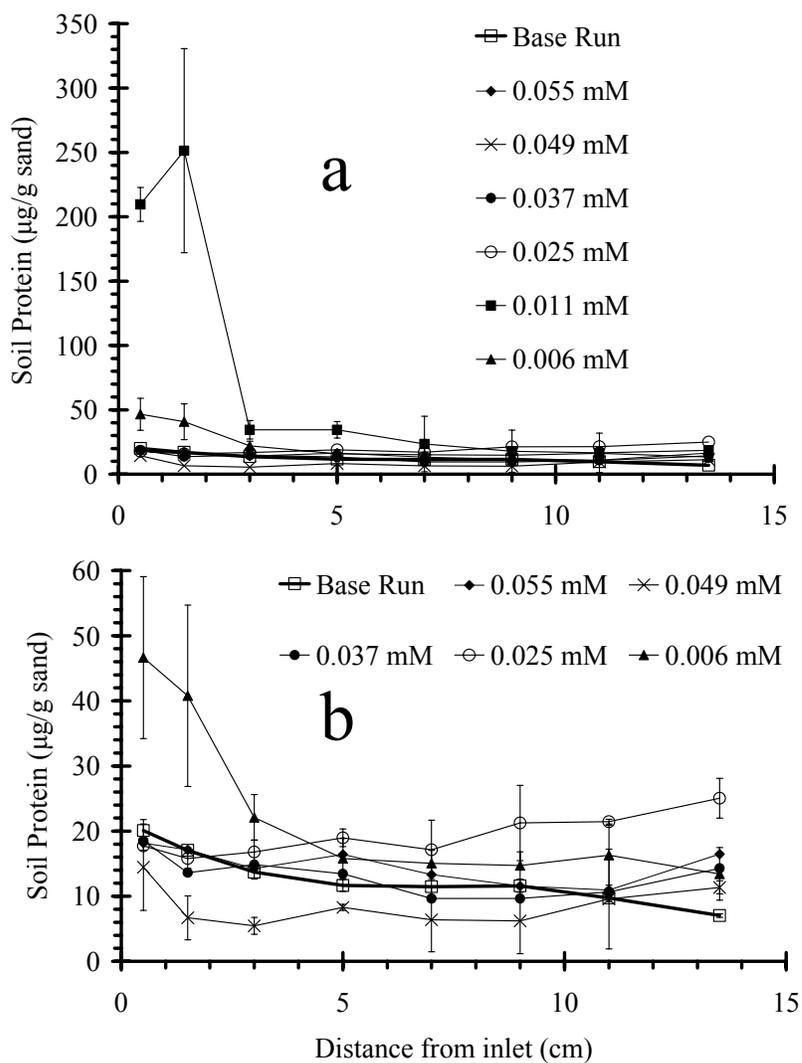
CHAPTER THREE

MODELING MICROBIAL REDUCTION OF Cr(VI) BY EMPLOYING THE DUAL ENZYME KINETIC MODEL

This chapter is presented as a paper that has been prepared for publication in a peer-reviewed journal.

Modeling Microbial Reduction of Cr(VI) by Employing the Dual Enzyme Kinetic Model

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ABSTRACT

Chromium(VI) contamination of soil and groundwater is a major environmental concern for some industrial sites and most of the US DOE's sites. Cr(VI) is toxic and relatively mobile in groundwater. Bioreduction of Cr(VI) to less toxic and less mobile Cr(III) is considered to be a feasible option. Among the Cr(VI) reducing bacteria *Shewanella oneidensis* MR-1 (MR-1) is the most widely studied and relatively effective. The reduction mechanism of Cr(VI) by MR-1 is described by a nonlinear dual enzyme kinetic model. Currently, no contaminant transport model is available that can simulate the fate and transport of Cr(VI) in groundwater by employing the dual enzyme kinetic model. The objective of this research work was to modify the three dimensional transport model RT3D by incorporating the dual enzyme kinetic model. The model developed through this modification was calibrated and validated by comparing model predictions with data obtained by performing sand column experiments in the laboratory. The model has been found to be reasonably accurate and reliable.

Keywords: Bioreduction, Chromium(VI), modeling, RT3D, Sand column, MR-1, Kinetic model.

INTRODUCTION

Chromium (Cr) is a redox-sensitive transition metal and it has several oxidation states ranging from -2 to +6 (Love, 1983) with trivalent chromium (Cr(III)) as the most stable form. Cr(III) can be found in nature as chromite (Cary, 1982). Cr can also

sometimes exist in +6 oxidation state as relatively stable oxyanions, such as, chromate and dichromate (Bartlett and Kimble, 1976). Hexavalent chromium (Cr(VI)) is mainly produced by anthropogenic activities and enters the environment as waste generated by many industrial and commercial processes, such as, leather/pelt tanning, aluminum anodizing, and other metal cleaning, pre-plating, and electroplating operations (Patterson, 1985). Cr(VI) compounds are also used as additives in production of pigments, catalysts, corrosion inhibitors, and wood preservatives (Cohen and Costa, 2000). The widespread consumption of Cr(VI) has resulted in chromium contaminated soil and groundwater in many industrial sites including United States Department of Energy (DOE) facilities (Riley et al., 1992). Cr(VI) is considered very toxic and is known to have carcinogenic and mutagenic effects (Langard, 1983; Cieslak-Golonka, 1996). Consequently, it was ranked 17th in the "2003 CERCLA Priority List of Hazardous Substances" (ATSDR, 2003). In addition, Cr(VI) species are highly soluble and mobile in aqueous systems and can spread over a large area through transport in groundwater (Bartlett and Kimble, 1976). Therefore, Cr(VI) in soil and groundwater can be considered a major environmental concern for some industrial and most of DOE's sites.

Most of the Cr(III) compounds found in the environment are slightly soluble, and much less toxic than Cr(VI) compounds (Ross et al., 1981). In fact, it is a popular dietary supplement for humans and is needed in trace amounts to augment the action of insulin (Mertz, 1979). Additionally, Cr(III) compounds are reported to be less mobile

which is a result of greater partitioning on to soil (Barnhart, 1997). Considering its slow mobility, reduced toxicity and solubility; transformation of Cr(VI) to Cr(III) can be considered an effective technology for the remediation Cr(VI) contaminated soil and groundwater.

Currently, a number of *in situ* and *ex situ* alternatives are available for the treatment of Cr(VI) contaminated soil and groundwater. The e*x situ* methods may involve soil excavation and washing or off-site disposal, or groundwater pumping followed by chemical or electrochemical reduction and precipitation or ion exchange (Patterson, 1985). These *ex situ* physico-chemical methods are relatively expensive because they are either energy intensive or require large quantities of chemical reagents and sometimes generate secondary wastes that require subsequent disposal (Chen and Hao, 1997; Guha et al., 2001). The *in situ* remediation of Cr(VI) contaminated soils may include heap leaching followed by leachate collection and treatment (Hanson et al., 1993); and permeable Fe^0 reactive walls (Blowes et al., 1997). Significant operational difficulties may arise when these methods are employed. Further, these methods suffer from high capital and operational costs and low efficiency. Therefore, the feasibility of these methods in remediating Cr(VI) contaminated soil and groundwater is yet to be established.

Consequently, the recent research focus has been on the microbial transformation of Cr(VI) to Cr(III). Microbial transformation of Cr(VI) to Cr(III) is considered a viable

alternative and a large volume of literature is available in support of it (Gvozdyak.,
1986; Bopp and Erlich, 1988; Wang et al., 1989; Llovera et al., 1993; Lovley and
Phillips, 1994; Wang and Shen 1995; Garbisu et al., 1998; Viamajala et al., 2002).
Cr(VI) reducing microbes appear to be ubiquitous in the subsurface. They are found in
both Cr(VI) contaminated and uncontaminated environments (Horitsu et al., 1978;
Turick et al., 1996; Schmieman et al., 1998; Sani et al., 2002). Some facultative
anaerobic microbes can utilize Cr(VI) as a terminal electron acceptor for their growth
(Romanenko and Koren'kov, 1977; Wang et al., 1989; Viamajala et al., 2002) while
others can reduce Cr(VI) co-metabolically (Bopp and Erlich, 1988; Shen and Wang,
1993). Microbial reduction of Cr(VI) to Cr(III) can, therefore, be considered a novel
remediation alternative to the conventional physico-chemical treatments which are
expensive and produce secondary wastes.

Among the Cr(VI) reducing microbes, the *Shewanella* species are perhaps the
most widely studied (Venkateswaran et al., 1999). *Shewanella oneidensis* MR-1 (MR-
1), a gram negative facultative anaerobe, is an important model organism for
bioremediation studies because of its diverse respiratory capabilities. Viamajala et al.
(2002) reported that MR-1 can effectively reduce Cr(VI) for a wide range of
concentrations and the reduction is accomplished by more than one mechanisms.
Viamajala et al. (2003) developed a nonlinear dual-enzyme kinetic model to simulate the
multi-mechanism reduction of Cr(VI) by MR-1. The model is based on the assumption
that two enzymes – a fast acting but quickly deactivating and a slow acting but stable,

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are responsible for reducing Cr(VI) to Cr(III) and the enzymatic reactions are simultaneous.

A wide range of microbes can reduce Cr(VI) to Cr(III). However, MR-1 is the most widely studied and is relatively effective in reducing Cr(VI) under anaerobic conditions. Laboratory experiments can be conducted in batch reactors to evaluate the feasibility and kinetics of microbial reduction of Cr(VI). A numerical model, however, is required to predict the fate and transport Cr(VI), in general, and effectiveness of microbial reduction of Cr(VI), in particular, for varying site specific conditions. Further, a numerical model can assist in developing remedial strategies.

There are a number of numerical models such as MT3D (Zheng, 1990), RT3D (Clement, 1997), and CTRAN/W (Geo-Slope International, 2004) capable of simulating fate of transport of contaminants in the subsurface. These models, however, are not designed to predict microbial reduction of Cr(VI) by employing the dual enzyme kinetic model.

The objective of this paper is to present a numerical model to predict the fate and transport of Cr(VI) in the groundwater employing the dual enzyme kinetic model and to evaluate its accuracy and the predictive ability by comparing model predictions with data obtained by performing flow-through sand column experiments.

MODEL FORMULATION

Advection and dispersion are the main modes of transport of Cr(VI) in the groundwater. Cr(VI) does not partition on to sand. Therefore, retardation may not play any role in defining its fate and transport. However, reaction – the microbial reduction can significantly influence the fate and transport of Cr(VI).

The Dual-Enzyme Kinetic Model

The dual-enzyme kinetic model proposed by Viamajala et al. (2003) can be summarized by the following parallel reactions.

$$Cr(VI) + E_d \rightarrow E_d^* + Cr^* \tag{1}$$

$$Cr(VI) + E_s \rightarrow E_s + Cr^* \tag{2}$$
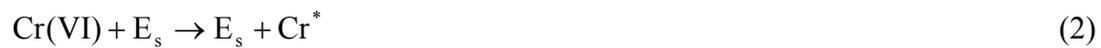
In the above equations, E_d is the "deactivating enzyme" which is fast acting but converts to the inactive form E_d^* while reacting with Cr(VI); Cr^* is the reduced product of Cr(VI) and assumed to be Cr(III); and E_s is the "stable enzyme" which is slow acting but remains in active form.

Viamajala et al. (2003) proposed the following rate expression for Cr(VI) reduction described by the preceding equations.

$$r_{Cr(VI)} = -\frac{d[Cr(VI)]}{dt} = k_1 [E_d]_s [X] + k_2^{\bullet} [E_s]_s [X] \qquad (3)$$

In Eq. 3, [Cr(VI)] = concentration of Cr(VI) [ML^{-3}], k_1 = rate constant for Cr(VI) reduction due to the deactivating enzyme [T^{-1}], $k_2^{\bullet}$ = rate constant for Cr(VI) reduction due to the stable enzyme [T^{-1}], $[E_d]_s$ = specific deactivating enzyme concentration (MM^{-1}), $[E_s]_s$ = specific stable enzyme concentration (MM^{-1}), and [X] = total microbial (MR^{-1}), concentration [ML^{-3}].

Microbes can be attached to the sand solids and can also be suspended in the pore water. Total microbial concentration accounts for both suspended and attached phase microbes as given below.

$$[X] = [X_s] + \frac{\rho_b}{\varepsilon}[X_a] \qquad (4)$$

Here, ρ_b = bulk density of the porous medium [ML^{-3}], ε = sand porosity, $[X_s]$ = microbial concentration in the suspended phase [ML^{-3}], and $[X_a]$ = microbial concentration in the attached phase [MM^{-1}].

The specific deactivating enzyme concentration declines with time during the reaction. Deactivating enzyme concentration, $[E_d]$ at any time is proportional to the microbial concentration and is given by $[E_d] = [E_d]_s [X]$. The specific stable enzyme concentration, on the contrary, does not change with time. The product $k_2^{\bullet} [E_s]_s$,

therefore, is a constant and can be lumped together as k_2. Therefore, the Cr(VI) reaction

rate expression can be written in the following form.

$$r_{Cr(VI)} = -\frac{d[Cr(VI)]}{dt} = k_1\left([E_{ds}] + \frac{\rho_b}{\varepsilon}[E_{da}]\right) + k_2\left([X_s] + \frac{\rho_b}{\varepsilon}[X_a]\right) \qquad (5)$$

Where, $[E_{ds}]$ = deactivating enzyme concentration in the suspended phase $[ML^{-3}]$,

and $[E_{da}]$ = deactivating enzyme concentration in the attached phase $[MM^{-1}]$.

Microbial Growth Kinetics and Substrate Utilization

Microbial growth kinetics can be simulated by the modified Monod model

proposed by Yamamoto et al. (1993) that accounts for Cr(VI) inhibition. Yamamoto et

al. (1993) proposed the following competitive inhibition model.

$$\mu = \frac{1}{[X]}\frac{d[X]}{dt} = \frac{\mu_m[S]}{K_m + [S]}\frac{1}{1 + \frac{[Cr(VI)]}{K_i}} \qquad (6)$$

In Eq. 6, μ = specific growth rate $[T^{-1}]$, μ_m = maximum specific growth rate

constant $[T^{-1}]$, $[S]$ = concentration of the limiting substrate $[ML^{-3}]$, K_m = Michaelis

constant or saturation constant for the substrate $[ML^{-3}]$, and K_i = growth inhibition

constant for Cr(VI) $[ML^{-3}]$.

The substrate utilization rate, r_s, can be related to the specific growth rate by

employing the growth yield, Y $[MM^{-1}]$, as follows.

$$r_s = -\frac{d[S]}{dt} = \frac{1}{Y}\mu[X] = \frac{1}{Y}\frac{\mu_m[S]}{K_m + [S]}\frac{1}{1 + \frac{[Cr(VI)]}{K_i}}\left([X_s] + \frac{\rho_b}{\varepsilon}[X_a]\right) \tag{7}$$

Microbial Attachment and Detachment

Physiological condition of the microbes and environmental factors may cause them to continuously attach to or detach from the sand surface. The attachment and the detachment processes can be modeled by first order rate expressions as proposed by Peyton et al. (1995) and Murphy and Ginn (2000) as given below.

$$r_a = K_a[X_s] \tag{8}$$

$$r_d = K_d[X_a] \tag{9}$$

Here, r_a = rate at which suspended phase microbes attach to the sand surface $[ML^{-3}T^{-1}]$, r_d = rate at which attached microbes detach from the sand surface $[MM^{-1}T^{-1}]$, K_a = attachment coefficient $[T^{-1}]$, and K_d = detachment coefficient $[T^{-1}]$.

Model Equations

A mass balance employing the dual-enzyme kinetic model, microbial growth kinetics, and the attachment-detachment rate expressions and assuming a first-order decay of the deactivating enzyme lead to the following equations for the fate and transport of Cr(VI), substrate, the deactivating enzyme, and the microbes – both in attached and in suspended phases for anaerobic conditions.

$$\frac{\partial[Cr(VI)]}{\partial t} = -\frac{\partial}{\partial x_i}\left(v_i[Cr(VI)]\right) + \frac{\partial}{\partial x_i}\left(D_{ij}\frac{\partial[Cr(VI)]}{\partial x_j}\right) - k_1\left([E_{ds}] + \frac{\rho_b}{\epsilon}[E_{da}]\right)\frac{[Cr(VI)]}{A + [Cr(VI)]}$$
$$- k_2\left([X_s] + \frac{\rho_b}{\epsilon}[X_a]\right)\frac{[Cr(VI)]}{A + [Cr(VI)]}$$

(10)

$$\frac{\partial[S]}{\partial t} = -\frac{\partial}{\partial x_i}\left(v_i[S]\right) + \frac{\partial}{\partial x_i}\left(D_{ij}\frac{\partial[S]}{\partial x_j}\right) - \frac{1}{Y}\frac{\mu_m[S]}{K_m + [S]}\frac{1}{1 + \frac{[Cr(VI)]}{K_i}}\left([X_s] + \frac{\rho_b}{\epsilon}[X_a]\right)$$

(11)

$$\frac{\partial[X_s]}{\partial t} = -\frac{\partial}{\partial x_i}\left(v_i[X_s]\right) + \frac{\partial}{\partial x_i}\left(D_{ij}\frac{\partial[X_s]}{\partial x_j}\right) + \left[\frac{\mu_m[S]}{K_m + [S]}\frac{1}{1 + \frac{[Cr(VI)]}{K_i}} - k_d\right][X_s]$$
$$- K_a[X_s] + \frac{K_d\rho_b}{\epsilon}[X_a]$$

(12)

$$\frac{\partial[X_a]}{\partial t} = \left[\frac{\mu_m[S]}{K_m + [S]}\frac{1}{1 + \frac{[Cr(VI)]}{K_i}} - k_d\right][X_s] + \frac{K_a\epsilon}{\rho_b}[X_a] - K_d[X_a]$$

(13)

$$\frac{\partial[E_{ds}]}{\partial t} = -\frac{\partial}{\partial x_i}\left(v_i[E_{ds}]\right) + \frac{\partial}{\partial x_i}\left(D_{ij}\frac{\partial[E_{ds}]}{\partial x_j}\right) + \left[\frac{\mu_m[S]}{K_m + [S]}\frac{1}{1 + \frac{[Cr(VI)]}{K_i}}\right][X_s][E_d]_0 - k_d[E_{ds}]$$
$$- K_a[E_{ds}] + \frac{K_d\rho_b}{\epsilon}[E_{da}] - k_{dc}[E_{ds}]\frac{[Cr(VI)]}{A + [Cr(VI)]}$$

(14)

$$\frac{\partial [E_{da}]}{\partial t} = \left[\frac{\mu_m [S]}{K_m + [S]} \frac{1}{1 + \frac{[Cr(VI)]}{K_i}} \right] [X_a][E_d]_0 - k_d [E_{da}] + \frac{K_a \varepsilon}{\rho_b} [E_{ds}] - K_d [E_{da}]$$

$$- k_{dc} [E_{da}] \frac{[Cr(VI)]}{A + [Cr(VI)]}$$

(15)

Where, D_{ij} = hydrodynamic dispersion coefficient tensor $[L^2 T^{-1}]$, v_i = pore water

velocity in the direction of x_i $[LT^{-1}]$, k_d = microbial death rate constant $[T^{-1}]$, k_{dc} = first-

order decay constant for the deactivating enzyme $[T^{-1}]$, A = a very small constant ($\approx$

0.0001) introduced in the model so that the Cr(VI) reduction kinetic becomes negligible

during the Cr(VI) free growth period, $[E_d]_0$ = specific deactivating enzyme

concentration related to new growth $[MM^{-1}]$, t = time [T], and x_i = spatial coordinate [L].

SOLUTION TECHNIQUE

The numerical model Reactive Transport in 3-Dimensions (RT3D) developed by

Clement (1997) has been modified to solve the model equations. RT3D is a generalized

3-dimesional model for simulating multi-solute transport with reaction. It uses a

reaction Operator-Split (OS) numerical strategy to solve any number of transport

equations which may be coupled via non linear reaction expressions, and is well-suited

for simulating natural attenuation and bioremediation. Details of the model can be found

elsewhere (Clement, 1997). Flow modeling is a prerequisite for transport modeling.

The U.S. Geological Survey's modular finite-difference ground-water flow model

(MODFLOW) has been adapted to simulate the flow. MODFLOW can be employed to

simulate flow in heterogeneous anisotropic aquifers for a variety of conditions accounting for sources and sinks.

The Groundwater Modeling System (GMS) was used to develop input files and to process output generated by the models. GMS has a comprehensive graphical user environment for performing various groundwater simulations. Several types of models are supported in GMS and facilities are provided to share information between different models and data types. Tools are also provided for mesh and grid generation, and post-processing of results. GMS provides a powerful user interface to both MODFLOW and RT3D.

SAND COLUMN EXPERIMENTS

Sand column experiments were conducted to develop data to evaluate the accuracy of the model developed. A schematic of the sand column system employed for the reduction of Cr(VI) to Cr(III) by MR-1 is presented in Fig. 10. The column is made of stainless steel. Its internal diameter is 2.1 cm and length is 15 cm. The column was equipped with flow a distributor and a stainless steel frit at the entrance and the exit to minimize short-circuiting. Flow to the column was delivered through a multi-channel syringe pump. The syringe contained sterile simulated groundwater medium (SGM) (Petersen et al. 1994) amended with lactate as the electron donor and fumarate as the terminal electron acceptor. A sterile 0.2 μm filter at the end of the syringe prevented bacterial movement from the reactor to the syringe feed solution. Stainless steel tubing

of 1.02 mm inside diameter was used to connect different components of the column system.

Column Startup

Components of the columns were autoclaved at 121°C for 15 min. Quartz sand of average size 840 μm was washed with 5% HNO$_3$ and combusted at 550°C for 30 min to sterilize and remove any residual metal and organic matter. A cryovial containing 1.8 mL pure culture of MR-1, frozen in 20% glycerol at $-$ 85°C, was thawed and grown aerobically in a 165 mL serum bottle containing 50 mL of the growth medium which was prepared with SGM amended with 15 mM (1335 $\frac{mg}{L}$) lactate as the electron donor and 12 mM (1368 $\frac{mg}{L}$) fumarate as the electron acceptor. The growth medium also contained the following micro-nutrients: 100 $\frac{mg}{L}$ casamino acid, 20 $\frac{mg}{L}$ of L-serine, L-arginine, and L-glutamic acid to stimulate growth. After 24 hours of incubation at 30°C, 1 mL of the grown culture was transferred to 50 mL fresh medium to facilitate the removal of residual glycerol. The strain was grown for another 24 hours to mark the end of log growth phase. Approximately 100 g of pretreated sterile sand was then inoculated with 50 mL of MR-1 culture and mixed thoroughly in a 250 mL sterile Erlenmeyer flask. The inoculated sand was aseptically poured into the sterile column as slurry in a laminar flow hood. The column was gently vortexed at several stages to ensure uniform packing. The column inoculated with MR-1 was then capped and connected to the

sterile tubing and syringes of the flow through system. Porosity, ε, of the packed column

was 0.43 and the bulk density of the sand was 1.48 $\frac{\text{gm}}{\text{cm}^3}$.

At the beginning of each experiment, the column was flushed with two pore

volumes of SGM containing no lactate or fumarate to remove unattached biomass from

inter-particle pore spaces. Concentration of MR-1 in the attached phase, measured as cell

protein, in the packed column was estimated to be $5 \times 10^{-6} \frac{\text{mg protein}}{\text{mg soil}}$. The

corresponding deactivating enzyme concentration was estimated to

be $5 \times 10^{-8} \frac{\text{mg enzyme}}{\text{mg soil}}$.

The SGM amended with the nutrients necessary to support bacterial growth was

then introduced into the column. The initial feed solution contained SGM amended with

3 mM lactate as the electron donor, and 2 mM fumarate as the electron acceptor - the

limiting nutrient. The initial feed did not contain any Cr(VI) to promote bacterial

growth. After a growth period of 8 days, Cr(VI) was added to the feed as chromate at a

predetermined concentration.

Two experiments were performed at influent Cr(VI) concentrations of 0.049 and

0.055 mM (2.54 and 2.85 $\frac{\text{mg}}{\text{L}}$). The rate of flow to the column was constant at 24

mL/day which provided a flow velocity of 16 cm/day. The range of Cr(VI)

concentrations and the flow velocity were typical to be found at Hanford Site, Washington, where the Site is contaminated with Cr(VI). A sterile column without MR-1 served as an un-inoculated control.

Sampling

Column effluent samples were collected in vials maintained in an ice bath to minimize growth and degradation during sampling. The samples were filtered by employing 0.2 μm syringe filters (Millipore, Bedford, MA) to remove suspended cells and filterable precipitates. The column influent and the filtered effluent samples were refrigerated at $4^{\circ}C$ for subsequent analysis for Cr(VI) and anions including fumarate.

Analytical Methods

Hexavalent Chromium

Cr(VI) concentrations were determined by the diphenylcarbazide method using Hach Chroma Ver 3 reagent (Hach Company, Loveland, CO) adapted for use in a Costar 48-well tissue culture plate (Corning Inc., NY) (Schmieman et al., 1998). A pink color was developed when Cr(VI) was present, and the absorbance was measured on a 48-well bioassay reader (HTS7000 Bio Assay Reader, Perkin Elmer, Norwalk, CT) at a wavelength of 540 nm. The method provided a detection limit of 0.001 mM.

Anions

All anions - lactate, acetate, fumarate, succinate, and chloride; concentrations were measured by ion chromatography, IC, (Model DX 500, Dionex Corporation, Sunnyvale, CA). The IC had a 4-mm AS11 HC column, a 4-mm AG11 HC guard column, a CD 20 conductivity detector, and an AS3500 autosampler. Peaknet software 5.1 was used to process the chromatograms. The lower detection limit for lactate and acetate was 0.1 mM and for chloride, fumarate and succinate was 0.05 mM.

PARAMETER ESTIMATION

Batch experiments were conducted to evaluate the Cr(VI) reaction kinetics. A description of the experimental method can be found in Viamajala et al. (2003). The kinetic parameters estimated from the batch data are presented in Table 3. A tracer test, using chloride (Cl⁻), was conducted to evaluate extent of dispersion in the sand column. The tracer test data was analyzed to find the dispersion coefficient by employing the method suggested by Fetter et al. (1999). The calculated dispersion coefficient is

$1.21 \times 10^{-4} \, \dfrac{m^2}{day}$. MR-1 attachment coefficient, K_a, was computed in accordance with the filtration theory for deep bed filters proposed by Tien et al. (1979) and later employed by Hornberger et al. (1992) to describe bacterial attachment process in porous media. The estimated value was 6.72 day^{-1}. The detachment coefficient, K_d was estimated to be 0.55 day^{-1} by evaluating the effluent cell protein data from the sand column experiment. MR-1 decay rate, k_d, was taken to be 0.06 day^{-1} which falls within the range of values

reported in literature (Clement et al., 1997). Cr(VI) significantly inhibits the growth of

MR-1 even at low concentrations of 0.015 mM Cr(VI). Therefore, the inhibition

constant, K_i should be small and was assumed to be 0.000285 mM Cr(VI) in order to

calibrate the model with experimental data.

RESULTS AND DISCUSSION

The model developed to simulate bioreduction of Cr(VI) to Cr(III) employs the

flow model MODFLOW and the modified transport model RT3D as discussed earlier.

Numerical adequacy of MODFLOW and RT3D are well established. Numerical

solution of contaminant transport equation usually suffers from "artificial" dispersion

and oscillation. The solution techniques adapted in RT3D minimizes both artificial

dispersion and oscillation. Numerical tests revealed that for Peclet (Pe) number 100 and

for Courant (Cn) number ≤ 1, the model results were very similar to that for Pe = 2,

which is the frequently cited criterion for transport models to provide oscillation free

predictions. Numerical accuracy of a model does not always ensure its ability to predict

laboratory or field observations. The predictive ability of a model can be tested through

the process of calibration and validation by using laboratory data.

Calibration and Validation

Hydraulic and kinetic parameters, discussed earlier, were evaluated carefully by

performing laboratory experiments. Specific deactivating enzyme concentration related

to new microbial growth, $[E_d]_0$, and the inhibition constant, K_i, could not be estimated

with acceptable degree of accuracy. Therefore, these two parameters were used as

calibration parameters. $[E_d]_0 = 0.01 \dfrac{mg\,enzyme}{mg\,protein}$ and $K_i = 2.85 \times 10^{-4}$ mM provided the

best match of the model prediction for Cr(VI) and fumarate with the experimental data

obtained for an influent Cr(VI) concentration of 0.049 mM (2.54 mg/L) as shown in

Figs. 11 and 12. The observed deviation can be explained by the fact that the kinetic

parameters were determined by performing batch experiments. The column

environment can be significantly different than that of the batch. In fact, in a column

most of the microbes are in the attached phase whereas in a batch reactor they are in the

suspended phase. This environmental difference can lead to difference in reaction rates

(Kelly et al., 1996; Park et el, 2001).

The calibrated model was then employed to predict effluent concentrations of

Cr(VI) and fumarate for the experiment conducted with an influent concentration of

0.055 mM (2.85 mg/L). Model predictions and the corresponding effluent

concentrations are plotted in Figs. 13 and 14. A reasonable fit is observed between the

model prediction and the experimental data.

The model developed in this study is intended as a tool to study microbial Cr(VI)

reduction in saturated porous media. It was applied to simulate Cr(VI) reduction by MR-

1 in laboratory scale soil columns. It can be used to design and predict Cr(VI)

bioreduction in scale-up systems. However, the model should be calibrated with meso-

scale experimental data prior to field application. In addition, in an actual field case, the model would likely require system specific modification to suit the field conditions. Further research should be aimed at additional physical, chemical, and biological processes that influence Cr(VI) bioreduction in subsurface environments.

SUMMARY AND CONCLUSIONS

Bioreduction of Cr(VI) is considered an effective alternative to the currently practiced treatment technologies. Among the Cr(VI) reducing bacteria MR-1 is the most widely studied one and relatively effective. Cr(VI) reduction mechanism by MR-1 is described by a dual enzyme kinetic model. The three-dimensional transport model RT3D has been modified to include the dual enzyme kinetic model of Cr(VI) reduction by MR-1. The resulting model has been calibrated and validated by utilizing data generated in the laboratory. The model is stable and was found to be reasonably accurate and reliable for $Pe \leq 100$ and $Cn \leq 1$.

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FIGURE CAPTION

Fig. 10. Schematic of the flow through sand column experimental system

Fig. 11. Cr(VI) breakthrough at the column outlet for an influent Cr(VI) concentration of 0.049 mM. Arrow represents the time when Cr(VI) was introduced to the feed.

Fig. 12. Fumarate breakthrough at the column outlet for an influent Cr(VI) concentration of 0.049 mM.

Fig. 13. Cr(VI) breakthrough at the column outlet for an influent Cr(VI) concentration of 0.055 mM. Arrow represents the time when Cr(VI) was introduced to the feed.

Fig. 14. Fumarate breakthrough at the column outlet for an influent Cr(VI) concentration of 0.055 mM.

TABLE 3: Kinetic parameters for Cr(VI)

Parameter	Value
k_1	$158.49 \ \text{day}^{-1}$
k_2	$0.0632 \ \text{day}^{-1}$
k_{dc}	$11.69 \ \text{day}^{-1}$
μ_m	$3.648 \ \text{day}^{-1}$
K_m	$0.088 \ \text{mM}$
Y	0.0153

FIGURE 10. Schematic of the flow through sand column experimental system

Column

Effluent
Carboy

Syringe feed

Filter

Syringe pump
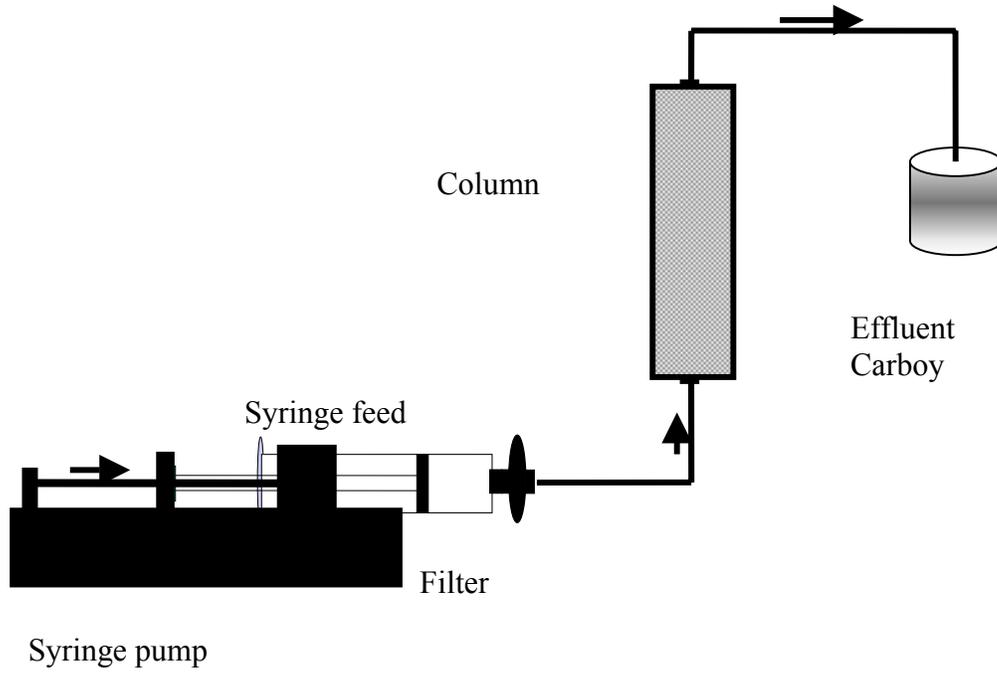
FIGURE 11. Cr(VI) breakthrough for an influent Cr(VI) concentration of 0.049 mM
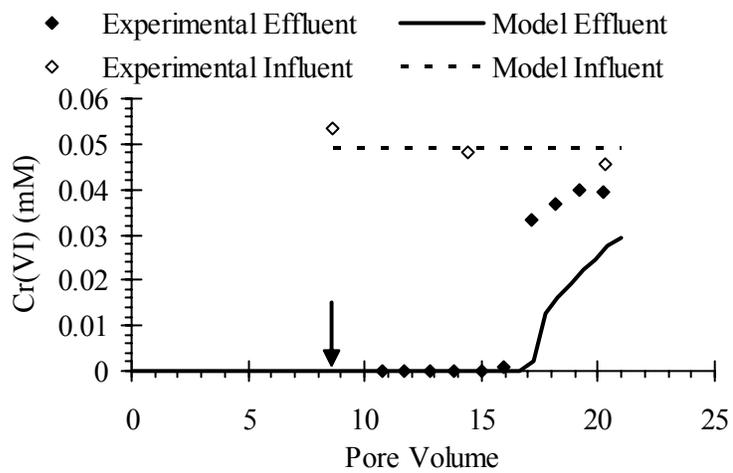
FIGURE 12. Fumarate breakthrough for an influent Cr(VI) concentration of 0.049 mM
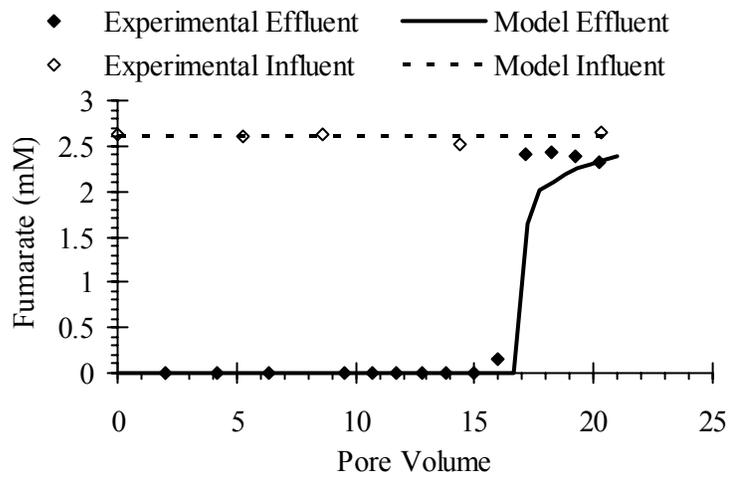
FIGURE 13. Cr(VI) breakthrough for an influent Cr(VI) concentration of 0.055 mM
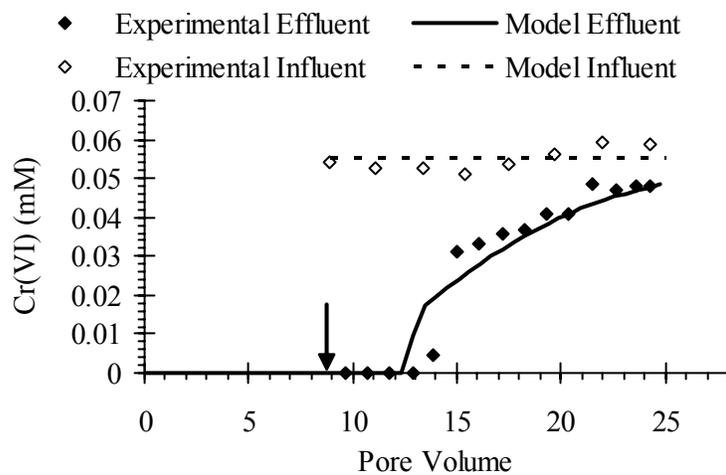
FIGURE 14. Fumarate breakthrough for an influent Cr(VI) concentration of 0.055 mM
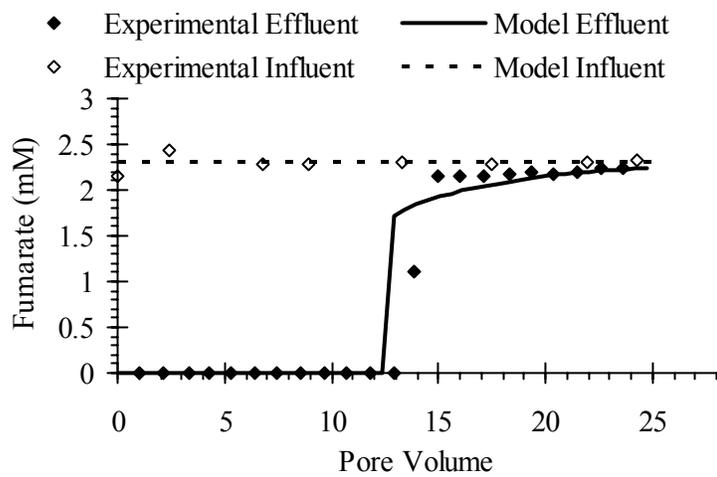
CHAPTER FOUR

FUTURE WORK

This dissertation focused on the kinetics of biological Cr(VI) reduction in a fixed film continuous flow environment. Both experimental data collection and numerical modeling were used to evaluate the rate and extent of Cr(VI) bioreduction. In the light of previous and on-going studies regarding bacterial Cr(VI) reduction, the following recommendations are made for future work.

1) Cr(VI) breakthrough was followed by a residual Cr(VI) reduction ranging from 20 – 45%. It was hypothesized based on a dual enzyme kinetic model, that a stable enzyme is responsible for the residual reduction. Further continuous flow experiments should be performed to define the stable enzyme(s) activity over a longer period of time.

2) Cr(VI) breakthrough time was found to be non-linear with influent Cr(VI) concentration. Future investigations should be aimed at delineating the apparent non-linear relationship between breakthrough time and influent Cr(VI) concentration.

3) To extend the results of Cr(VI) bioreduction by MR-1 in laboratory scale 1D sand columns, further experiments should be carried out in meso-scale

(2D/3D) continuous flow fixed film bioreactors. A lysimeter could also be used to study *in-situ* bioreduction of Cr(VI). This will provide more informative results prior to pilot and/or field scale application.

4)   *Shewanella oneidensis* MR-1, a widely known metal reducer, was used for Cr(VI) bioreduction in this study. It may be worthwhile to evaluate the potential for other microorganisms to reduce Cr(VI). Although MR-1 reduced Cr(VI) at a relatively high rate, they were susceptible to Cr(VI) toxicity and their growth was significantly inhibited by the presence of Cr(VI), even at relatively low concentrations (> 0.75 mg/L). This concentration is lower than typical concentrations found in DOE's Cr(VI)-contaminated sites. For example, the Hanford Site had a reported maximum chromium concentration of approximately 2 mg/L during May 1996. Additionally, mixed cultures should be investigated since the subsurface is a mixed culture environment.

5)   Soluble forms of Cr(III) complexes that may have been produced by microbial metabolism were detected in the column effluent. Further research should be directed toward characterizing these soluble Cr(III) complexes and their stability in the environment. This is necessary because water quality standards for chromium is measured as *total soluble Cr* that includes both Cr(VI) and Cr(III).

6)   The success of *in-situ* bioremediation depends on the amount of biomass present in the subsurface. The effect of other electron donors and acceptors

should be studied to optimize microbial growth for Cr(VI) reduction. Studies may also be directed to distribute biomass evenly over the whole area since microbes tend to grow over a small area near the nutrient injection well.

7) The developed model should be validated with meso-scale continuous flow experiments prior to field scale application.

APPENDICES

Appendix A.

Mass Balance Equations

This Appendix contains the derivation of the mass balance equations for the all mobile and immobile species including the fate and transport of chromium. The advection and dispersion terms of the equations are similar for all mobile species but the reaction terms are different. Reaction kinetics were incorporated in RT3D as a user defined reaction.

Small representative control volume:
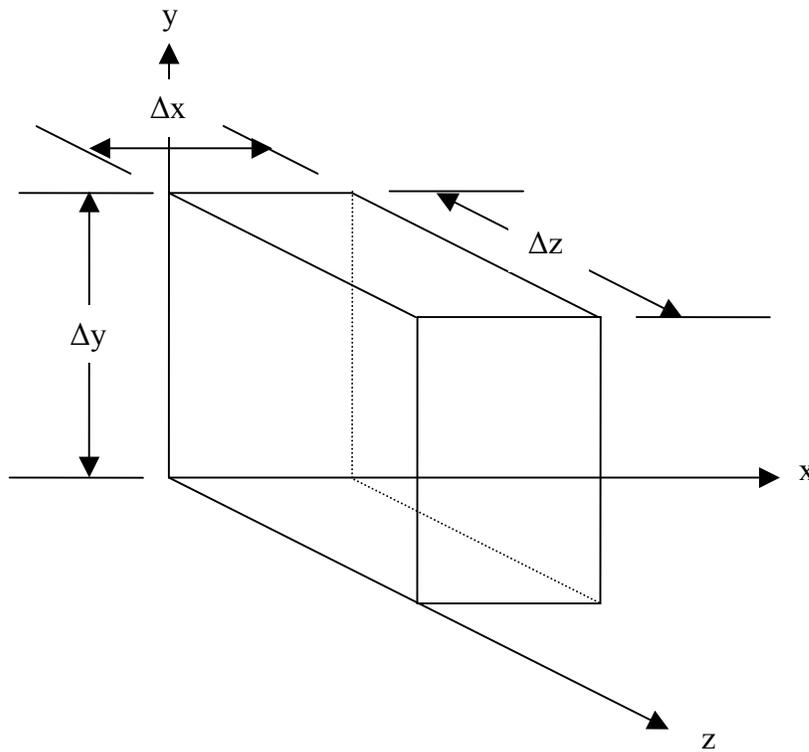
Total volume, $\Delta V_T = \Delta x \Delta y \Delta z$

Aqueous or pore volume, $\Delta V_s = \varepsilon \Delta x \Delta y \Delta z$; Where, ε = porosity

Mass Balance for Substrate:

v = groundwater flow velocity $[LT^{-1}]$

D = hydrodynamic dispersion coefficient $[L^2 T^{-1}]$

C = concentration of substrate $[ML^{-3}]$

$$vC - D\frac{\partial C}{\partial x} \qquad\qquad vC - D\frac{\partial C}{\partial x} + \frac{\partial}{\partial x}\left[vC - D\frac{\partial C}{\partial x}\right]\Delta x$$
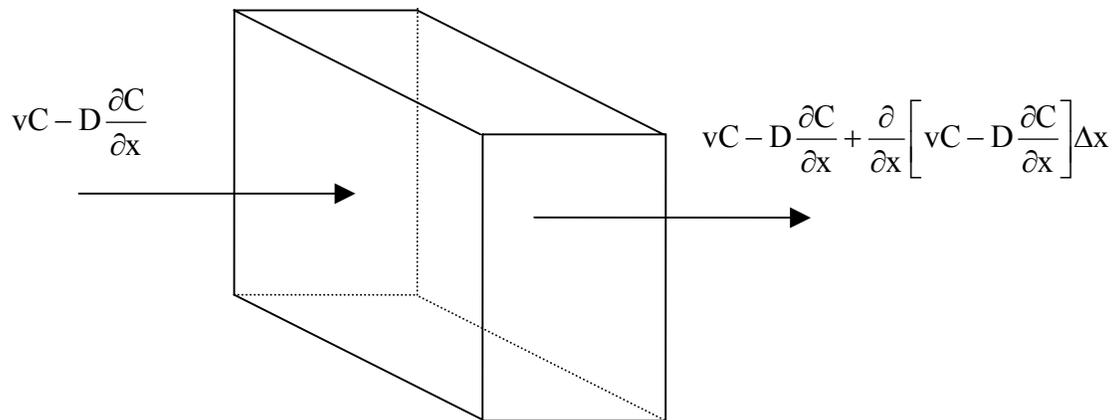
Overall rate of change = in – out – removal

$$= \text{in} - \text{out} - [\text{aqueous phase removal} + \text{attached phase removal}]$$

$$[\varepsilon \Delta x \Delta y \Delta z]\frac{\partial C}{\partial t} = \left[vC - D\frac{\partial C}{\partial x}\right][\varepsilon \Delta y \Delta z] - \left\{vC - D\frac{\partial C}{\partial x} + \frac{\partial}{\partial x}\left[vC - D\frac{\partial C}{\partial x}\right]\Delta x\right\}[\varepsilon \Delta y \Delta z]$$
$$- [r_s \Delta V_s + r_a \Delta V_a] \qquad (1)$$

Where:

ΔV_a = attached phase biomass volume = $\dfrac{X_a[\rho_b \Delta x \Delta y \Delta z]}{G_s \rho_w}$

X_a = attached phase biomass concentration [MM^{-1}]

ρ_b = bulk density of packing material [ML^{-3}]

G_s = specific gravity of biomass

ρ_w = density of water [ML^{-3}]

r_s = suspended phase reaction rate [T^{-1}]

r_a = attached phase reaction rate [T^{-1}]

From equation (1)

$$[\varepsilon \Delta x \Delta y \Delta z]\frac{\partial C}{\partial t} = \left[vC - D\frac{\partial C}{\partial x} \right][\varepsilon \Delta y \Delta z] - \left\{ vC - D\frac{\partial C}{\partial x} + \frac{\partial}{\partial x}\left[vC - D\frac{\partial C}{\partial x} \right]\Delta x \right\}[\varepsilon \Delta y \Delta z]$$
$$- \left[r_s \Delta V_s + r_a \Delta V_a \right]$$

$$[\varepsilon \Delta x \Delta y \Delta z]\frac{\partial C}{\partial t} = \left[vC - D\frac{\partial C}{\partial x} \right][\varepsilon \Delta y \Delta z] - \left[vC - D\frac{\partial C}{\partial x} \right][\varepsilon \Delta y \Delta z]$$
$$- \frac{\partial}{\partial x}\left[vC - D\frac{\partial C}{\partial x} \right]\Delta x[\varepsilon \Delta y \Delta z] - \left[r_s \Delta V_s + r_a \Delta V_a \right]$$

$$[\varepsilon \Delta x \Delta y \Delta z]\frac{\partial C}{\partial t} = -\frac{\partial}{\partial x}\left[vC - D\frac{\partial C}{\partial x} \right]\Delta x[\varepsilon \Delta y \Delta z] - \left[r_s \Delta V_s + r_a \Delta V_a \right]$$

$$\frac{\partial C}{\partial t} = -\frac{\partial}{\partial x}\left[vC - D\frac{\partial C}{\partial x} \right]\Delta x[\varepsilon \Delta y \Delta z]\frac{1}{[\varepsilon \Delta x \Delta y \Delta z]} - \left[r_s \Delta V_s + r_a \Delta V_a \right]\frac{1}{[\varepsilon \Delta x \Delta y \Delta z]}$$

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \left[r_s \Delta V_s + r_a \Delta V_a \right]\frac{1}{[\varepsilon \Delta x \Delta y \Delta z]}$$

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \left[r_s \varepsilon \Delta x \Delta y \Delta z + r_a \frac{X_a[\rho_b \Delta x \Delta y \Delta z]}{G_s \rho_w} \right]\frac{1}{[\varepsilon \Delta x \Delta y \Delta z]}$$

$$\frac{\partial C}{\partial t} = -v\frac{\partial C}{\partial x} + D\frac{\partial^2 C}{\partial x^2} - \left[r_s + r_a \frac{X_a \rho_b}{\varepsilon G_s \rho_w} \right] \tag{2}$$

Equation (2) is the generalized Advection-Dispersion equation accounted for reaction.

Fate and transport of Fumarate:

Suspended phase reaction rate

$$r_s = \frac{1}{Y_s} \left[\frac{\mu_{m,s} X_s F}{K_{m,s} + F} \frac{1}{1 + \dfrac{Cr}{K_i}} \right]$$

Where:

Y_s = yield at suspended phase [MM^{-1}]

$\mu_{m,s}$ = maximum specific growth rate at suspended phase [T^{-1}]

$K_{m,s}$ = suspended phase saturation constant [ML^{-3}]

X_s = suspended phase biomass concentration [ML^{-3}]

F = concentration of fumarate [ML^{-3}]

Cr = concentration of chromium [ML^{-3}]

K_i = growth inhibition constant [ML^{-3}]

Attached phase reaction rate

$$r_a = \frac{1}{Y_a} \left[\frac{\mu_{m,a} X_f F}{K_{m,a} + F} \frac{1}{1 + \dfrac{Cr}{K_i}} \right]$$

Where:

Y_a = yield at attached phase [MM^{-1}]

$\mu_{m,a}$ = maximum specific growth rate at attached phase [T^{-1}]

$K_{m,a}$ = attached phase saturation constant [ML^{-3}]

X_f = attached phase biomass concentration [ML^{-3}]

$$= \frac{\text{Biomass}}{\text{Volume}} = \frac{X_a[\rho_b \Delta x \Delta y \Delta z]}{\left[\dfrac{X_a[\rho_b \Delta x \Delta y \Delta z]}{G_s \rho_w} \right]} = G_s \rho_w$$

Assuming, $Y_s = Y_a = Y$

$\mu_{m,s} = \mu_{m,a} = \mu_m$

$K_{m,s} = K_{m,a} = K_m$

From equation (2)

$$\frac{\partial F}{\partial t} = -v\frac{\partial F}{\partial x} + D\frac{\partial^2 F}{\partial x^2} - \left[r_s + r_a \frac{X_a \rho_b}{\varepsilon G_s \rho_w} \right]$$

$$\frac{\partial F}{\partial t} = -v\frac{\partial F}{\partial x} + D\frac{\partial^2 F}{\partial x^2} - \left\{ \frac{1}{Y}\left[\frac{\mu_m X_s F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} \right] + \frac{1}{Y}\left[\frac{\mu_m X_f F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} \right]\frac{X_a \rho_b}{\varepsilon G_s \rho_w} \right\}$$

$$\frac{\partial F}{\partial t} = -v\frac{\partial F}{\partial x} + D\frac{\partial^2 F}{\partial x^2} - \left\{ \frac{1}{Y}\left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} \right]X_s + \frac{1}{Y}\left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} \right]G_s \rho_w \frac{X_a \rho_b}{\varepsilon G_s \rho_w} \right\}$$

$$\frac{\partial F}{\partial t} = -v\frac{\partial F}{\partial x} + D\frac{\partial^2 F}{\partial x^2} - \frac{1}{Y}\left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]\left[X_s + \frac{X_a \rho_b}{\epsilon}\right] \qquad (3)$$

Fate and transport of Chromium:

Suspended phase reaction rate

$$r_s = k_1 E_{ds} + k_2 X_s$$

Where:

k_1 = rate constant for Cr(VI) reduction due to the deactivating enzyme [T^{-1}]

E_{ds} = deactivating enzyme concentration in the suspended phase [ML^{-3}]

$k_2 = k_2^{\bullet}[E_s]_s$

$k_2^{\bullet}$ = rate constant for Cr(VI) reduction due to the stable enzyme [T^{-1}]

$[E_s]_s$ = specific stable enzyme concentration (MM^{-1})

Attached phase reaction rate

$$r_a = k_1 E_{df} + k_2 X_f$$

Where:

E_{df} = deactivating enzyme concentration in the attached phase [ML^{-3}]

$$= \frac{\text{Enzyme}}{\text{Volume}} = \frac{E_{da}[\rho_b \Delta x \Delta y \Delta z]}{\left[\dfrac{X_a[\rho_b \Delta x \Delta y \Delta z]}{G_s \rho_w}\right]} = \frac{E_{da}}{X_a} G_s \rho_w$$

From equation (2)

$$\frac{\partial Cr}{\partial t} = -v\frac{\partial Cr}{\partial x} + D\frac{\partial^2 Cr}{\partial x^2} - \left[k_1 E_{ds} + k_2 X_s + \{k_1 E_{df} + k_2 X_f\}\frac{X_a \rho_b}{\varepsilon G_s \rho_w}\right]$$

$$\frac{\partial Cr}{\partial t} = -v\frac{\partial Cr}{\partial x} + D\frac{\partial^2 Cr}{\partial x^2} - \left[k_1 E_{ds} + k_2 X_s + k_1 \frac{E_{da}}{X_a} G_s \rho_w \frac{X_a \rho_b}{\varepsilon G_s \rho_w} + k_2 G_s \rho_w \frac{X_a \rho_b}{\varepsilon G_s \rho_w}\right]$$

$$\frac{\partial Cr}{\partial t} = -v\frac{\partial Cr}{\partial x} + D\frac{\partial^2 Cr}{\partial x^2} - k_1\left[E_{ds} + \frac{\rho_b}{\varepsilon}E_{da}\right] - k_2\left[X_s + \frac{\rho_b}{\varepsilon}X_a\right] \qquad (4)$$

Mass balance for biomass at suspended phase
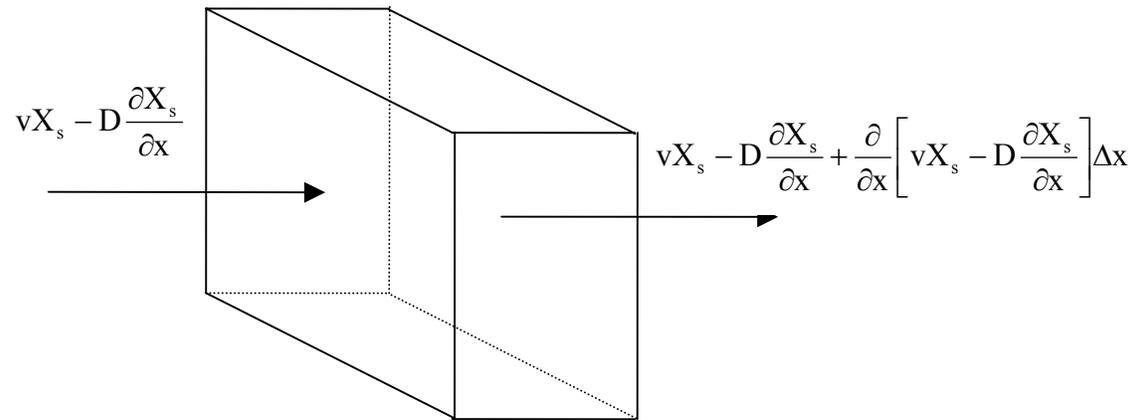
Overall rate of change = in – out – attachment + detachment + net growth

$$\left[\varepsilon\Delta x\Delta y\Delta z\right]\frac{\partial X_s}{\partial t} = \left[vX_s - D\frac{\partial X_s}{\partial x}\right]\left[\varepsilon\Delta y\Delta z\right]$$

$$-\left\{vX_s - D\frac{\partial X_s}{\partial x} + \frac{\partial}{\partial x}\left[vX_s - D\frac{\partial X_s}{\partial x}\right]\Delta x\right\}\left[\varepsilon\Delta y\Delta z\right]$$

$$+\left\{-r_{att} + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]X_s\right\}\left[\varepsilon\Delta x\Delta y\Delta z\right] + r_{det}\left[\rho_b\Delta x\Delta y\Delta z\right]$$

Where:

r_{att} = rate of attachment $[T^{-1}]$

r_{det} = rate of detachment

k_d = decay constant for biomass

$$\left[\varepsilon\Delta x\Delta y\Delta z\right]\frac{\partial X_s}{\partial t} = -\frac{\partial}{\partial x}\left[vX_s - D\frac{\partial X_s}{\partial x}\right]\Delta x\left[\varepsilon\Delta y\Delta z\right]$$

$$+\left\{-r_{att} + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]X_s\right\}\left[\varepsilon\Delta x\Delta y\Delta z\right] + r_{det}\left[\rho_b\Delta x\Delta y\Delta z\right]$$

$$\frac{\partial X_s}{\partial t} = -v\frac{\partial X_s}{\partial x} + D\frac{\partial^2 X_s}{\partial x^2} - r_{att} + r_{det}\frac{\rho_b}{\varepsilon} + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]X_s$$

If $r_{att} = K_{att}X_s$

$r_{det} = K_{det}X_a$

Where:

K_{att} = attachment coefficient $[T^{-1}]$

K_{det} = detachment coefficient $[T^{-1}]$

$$\frac{\partial X_s}{\partial t} = -v\frac{\partial X_s}{\partial x} + D\frac{\partial^2 X_s}{\partial x^2} - K_{att}X_s + K_{det}\frac{\rho_b}{\varepsilon}X_a + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]X_s \qquad (5)$$

Mass balance for biomass at attached phase

Overall rate of change = attachment – detachment + net growth

$$[\rho_b \Delta x \Delta y \Delta z]\frac{\partial X_a}{\partial t} = r_{att}[\varepsilon \Delta x \Delta y \Delta z] - r_{det}[\rho_b \Delta x \Delta y \Delta z]$$

$$+ \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]X_f\frac{X_a[\rho_b \Delta x \Delta y \Delta z]}{G_s \rho_w}$$

$$\frac{\partial X_a}{\partial t} = r_{att}\frac{\varepsilon}{\rho_b} - r_{det} + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]G_s \rho_w\frac{X_a}{G_s \rho_w}$$

$$\frac{\partial X_a}{\partial t} = K_{att}\frac{\varepsilon}{\rho_b}X_s - K_{det}X_a + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}} - k_d\right]X_a \qquad (6)$$

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Mass balance for deactivating enzyme at suspended phase
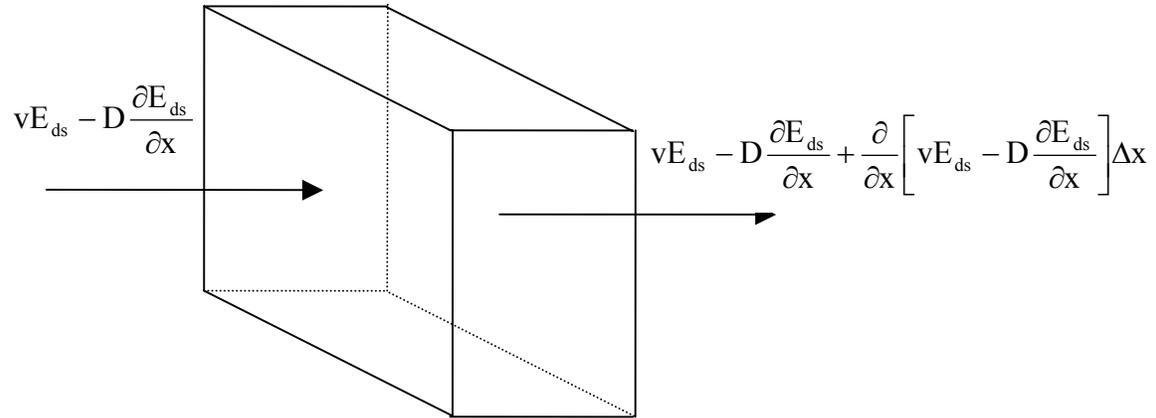
$vE_{ds} - D\dfrac{\partial E_{ds}}{\partial x}$ (on left side of box)

$vE_{ds} - D\dfrac{\partial E_{ds}}{\partial x} + \dfrac{\partial}{\partial x}\left[vE_{ds} - D\dfrac{\partial E_{ds}}{\partial x}\right]\Delta x$ (on right side of box)

Overall rate of change = in – out – attachment + detachment + net production

$$[\varepsilon\Delta x\Delta y\Delta z]\frac{\partial E_{ds}}{\partial t} = \left[vE_{ds} - D\frac{\partial E_{ds}}{\partial x}\right][\varepsilon\Delta y\Delta z]$$

$$-\left\{vE_{ds} - D\frac{\partial E_{ds}}{\partial x} + \frac{\partial}{\partial x}\left[vE_{ds} - D\frac{\partial E_{ds}}{\partial x}\right]\Delta x\right\}[\varepsilon\Delta y\Delta z] - r_{att}[\varepsilon\Delta x\Delta y\Delta z]$$

$$+ r_{det}[\rho_b\Delta x\Delta y\Delta z] + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]X_s E_{d0}[\varepsilon\Delta x\Delta y\Delta z] - k_d E_{ds}[\varepsilon\Delta x\Delta y\Delta z]$$

$$- k_{dc} E_{ds}[\varepsilon\Delta x\Delta y\Delta z]$$

Where:

E_{d0} = specific deactivating enzyme concentration related to new growth [MM^{-1}]

k_{dc} = first-order decay constant for the deactivating enzyme [T^{-1}]

$$\left[\varepsilon\Delta x\Delta y\Delta z\right]\frac{\partial E_{ds}}{\partial t} = -\frac{\partial}{\partial x}\left[vE_{ds} - D\frac{\partial E_{ds}}{\partial x}\right]\Delta x\left[\varepsilon\Delta y\Delta z\right] - r_{att}\left[\varepsilon\Delta x\Delta y\Delta z\right] + r_{det}\left[\rho_b\Delta x\Delta y\Delta z\right]$$

$$+ \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]X_s E_{d0}\left[\varepsilon\Delta x\Delta y\Delta z\right] - k_d E_{ds}\left[\varepsilon\Delta x\Delta y\Delta z\right] - k_{dc} E_{ds}\left[\varepsilon\Delta x\Delta y\Delta z\right]$$

$$\frac{\partial E_{ds}}{\partial t} = -v\frac{\partial E_{ds}}{\partial x} + D\frac{\partial^2 E_{ds}}{\partial x^2} - K_{att}E_{ds} + K_{det}\frac{\rho_b}{\varepsilon}E_{da}$$

$$+ \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]X_s E_{d0} - k_d E_{ds} - k_{dc} E_{ds} \tag{7}$$

Mass balance for deactivating enzyme at attached phase

Overall rate of change = attachment – detachment + net production

$$\left[\rho_b\Delta x\Delta y\Delta z\right]\frac{\partial E_{da}}{\partial t} = r_{att}\left[\varepsilon\Delta x\Delta y\Delta z\right] - r_{det}\left[\rho_b\Delta x\Delta y\Delta z\right] - k_{dc}E_{da}\left[\rho_b\Delta x\Delta y\Delta z\right]$$

$$+ \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]X_f E_{d0}\frac{X_a\left[\rho_b\Delta x\Delta y\Delta z\right]}{G_s\rho_w} - k_d E_{da}\left[\rho_b\Delta x\Delta y\Delta z\right]$$

$$\frac{\partial E_{da}}{\partial t} = r_{att}\frac{\varepsilon}{\rho_b} - r_{det} - k_{dc}E_{da} + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]G_s\rho_w E_{d0}\frac{X_a}{G_s\rho_w} - k_d E_{da}$$

$$\frac{\partial E_{da}}{\partial t} = K_{att}\frac{\varepsilon}{\rho_b}E_{ds} - K_{det}E_{da} - k_{dc}E_{da} + \left[\frac{\mu_m F}{K_m + F}\frac{1}{1+\dfrac{Cr}{K_i}}\right]X_a E_{d0} - k_d E_{da} \tag{8}$$

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Appendix B.

FORTRAN Code

This Appendix contains the FORTRAN source code of user defined reaction kinetics for Cr(VI) bioreduction. A DLL file from this code was created and incorporated into reaction modules of RT3D.

```
C*********************************************************
C USER DEFINED KINETICS FOR Cr(VI) BIOTRANSFORMATION
C*********************************************************
C
      SUBROUTINE Rxns(ncomp,nvrxndata,j,i,k,y,dydt,
     &          poros,rhob,reta,rc,nlay,nrow,ncol,vrc)
C
C*Block 1:*********************************************
C
C     List of calling arguments
C     ncomp - Total number of components
C     nvrxndata - Total number of variable reaction
C                 parameters to be input via RCT file
C     J, I, K - node location (used if reaction parameters
C               are spatially variable)
C     y - Concentration value of all component at the node
C         [array variable y(ncomp)]
```

```
C    dydt - Computed RHS of your differential equation

C          [array variable dydt(ncomp)]

C    poros - porosity of the node

C    reta -  Retardation factor [ignore dummy reta values

C          of immobile species]

C    rhob -  bulk density of the node

C    rc - Stores spatially constant reaction parameters

C        (can dimension upto 100 values)

C    nlay, nrow, ncol - Grid size (used only for

C                      dimensioning purposes)

C    vrc - Array variable that stores spatially variable

C        reaction parameters

C*End of Block 1****************************************

C

C*Block 2:**********************************************

C*    *Please do not modify this standard interface block*

      !MS$ATTRIBUTES DLLEXPORT :: rxns

      IMPLICIT NONE

      INTEGER ncol,nrow,nlay

      INTEGER ncomp,nvrxndata,j,i,k

      INTEGER, SAVE :: First_time=1

      DOUBLE PRECISION y,dydt,poros,rhob,reta

      DOUBLE PRECISION rc,vrc

      DIMENSION y(ncomp),dydt(ncomp),rc(100)

      DIMENSION vrc(ncol,nrow,nlay,nvrxndata),reta(ncomp)

C*End of block 2****************************************
```

```
C
C*Block 3:*******************************************
C      *Declaring problem-specific new variables here*
C
C      Variable names
C      cr – Chromium(VI)
C      ea – Electron acceptor (Fumarate)
C      xl – Aqueous phase protein
C      xs – Attached phase protein
C      e1l – Aqueous phase deactivating enzyme
C      e1s – Attached phase deactivating enzyme
C
C      Parameter list
C      k1 - Zero order rate constant for deactivating enzyme
C      rs0 – Constant for stable enzyme = k2.[Es]; k2 = zero
C             order rate const for stable enzyme; [Es] =
C             stable enzyme concentration
C      kdprime - Coefficient analogous to 1st order
C                deactivation constant = k1.Kc; Kc = A
C                stoichiometric coefficient analogous to
C                amount of deactivating enzyme needed per
C                unit amount of Cr(VI) reduced
C      kmcr – A small value (0.0001) to account for Cr(VI)
C              free growth period
C      ki – Growth inhibition constant due to Cr(VI)
C      mumax - Maximum specific growth rate constant
```

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```
C     kmea - Half saturation constant for electron acceptor

C     yld - Bacterial growth yield

C     kd - Microbial death rate constant

C     ed0 - Specific deactivating enzyme concentration at

C          the beginning

C     katt - attachment coefficient

C     kdet - detachment coefficient

C

C     INTEGER
      DOUBLE PRECISION cr, ea, xl, xs, ell, els
      DOUBLE PRECISION k1, rs0, kdprime, kmcr, ki
      DOUBLE PRECISION mumax, kmea, yld, kd, ed0
      DOUBLE PRECISION katt, kdet

C

C*End of block 3*****************************************

C

C*Block 4:**********************************************

C       *Initilize reaction parameters here, if required*

      IF (First_time .EQ. 1) THEN

         k1 = rc(1)

         rs0 = rc(2)

         kdprime = rc(3)

         mumax = rc(4)

         kmea = rc(5)

         kmcr = rc(6)

         ki = rc(7)
```

```
          yld = rc(8)

          katt = rc(9)

          kdet = rc(10)

          ed0 = rc(11)

          kd = rc(12)

C

          First_time = 0 !reset First_time to skip this

C                         block later

      END IF

C

C*End of block 4****************************************

C

C*Block 5:*********************************************

C     *Assign or compute values for new variables, if

C      required*

       cr = y(1)

       ea = y(2)

       xl = y(3)

       xs = y(4)

      ell = y(5)

      els = y(6)

C

C*End of block 5****************************************

C

C*Block 6:*********************************************

C     *Differential Reaction Equations*
```

```
C

      dydt(1) = - k1*(e1l+rhob*e1s/poros)*(cr/(kmcr+cr))
     +             - rs0*(xl+rhob*xs/poros)*(cr/(kmcr+cr))
C

      dydt(2) = - 1.0/yld*(mumax*ea/(kmea+ea))*
     +            (1.0/(1.0+cr/ki))*(xl+rhob*xs/poros)
C

      dydt(3) = ((mumax*ea/(kmea+ea))*(1.0/(1.0+cr/ki))-
     +            kd)*xl - katt*xl + kdet*rhob*xs/poros
C

      dydt(4) = ((mumax*ea/(kmea+ea))*(1.0/(1.0+cr/ki))-
     +            kd)*xs + katt*poros*xl/rhob - kdet*xs
C

      dydt(5) = (mumax*ea/(kmea+ea))*(1.0/(1.0+cr/ki))*xl*
     +            ed0 - kd*e1l - katt*e1l + kdet*rhob*
     +            e1s/poros - kdprime*e1l*(cr/(kmcr+cr))
C

      dydt(6) = (mumax*ea/(kmea+ea))*(1.0/(1.0+cr/ki))*xs*
     +            ed0 - kd*e1s + katt*poros*e1l/rhob -
     +            kdet*e1s - kdprime*e1s*(cr/(kmcr+cr))
C
C*End of block 6***************************************
C

      RETURN

      END
```

Appendix C.

Data

This Appendix contains representative data for all conducted experiments. It was not possible to include all data collected during the course of this research, such as chromatograms and computer simulations, however, the most relevant data has been summarized in tabular format.

TABLE 4. Influent and Effluent Cr(VI) absorbance data from an experiment with 0.055 mM influent Cr(VI) concentration

Column Start Time: 4/17/2003 11:00 hrs

Filtered Influent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
4/25/2003 18:00	8.29	0.4595	2.81
4/27/2003 17:45	10.28	0.4464	2.72
4/27/2003 18:00	10.29	0.4518	2.76
4/29/2003 20:45	12.41	0.4507	2.75
4/29/2003 21:00	12.42	0.4496	2.74
5/1/2003 17:45	14.28	0.4341	2.64
5/1/2003 18:00	14.29	0.4393	2.67
5/3/2003 17:45	16.28	0.4502	2.74
5/3/2003 18:00	16.29	0.4634	2.83
5/5/2003 17:45	18.28	0.4515	2.75
5/5/2003 18:00	18.29	0.5017	3.09
5/7/2003 20:45	20.41	0.4964	3.05
5/7/2003 21:00	20.42	0.5076	3.12
5/10/2003 0:15	22.55	0.4974	3.06

Filtered Effluent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
4/26/2003 10:00	8.96	0.0338	0.00
4/27/2003 10:00	9.96	0.0337	0.00
4/28/2003 10:00	10.96	0.0348	0.00
4/29/2003 10:45	11.99	0.0365	0.00
4/30/2003 9:00	12.92	0.0732	0.25
5/1/2003 10:00	13.96	0.2813	1.63
5/2/2003 9:00	14.92	0.2982	1.74
5/3/2003 10:00	15.96	0.3174	1.87
5/4/2003 11:00	17.00	0.323	1.90
5/5/2003 10:00	17.96	0.358	2.13
5/6/2003 10:00	18.96	0.3559	2.12
5/7/2003 10:30	19.98	0.4176	2.53

Date & Time	Days	Absorbance	Cr(VI) mg/L
5/8/2003 12:00	21.04	0.4048	2.44
5/9/2003 9:30	21.94	0.4144	2.51
5/10/2003 0:15	22.55	0.4127	2.50

TABLE 5. Aqueous phase cell count as colony forming units (CFUs) in the column

effluent from an experiment with 0.055 mM influent Cr(VI) concentration

Date & Time	Days	CFUs	Dilution Factor	CFU/mL
4/18/2003 9:45	0.95	183	1.00E-04	18300000
4/20/2003 12:45	3.07	64	1.00E-04	6400000
4/22/2003 9:45	4.95	185	1.00E-03	1850000
4/24/2003 9:45	6.95	50	1.00E-03	500000
4/26/2003 10:00	8.96	43	1.00E-03	430000
4/28/2003 10:00	10.96	44	1.00E-04	4400000
4/30/2003 9:00	12.92	189	1.00E-04	18900000
5/2/2003 9:00	14.92	46	1.00E-03	460000
5/4/2003 11:00	17.00	5	1.00E-03	50000
5/6/2003 10:00	18.96	35	1.00E-02	35000
5/8/2003 12:00	21.04	43	1.00E-03	430000

TABLE 6. Lactate (L), Acetate (A), Fumarate (F) and Succinate (S) data from IC chromatograms for an experiment with 0.055 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
4/17/2003 11:00	0.00	1082029	0	0	1694692	304	0	0	246
4/19/2003 17:45	2.28	1116575	0	0	1927007	314	0	0	276
4/23/2003 17:45	6.28	843131	0	0	1812763	239	0	0	261
4/25/2003 17:45	8.28	842048	0	0	1805658	238	0	0	260
4/29/2003 20:45	12.41	850431	0	0	1830988	241	0	0	264
5/3/2003 17:45	16.28	838425	0	0	1806053	237	0	0	261
5/7/2003 20:45	20.41	1007568	0	0	1823570	284	0	0	263
5/10/2003 0:15	22.55	1013274	0	0	1835434	285	0	0	264

Filtered Effluent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
4/18/2003 9:45	0.95	474565	565285	1386631	0	138	108	267	0
4/19/2003 10:45	1.99	642648	466926	1276410	0	184	90	246	0
4/20/2003 12:45	3.07	704538	345850	1230236	0	201	67.9	237	0
4/21/2003 10:00	3.96	451367	346256	1165608	0	131	68	224	0
4/22/2003 9:45	4.95	460344	340418	1166722	0	134	66.9	225	0
4/23/2003 9:45	5.95	457523	335258	1178280	0	133	66	227	0
4/24/2003 9:45	6.95	451349	357625	1169171	0	131	70.1	225	0
4/25/2003 9:45	7.95	449202	340569	1170880	0	131	67	225	0
4/26/2003 10:00	8.96	399674	404104	1150310	0	117	78.5	221	0
4/27/2003 10:00	9.96	429395	347770	1131687	0	125	68.3	218	0
4/28/2003 10:00	10.96	409519	343424	1110279	0	120	67.5	213	0
4/29/2003 10:45	11.99	423691	316884	1117005	0	124	62.6	215	0
4/30/2003 9:00	12.92	600590	144300	661812	790404	172	31.2	126	127
5/1/2003 10:00	13.96	804188	0	56478	1692066	228	0	7.7	246
5/2/2003 9:00	14.92	804223	0	53902	1691190	228	0	7.2	245
5/3/2003 10:00	15.96	793057	22976	66768	1697653	225	9.13	9.71	246
5/4/2003 11:00	17.00	743354	0	84126	1702869	211	0	13.1	247
5/5/2003 10:00	17.96	893107	0	93085	1722326	252	0	14.9	250
5/6/2003 10:00	18.96	885490	0	103867	1706572	250	0	17	247
5/7/2003 10:30	19.98	986325	0	99337	1735247	278	0	16.1	251
5/8/2003 12:00	21.04	978225	0	91840	1758191	276	0	14.6	254
5/9/2003 9:30	21.94	995734	0	94990	1773662	281	0	15.2	256

TABLE 7. Total Cr data from ICP-MS for an experiment with 0.055 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
4/25/2003 18:00	8.29	2.81	3.00	0.19
4/27/2003 17:45	10.28	2.72	2.86	0.14
4/27/2003 18:00	10.29	2.76	2.90	0.15
4/29/2003 20:45	12.41	2.75	2.83	0.08
4/29/2003 21:00	12.42	2.74	2.95	0.21
5/1/2003 17:45	14.28	2.64	2.94	0.30
5/1/2003 18:00	14.29	2.67	2.87	0.19
5/3/2003 17:45	16.28	2.74	2.85	0.11
5/3/2003 18:00	16.29	2.83	2.98	0.15
5/5/2003 17:45	18.28	2.75	2.67	0.00
5/5/2003 18:00	18.29	3.09	3.13	0.04
5/7/2003 20:45	20.41	3.05	3.12	0.07
5/7/2003 21:00	20.42	3.12	3.38	0.25
5/10/2003 0:15	22.55	3.06	3.36	0.31

Filtered Effluent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
4/26/2003 10:00	8.96	0.00	0.04	0.04
4/27/2003 10:00	9.96	0.00	0.66	0.66
4/28/2003 10:00	10.96	0.00	1.04	1.04
4/29/2003 10:45	11.99	0.00	1.46	1.46
4/30/2003 9:00	12.92	0.25	1.71	1.45
5/1/2003 10:00	13.96	1.63	2.39	0.77
5/2/2003 9:00	14.92	1.74	2.39	0.65
5/3/2003 10:00	15.96	1.87	2.45	0.58
5/4/2003 11:00	17.00	1.90	2.51	0.61
5/5/2003 10:00	17.96	2.13	2.73	0.60
5/6/2003 10:00	18.96	2.12	2.71	0.59
5/7/2003 10:30	19.98	2.53	3.03	0.50
5/8/2003 12:00	21.04	2.44	2.96	0.52
5/9/2003 9:30	21.94	2.51	3.00	0.49

Unfiltered Effluent Samples

Date & Time	Days	Total Cr mg/L
4/27/2003 10:00	9.96	2.16
4/29/2003 10:45	11.99	2.40
5/1/2003 10:00	13.96	2.54
5/3/2003 10:00	15.96	2.53
5/5/2003 10:00	17.96	2.85
5/7/2003 10:30	19.98	3.03
5/9/2003 9:30	21.94	2.95

TABLE 8. Sand total Cr data from ICP-MS for an experiment with 0.055 mM influent

Cr(VI) concentration

Segment	Distance	Total Cr mg/L	Cr mg	Sand g	Total Cr μg/g sand
0-1 cm	0.5	5.59	0.0056	1.11	5.03
1-2 cm	1.5	4.02	0.0040	0.89	4.52
2-4 cm	3.0	3.40	0.0034	1.75	1.94
4-6 cm	5.0	3.90	0.0039	0.87	4.48
6-8 cm	7.0	5.78	0.0058	1.27	4.55
8-10 cm	9.0	4.91	0.0049	0.88	5.58
10-12 cm	11.0	7.25	0.0072	1.42	5.10
12-15 cm	13.5	7.81	0.0078	1.47	5.31

TABLE 9. Sand protein data for an experiment with 0.055 mM influent Cr(VI) concentration

Depth range	Replica	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-15 cm
Distance		0.5	1.5	3.0	5.0	7.0	9.0	11.0	13.5
Absorbance	1	0.4257	0.3725	0.4432	0.4337	0.5123	0.4538	0.4356	0.4774
	2	0.4654	0.4539	0.4695	0.4426	0.4838	0.4839	0.4528	0.4641
	3	0.3993	0.4689	0.4953	0.4342	0.5562	0.5072	0.5208	0.4839
Protein	1	22.80	16.15	24.99	23.80	33.63	26.31	24.04	29.26
(mg/L)	2	27.76	26.33	28.28	24.91	30.06	30.08	26.19	27.60
	3	19.50	28.20	31.50	23.86	39.11	32.99	34.69	30.08
Protein before	1	22.80	16.15	24.99	23.80	33.63	26.31	24.04	29.26
dilution (mg/L)	2	27.76	26.33	28.28	24.91	30.06	30.08	26.19	27.60
	3	19.50	28.20	31.50	23.86	39.11	32.99	34.69	30.08
Protein (mg)	1	0.0228	0.0162	0.0250	0.0238	0.0336	0.0263	0.0240	0.0293
	2	0.0278	0.0263	0.0283	0.0249	0.0301	0.0301	0.0262	0.0276
	3	0.0195	0.0282	0.0315	0.0239	0.0391	0.0330	0.0347	0.0301
Sand weight	1	1.34	0.90	1.91	1.57	1.93	1.73	1.63	1.69
(gm)	2	1.47	1.61	1.94	1.57	1.60	1.95	1.63	1.63
	3	1.05	1.65	2.09	1.31	1.99	1.83	2.34	1.99
Sand protein	1	17.01	17.94	13.08	15.16	1.46	1.23	1.92	17.32
(mg/g)	2	18.89	16.35	14.57	15.87	18.79	15.42	16.07	16.93
	3	18.57	17.09	15.07	18.22	19.65	18.03	14.82	15.11
Average		18.16	17.13	14.24	16.41	13.30	11.56	10.94	16.45
S.D.[1]		1.00	0.80	1.04	1.60	10.26	9.04	7.83	1.18
C.I.[2]		1.13	0.90	1.17	1.81	11.61	10.23	8.86	1.33

[1]Standard deviation, [2]Confidence interval (95%)

TABLE 10. Influent and Effluent Cr(VI) absorbance data from an experiment with 0.049

mM influent Cr(VI) concentration

Column Start Time: 11/28/2002 14:45 hrs

Filtered Influent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
12/6/2002 15:00	8.01	0.4864	2.77
12/12/2002 0:15	13.40	0.4183	2.35
12/12/2002 0:30	13.41	0.4670	2.65
12/17/2002 12:00	18.89	0.4197	2.36

Filtered Effluent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
12/8/2002 14:00	9.97	0.0343	0.00
12/9/2002 12:00	10.89	0.0316	0.00
12/10/2002 13:00	11.93	0.0341	0.00
12/11/2002 11:15	12.85	0.0350	0.00
12/12/2002 13:00	13.93	0.0347	0.00
12/13/2002 11:15	14.85	0.0404	0.04
12/14/2002 13:00	15.93	0.3154	1.72
12/15/2002 13:00	16.93	0.3454	1.91
12/16/2002 11:30	17.86	0.3738	2.08
12/17/2002 11:00	18.84	0.3697	2.05

TABLE 11. Aqueous phase cell count as colony forming units (CFUs) in the column

effluent from an experiment with 0.049 mM influent Cr(VI) concentration

Date & Time	Days	CFUs	Dilution Factor	CFU/mL
11/30/2002 13:00	1.93	148	1.00E-03	1480000
12/2/2002 12:45	3.92	102	1.00E-03	1020000
12/4/2002 13:00	5.93	182	1.00E-03	1820000
12/9/2002 12:00	10.89	26	1.00E-03	260000

TABLE 12. Lactate (L), Acetate (A), Fumarate (F) and Succinate (S) data from IC

chromatograms for an experiment with 0.049 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
11/28/2002 14:45	0.00	907101	0	0	1797284	315	0	0	300
12/3/2002 12:00	4.89	891454	0	0	1767986	309	0	0	295
12/3/2002 12:15	4.90	903240	0	0	1800206	313	0	0	301
12/6/2002 14:45	8.00	915496	0	0	1820566	317	0	0	304
12/6/2002 15:00	8.01	883366	0	0	1764632	307	0	0	295
12/12/2002 0:15	13.40	844064	0	0	1696298	293	0	0	283
12/12/2002 0:30	13.41	868601	0	0	1742651	302	0	0	291
12/17/2002 13:00	18.93	904896	0	0	1807732	314	0	0	302

Filtered Effluent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
11/30/2002 12:00	1.89	479665	351598	1156746	0	171	73.7	178	0
12/2/2002 12:00	3.89	519958	326615	1076380	0	185	68.8	166	0
12/4/2002 12:00	5.89	544163	325577	1108712	0	193	68.6	171	0
12/7/2002 12:00	8.89	544589	323374	1130836	0	193	68.2	174	0
12/8/2002 14:00	9.97	485430	301977	1096135	0	173	64.1	169	0
12/9/2002 12:00	10.89	450943	293666	1051104	0	161	62.4	163	0
12/10/2002 13:00	11.93	462743	293003	1071438	0	165	62.3	166	0
12/11/2002 11:15	12.85	446991	290887	1087291	0	160	61.9	168	0
12/12/2002 13:00	13.93	419400	282144	1068961	0	151	60.2	165	0
12/13/2002 11:15	14.85	364986	278293	1011954	96924	132	59.5	157	18
12/14/2002 13:00	15.93	850698	0	43197	1644441	296	0	12.3	275
12/15/2002 13:00	16.93	846145	0	26640	1660365	294	0	9.8	277
12/16/2002 11:30	17.86	835626	0	23777	1635597	291	0	9.37	273
12/17/2002 11:00	18.84	822749	0	27876	1589423	286	0	9.98	266

TABLE 13. Total Cr data from ICP-MS for an experiment with 0.049 mM influent

Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
12/6/2002 15:00	8.01	2.77	3.44	0.67
12/12/2002 0:15	13.40	2.35	2.61	0.26
12/12/2002 0:30	13.41	2.65	3.03	0.38
12/17/2002 12:00	18.89	2.36	3.12	0.76

Filtered Effluent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
12/8/2002 14:00	9.97	0.00	0.54	0.54
12/9/2002 12:00	10.89	0.00	0.92	0.92
12/10/2002 13:00	11.93	0.00	1.19	1.19
12/11/2002 11:15	12.85	0.00	1.23	1.23
12/12/2002 13:00	13.93	0.00	1.40	1.40
12/13/2002 11:15	14.85	0.04	1.76	1.72
12/14/2002 13:00	15.93	1.72	2.54	0.82
12/15/2002 13:00	16.93	1.91	2.65	0.75
12/16/2002 11:30	17.86	2.08	2.89	0.81
12/17/2002 11:00	18.84	2.05	2.90	0.85

Unfiltered Effluent Samples

Date & Time	Days	Total Cr mg/L
12/9/2002 12:00	10.89	0.87
12/10/2002 13:00	11.93	1.20
12/11/2002 11:15	12.85	1.50
12/12/2002 13:00	13.93	1.40
12/13/2002 11:15	14.85	1.86
12/14/2002 13:00	15.93	2.62
12/15/2002 13:00	16.93	2.65
12/16/2002 11:30	17.86	2.70

Date & Time	Days	Total Cr mg/L
12/17/2002 11:00	18.84	2.86

TABLE 14. Sand total Cr data from ICP-MS for an experiment with 0.049 mM influent

Cr(VI) concentration

Segment	Distance	Total Cr mg/L	Cr mg	Sand g	Total Cr µg/g sand
0-1 cm	0.5	1.93	0.0019	0.78	2.47
1-2 cm	1.5	2.63	0.0026	0.87	3.02
2-4 cm	3.0	2.80	0.0028	1.27	2.21
4-6 cm	5.0	3.50	0.0035	1.74	2.01
6-8 cm	7.0	3.10	0.0031	1.18	2.63
8-10 cm	9.0	3.29	0.0033	1.13	2.91
10-12 cm	11.0	3.48	0.0035	1.43	2.44
12-15 cm	13.5	4.33	0.0043	2.02	2.14

TABLE 15. Sand protein data for an experiment with 0.049 mM influent Cr(VI) concentration

Depth range	Replica	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-15 cm
Distance		0.5	1.5	3.0	5.0	7.0	9.0	11.0	13.5
Absorbance	1	0.4248	0.3588	0.3896	0.4510	0.4194	0.4717	0.4689	0.4943
	2	0.5185	0.3941	0.3835	0.4334	0.4509	0.4617	0.5583	0.5334
	3	0.4617	0.4306	0.4137	0.4153	0.4766	0.4878	0.4961	0.5544
Protein	1	13.42	5.06	8.96	16.73	12.73	19.35	19.00	22.22
(mg/L)	2	25.28	9.53	8.19	14.51	16.72	18.09	30.32	27.16
	3	18.09	14.15	12.01	12.22	19.97	21.39	22.44	29.82
Protein before	1	13.42	5.06	8.96	16.73	12.73	19.35	19.00	22.22
dilution (mg/L)	2	25.28	9.53	8.19	14.51	16.72	18.09	30.32	27.16
	3	18.09	14.15	12.01	12.22	19.97	21.39	22.44	29.82
Protein (mg)	1	0.0134	0.0051	0.0090	0.0167	0.0127	0.0194	0.0190	0.0222
	2	0.0253	0.0095	0.0082	0.0145	0.0167	0.0181	0.0303	0.0272
	3	0.0181	0.0142	0.0120	0.0122	0.0200	0.0214	0.0224	0.0298
Sand weight	1	1.42	1.27	1.69	1.92	1.83	2.19	1.93	2.15
(gm)	2	1.21	1.55	1.86	1.75	2.07	2.37	2.05	2.3
	3	1.39	1.43	1.8	1.55	2.07	2.19	1.87	2.5
Sand protein	1	9.45	3.99	5.30	8.72	1.46	1.23	1.92	10.33
(mg/g)	2	20.89	6.15	4.40	8.29	8.08	7.63	14.79	11.81
	3	13.01	9.90	6.67	7.88	9.65	9.77	12.00	11.93
Average		14.45	6.68	5.46	8.30	6.40	6.21	9.57	11.36
S.D.[1]		5.86	2.99	1.14	0.42	4.35	4.44	6.77	0.89
C.I.[2]		6.63	3.38	1.29	0.47	4.92	5.03	7.66	1.01

[1]Standard deviation, [2]Confidence interval (95%)

TABLE 16. Influent and Effluent Cr(VI) absorbance data from an experiment with 0.037

mM influent Cr(VI) concentration

Column Start Time: 4/17/2003 11:00 hrs

Filtered Influent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
4/25/2003 18:00	8.29	0.3221	1.90
4/27/2003 17:45	10.28	0.301	1.76
4/27/2003 18:00	10.29	0.3111	1.82
4/29/2003 20:45	12.41	0.3105	1.82
4/29/2003 21:00	12.42	0.3103	1.82
5/1/2003 17:45	14.28	0.3051	1.79
5/1/2003 18:00	14.29	0.304	1.78
5/3/2003 17:45	16.28	0.3039	1.78
5/3/2003 18:00	16.29	0.3586	2.14
5/5/2003 17:45	18.28	0.3662	2.19
5/5/2003 18:00	18.29	0.3317	1.96
5/7/2003 20:45	20.41	0.3417	2.03
5/7/2003 21:00	20.42	0.342	2.03
5/10/2003 0:15	22.55	0.3413	2.02
5/10/2003 0:30	22.56	0.3373	2.00
5/12/2003 10:45	24.99	0.3353	1.98
5/12/2003 11:00	25.00	0.343	2.04
5/14/2003 18:45	27.32	0.3375	2.00
5/14/2003 19:00	27.33	0.3309	1.96
5/16/2003 17:00	29.25	0.3325	1.97

Filtered Effluent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
4/26/2003 10:00	8.96	0.0339	0.00
4/27/2003 10:00	9.96	0.031	0.00
4/28/2003 10:00	10.96	0.0321	0.00
4/29/2003 10:45	11.99	0.0352	0.00
4/30/2003 9:00	12.92	0.0354	0.00
5/1/2003 11:00	14.00	0.0357	0.00

Date & Time	Days	Absorbance	Cr(VI) mg/L
5/2/2003 9:00	14.92	0.0335	0.00
5/3/2003 10:00	15.96	0.0333	0.00
5/4/2003 11:00	17.00	0.0343	0.00
5/5/2003 13:00	18.08	0.0336	0.00
5/6/2003 10:00	18.96	0.0367	0.00
5/7/2003 10:30	19.98	0.0367	0.00
5/8/2003 12:00	21.04	0.0417	0.04
5/9/2003 9:30	21.94	0.0989	0.42
5/10/2003 8:30	22.90	0.1591	0.82
5/11/2003 10:00	23.96	0.1961	1.06
5/12/2003 10:00	24.96	0.1951	1.06
5/13/2003 10:00	25.96	0.1938	1.05
5/14/2003 10:00	26.96	0.1994	1.09
5/15/2003 10:00	27.96	0.1962	1.06
5/16/2003 10:00	28.96	0.207	1.14
5/16/2003 17:00	29.25	0.2115	1.17

TABLE 17. Aqueous phase cell count as colony forming units (CFUs) in the column

effluent from an experiment with 0.037 mM influent Cr(VI) concentration

Date & Time	Days	CFUs	Dilution Factor	CFU/mL
4/18/2003 9:45	0.95	401	1.00E-04	40100000
4/20/2003 12:45	3.07	58	1.00E-04	5800000
4/22/2003 9:45	4.95	260	1.00E-03	2600000
4/24/2003 9:45	6.95	107	1.00E-03	1070000
4/26/2003 10:00	8.96	46	1.00E-03	460000
4/28/2003 10:00	10.96	99	1.00E-03	990000
4/30/2003 9:00	12.92	79	1.00E-03	790000
5/2/2003 9:00	14.92	71	1.00E-03	710000
5/4/2003 11:00	17.00	78	1.00E-03	780000
5/6/2003 10:00	18.96	168	1.00E-04	16800000
5/8/2003 12:00	21.04	158	1.00E-04	15800000
5/10/2003 8:30	22.90	157	1.00E-03	1570000
5/12/2003 10:00	24.96	24	1.00E-03	240000
5/14/2003 10:00	26.96	112	1.00E-02	112000

TABLE 18. Lactate (L), Acetate (A), Fumarate (F) and Succinate (S) data from IC chromatograms for an experiment with 0.037 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
4/17/2003 11:00	0.00	1082029	0	0	1694692	309	0	0	247
4/19/2003 17:45	2.28	1094929	0	0	1714424	312	0	0	249
4/21/2003 17:45	4.28	867600	0	0	1629100	249	0	0	238
4/23/2003 17:45	6.28	836568	0	0	1615538	240	0	0	236
4/25/2003 17:45	8.28	841263	0	0	1623297	242	0	0	237
4/27/2003 18:00	10.29	847459	0	0	1832577	238	0	0	235
5/1/2003 17:45	14.28	888506	0	0	1902170	250	0	0	244
5/3/2003 17:45	16.28	840470	0	0	1820105	237	0	0	234
5/5/2003 17:45	18.28	956720	0	0	1880580	268	0	0	241
5/10/2003 0:15	22.55	1012341	0	0	1846108	283	0	0	237
5/12/2003 10:45	24.99	1016624	0	0	1844463	285	0	0	237
5/14/2003 18:45	27.32	993167	0	0	1805250	278	0	0	232
5/16/2003 17:00	29.25	1006884	0	0	1844958	282	0	0	237

Filtered Effluent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
4/18/2003 9:45	0.95	482760	497852	1435381	0	142	96.4	280	0
4/19/2003 10:45	1.99	640931	438002	1315807	0	186	85.4	256	0
4/20/2003 12:45	3.07	719198	366976	1251269	0	208	72.2	242	0
4/21/2003 10:00	3.96	460645	350875	1177588	0	135	69.2	228	0
4/22/2003 9:45	4.95	414660	397381	1148916	0	123	77.9	222	0
4/23/2003 9:45	5.95	453294	368023	1168747	0	133	72.4	226	0
4/24/2003 9:45	6.95	420810	376644	1147699	0	124	74	222	0
4/25/2003 9:45	7.95	447579	350116	1120131	0	132	69.1	216	0
4/26/2003 10:00	8.96	390711	376822	1092201	0	116	74	210	0
4/27/2003 10:00	9.96	429566	342569	1115524	0	127	67.7	215	0
4/28/2003 10:00	10.96	418993	323334	1080227	0	124	64.2	208	0
4/29/2003 10:45	11.99	421043	337178	1108163	0	124	66.7	214	0
4/30/2003 9:00	12.92	422244	338388	1098768	0	125	66.9	212	0
5/1/2003 11:00	14.00	400258	76658	1096186	0	119	18.5	211	0
5/2/2003 9:00	14.92			1075139	0			207	0
5/3/2003 10:00	15.96	374284	90839	1081335	0	111	21.1	208	0
5/4/2003 11:00	17.00	419987	333702	1082563	0	124	66.1	208	0
5/5/2003 13:00	18.08	560338	124380	1116509	0	163	27.3	215	0
5/6/2003 10:00	18.96			1106579	0			213	0
5/7/2003 10:30	19.98	520451	126752	959014	98065	152	27.8	184	26.5
5/8/2003 12:00	21.04	637888	400319	868119	212558	185	78.4	165	42.3
5/9/2003 9:30	21.94	842842	38381	503747	984363	242	11.4	91.8	149

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
5/10/2003 8:30	22.90	1005460	559543	287693	1368811	287	108	48.2	202
5/11/2003 10:00	23.96	902398	0	175652	1505216	259	0	25.6	221
5/12/2003 10:00	24.96	1075367	795602	171678	1528103	307	152	24.8	224
5/13/2003 10:00	25.96	979164	0	161973	1534875	280	0	22.8	225
5/14/2003 10:00	26.96	962006	427614	195803	1473042	275	83.4	29.6	216
5/15/2003 10:00	27.96	926070	0	237191	1443290	265	0	38	212
5/16/2003 10:00	28.96	929620	0	183391	1454196	266	0	27.1	214

TABLE 19. Total Cr data from ICP-MS for an experiment with 0.037 mM influent

Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
4/25/2003 18:00	8.29	1.90	1.94	0.04
4/27/2003 17:45	10.28	1.76	1.82	0.07
4/27/2003 18:00	10.29	1.82	2.00	0.18
4/29/2003 20:45	12.41	1.82	1.94	0.12
4/29/2003 21:00	12.42	1.82	1.94	0.12
5/1/2003 17:45	14.28	1.79	1.94	0.16
5/1/2003 18:00	14.29	1.78	1.92	0.14
5/3/2003 17:45	16.28	1.78	1.85	0.07
5/3/2003 18:00	16.29	2.14	2.29	0.15
5/5/2003 17:45	18.28	2.19	2.17	0.00
5/5/2003 18:00	18.29	1.96	2.08	0.12
5/7/2003 20:45	20.41	2.03	2.09	0.06
5/7/2003 21:00	20.42	2.03	2.11	0.08
5/10/2003 0:15	22.55	2.02	2.12	0.10
5/10/2003 0:30	22.56	2.00	2.02	0.02
5/12/2003 10:45	24.99	1.98	2.12	0.14
5/12/2003 11:00	25.00	2.04	2.08	0.05
5/14/2003 18:45	27.32	2.00	2.10	0.10
5/14/2003 19:00	27.33	1.96	2.08	0.12
5/16/2003 17:00	29.25	1.97	2.07	0.11

Filtered Effluent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
4/26/2003 10:00	8.96	0.00	0.04	0.04
4/27/2003 10:00	9.96	0.00	0.43	0.43
4/28/2003 10:00	10.96	0.00	0.52	0.52
4/29/2003 10:45	11.99	0.00	0.61	0.61
4/30/2003 9:00	12.92	0.00	0.63	0.63
5/1/2003 11:00	14.00	0.00	0.74	0.74
5/2/2003 9:00	14.92	0.00	0.77	0.77
5/3/2003 10:00	15.96	0.00	0.88	0.88

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
5/4/2003 11:00	17.00	0.00	0.88	0.88
5/5/2003 13:00	18.08	0.00	1.27	1.27
5/6/2003 10:00	18.96	0.00	1.32	1.32
5/7/2003 10:30	19.98	0.00	1.31	1.31
5/8/2003 12:00	21.04	0.04	1.17	1.12
5/9/2003 9:30	21.94	0.42	1.45	1.03
5/10/2003 8:30	22.90	0.82	1.49	0.67
5/11/2003 10:00	23.96	1.06	1.68	0.61
5/12/2003 10:00	24.96	1.06	1.57	0.51
5/13/2003 10:00	25.96	1.05	1.65	0.60
5/14/2003 10:00	26.96	1.09	1.69	0.60
5/15/2003 10:00	27.96	1.06	1.55	0.49
5/16/2003 10:00	28.96	1.14	1.66	0.52

Unfiltered Effluent Samples

Date & Time	Days	Total Cr mg/L
4/27/2003 10:00	9.96	0.57
4/29/2003 10:45	11.99	2.10
5/1/2003 11:00	14.00	1.18
5/3/2003 10:00	15.96	1.27
5/5/2003 13:00	18.08	1.46
5/7/2003 10:30	19.98	1.67
5/9/2003 9:30	21.94	1.56
5/13/2003 10:00	25.96	1.74
5/15/2003 10:00	27.96	1.71

TABLE 20. Sand total Cr data from ICP-MS for an experiment with 0.037 mM influent Cr(VI) concentration

Segment	Distance	Total Cr mg/L	Cr mg	Sand g	Total Cr μg/g sand
0-1 cm	0.5	1.74	0.0017	0.70	2.49
1-2 cm	1.5	2.75	0.0028	0.90	3.06
2-4 cm	3.0	3.19	0.0032	1.03	3.10
4-6 cm	5.0	3.48	0.0035	1.06	3.28
6-8 cm	7.0	4.76	0.0048	1.73	2.75
8-10 cm	9.0	5.73	0.0057	2.01	2.85
10-12 cm	11.0	4.54	0.0045	1.35	3.36
12-15 cm	13.5	4.89	0.0049	1.45	3.37

TABLE 21. Sand protein data for an experiment with 0.037 mM influent Cr(VI) concentration

Depth range	Replica	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-15 cm
Distance		0.5	1.5	3.0	5.0	7.0	9.0	11.0	13.5
Absorbance	1	0.4124	0.3794	0.4615	0.4133	0.4105	0.4646	0.4986	0.4868
	2	0.5008	0.4395	0.4677	0.4650	0.4568	0.5105	0.4817	0.4914
	3	0.5340	0.5863	0.4994	0.4820	0.4879	0.4784	0.5126	0.5503
Protein	1	16.16	12.13	22.15	16.27	15.93	22.52	26.67	25.23
(mg/L)	2	26.94	19.46	22.90	22.57	21.57	28.12	24.61	25.79
	3	30.99	37.37	26.77	24.65	25.37	24.21	28.38	32.98
Protein before	1	16.16	12.13	22.15	16.27	15.93	22.52	26.67	25.23
dilution (mg/L)	2	26.94	19.46	22.90	22.57	21.57	28.12	24.61	25.79
	3	30.99	37.37	26.77	24.65	25.37	24.21	28.38	32.98
Protein (mg)	1	0.0162	0.0121	0.0221	0.0163	0.0159	0.0225	0.0267	0.0252
	2	0.0269	0.0195	0.0229	0.0226	0.0216	0.0281	0.0246	0.0258
	3	0.0310	0.0374	0.0268	0.0246	0.0254	0.0242	0.0284	0.0330
Sand weight	1	1.16	1.13	1.45	1.57	1.35	1.51	1.58	1.38
(gm)	2	1.29	1.42	1.62	1.64	1.42	1.84	1.87	2.14
	3	1.49	2.28	1.76	1.52	2.07	1.95	1.68	2.63
Sand protein	1	13.93	10.74	15.27	10.36	1.46	1.23	1.92	18.28
(mg/g)	2	20.88	13.71	14.14	13.76	15.19	15.28	13.16	12.05
	3	20.80	16.39	15.21	16.21	12.25	12.41	16.89	12.54
Average		18.54	13.61	14.87	13.45	9.64	9.64	10.66	14.29
S.D.[1]		3.99	2.83	0.64	2.94	7.23	7.43	7.79	3.47
C.I.[2]		4.51	3.20	0.72	3.33	8.18	8.40	8.82	3.92

[1]Standard deviation, [2]Confidence interval (95%)

TABLE 22. Influent and Effluent Cr(VI) absorbance data from an experiment with 0.025 mM influent Cr(VI) concentration

Column Start Time: 7/26/2002 12:15 hrs

Filtered Influent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
8/3/2002 15:30	8.14	0.2575	1.37
8/8/2002 14:45	13.10	0.2452	1.29
8/8/2002 15:00	13.11	0.2213	1.15
8/13/2002 11:30	17.97	0.2317	1.21
8/13/2002 11:45	17.98	0.2961	1.60
8/17/2002 22:30	22.43	0.2689	1.44
8/17/2002 22:45	22.44	0.2428	1.28
8/23/2002 16:15	28.17	0.2364	1.24

Filtered Effluent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
8/4/2002 19:30	9.30	0.0361	0.00
8/5/2002 12:00	9.99	0.0341	0.00
8/6/2002 11:00	10.95	0.0331	0.00
8/7/2002 12:00	11.99	0.0313	0.00
8/8/2002 11:00	12.95	0.0356	0.00
8/9/2002 11:00	13.95	0.0328	0.00
8/10/2002 10:00	14.91	0.0365	0.00
8/11/2002 13:00	16.03	0.0337	0.00
8/12/2002 12:00	16.99	0.0342	0.00
8/13/2002 10:30	17.93	0.0344	0.00
8/14/2002 11:00	18.95	0.035	0.00
8/15/2002 11:00	19.95	0.0335	0.00
8/16/2002 11:30	20.97	0.0328	0.00
8/17/2002 9:00	21.86	0.0335	0.00
8/18/2002 11:00	22.95	0.0332	0.00
8/19/2002 11:00	23.95	0.0334	0.00
8/20/2002 11:30	24.97	0.0346	0.00
8/21/2002 12:00	25.99	0.0359	0.00

Date & Time	Days	Absorbance	Cr(VI) mg/L
8/22/2002 11:30	26.97	0.0355	0.00
8/23/2002 11:00	27.95	0.0356	0.00

TABLE 23. Aqueous phase cell count as colony forming units (CFUs) in the column

effluent from an experiment with 0.025 mM influent Cr(VI) concentration

Date & Time	Days	CFUs	Dilution Factor	CFU/mL
7/31/2002 11:00	4.95	55	1.00E-03	550000
8/2/2002 11:00	6.95	71	1.00E-03	710000
8/5/2002 12:00	9.99	175	1.00E-03	1750000
8/7/2002 12:00	11.99	258	1.00E-03	2580000
8/9/2002 11:00	13.95	118	1.00E-03	1180000
8/12/2002 12:00	16.99	187	1.00E-03	1870000
8/14/2002 11:00	18.95	218	1.00E-03	2180000
8/16/2002 11:30	20.97	35	1.00E-04	3500000

TABLE 24. Lactate (L), Acetate (A), Fumarate (F) and Succinate (S) data from IC

chromatograms for an experiment with 0.025 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
7/26/2002 12:15	0.00	886098	0	0	1831284	318	0	0	239
7/30/2002 15:30	4.14	783550	0	0	1639078	282	0	0	214
7/30/2002 15:45	4.15	868970	0	0	1804479	312	0	0	235
8/3/2002 15:15	8.13	892484	0	0	1836624	320	0	0	240
8/3/2002 15:30	8.14	879089	0	0	1813065	316	0	0	236
8/8/2002 14:45	13.10	878336	0	0	1845308	315	0	0	241
8/8/2002 15:00	13.11	843508	0	0	1768849	303	0	0	231
8/13/2002 11:30	17.97	883101	0	0	1929260	333	0	0	250
8/13/2002 11:45	17.98	827141	0	0	1810926	313	0	0	234
8/17/2002 22:30	22.43	930733	0	0	2001432	351	0	0	259
8/17/2002 22:45	22.44	841402	0	0	1841079	318	0	0	238
8/23/2002 16:15	28.17	871059	0	0	1895616	329	0	0	245

Filtered Effluent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
7/27/2002 11:00	0.95			1375831	0	0	0	267	0
7/28/2002 11:00	1.95	454577	335785	1198862	0	167	71.3	233	0
7/29/2002 11:00	2.95	494147	316491	1105096	0	181	67.6	215	0
7/30/2002 11:00	3.95	322468	297915	1093308	0	121	64	212	0
7/31/2002 11:00	4.95	363160	290750	1082037	0	135	62.6	210	0
8/1/2002 11:00	5.95			1085998	0			211	0
8/2/2002 11:00	6.95			1067917	0			208	0
8/3/2002 11:00	7.95			1083707	0			211	0
8/4/2002 19:30	9.30	458538	279186	1088746	0	168	60.3	212	0
8/5/2002 12:00	9.99	491248	275478	1039945	0	180	59.6	202	0
8/6/2002 11:00	10.95			1071606	0			208	0
8/7/2002 12:00	11.99	435652	265780	977868	0	160	57.7	190	0
8/8/2002 11:00	12.95	450611	332638	1056414	0	166	70.7	205	0
8/9/2002 11:00	13.95			1238941	0			212	0
8/10/2002 10:00	14.91	442614	365641	1245556	0	172	76.9	213	0
8/11/2002 13:00	16.03			1240437	0			213	0
8/12/2002 12:00	16.99			1237759	0			212	0
8/13/2002 10:30	17.93			1272068	0			218	0
8/14/2002 11:00	18.95	449527	331940	1216129	0	175	70.5	209	0
8/15/2002 11:00	19.95			1271286	0			218	0
8/16/2002 11:30	20.97			1271276	0			218	0
8/17/2002 9:00	21.86			1299633	0			222	0
8/18/2002 11:00	22.95			1281545	0			219	0

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
8/19/2002 11:00	23.95			1212381	0			208	0
8/20/2002 11:30	24.97			1159290	0			199	0
8/21/2002 12:00	25.99			1101197	0			189	0
8/22/2002 11:30	26.97			1142854	0			196	0
8/23/2002 11:00	27.95			835101	16478			145	3.04

TABLE 25. Total Cr data from ICP-MS for an experiment with 0.025 mM influent

Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
8/3/2002 15:30	8.14	1.37	1.10	0.00
8/8/2002 14:45	13.10	1.29	1.12	0.00
8/8/2002 15:00	13.11	1.15	1.19	0.05
8/13/2002 11:30	17.97	1.21	1.10	0.00
8/13/2002 11:45	17.98	1.60	1.30	0.00
8/17/2002 22:30	22.43	1.44	1.24	0.00
8/17/2002 22:45	22.44	1.28	1.05	0.00
8/23/2002 16:15	28.17	1.24	1.07	0.00

Filtered Effluent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
8/4/2002 19:30	9.30	0.00	0.04	0.04
8/5/2002 12:00	9.99	0.00	0.20	0.20
8/6/2002 11:00	10.95	0.00	0.27	0.27
8/7/2002 12:00	11.99	0.00	0.31	0.31
8/8/2002 11:00	12.95	0.00	0.35	0.35
8/9/2002 11:00	13.95	0.00	0.37	0.37
8/10/2002 10:00	14.91	0.00	0.36	0.36
8/11/2002 13:00	16.03	0.00	0.35	0.35
8/12/2002 12:00	16.99	0.00	0.40	0.40
8/13/2002 10:30	17.93	0.00	0.41	0.41
8/14/2002 11:00	18.95	0.00	0.40	0.40
8/15/2002 11:00	19.95	0.00	0.45	0.45
8/16/2002 11:30	20.97	0.00	0.50	0.50
8/17/2002 9:00	21.86	0.00	0.53	0.53
8/18/2002 11:00	22.95	0.00	0.57	0.57
8/19/2002 11:00	23.95	0.00	0.60	0.60
8/20/2002 11:30	24.97	0.00	0.56	0.56
8/21/2002 12:00	25.99	0.00	0.64	0.64
8/22/2002 11:30	26.97	0.00	0.68	0.68
8/23/2002 11:00	27.95	0.00	0.70	0.70

Unfiltered Effluent Samples

Date & Time	Days	Total Cr mg/L
8/5/2002 12:00	9.99	0.23
8/6/2002 11:00	10.95	0.34
8/8/2002 11:00	12.95	0.40
8/11/2002 13:00	16.03	0.40
8/13/2002 10:30	17.93	0.48
8/15/2002 11:00	19.95	0.61
8/18/2002 11:00	22.95	0.70
8/22/2002 11:30	26.97	0.73

TABLE 26. Sand total Cr data from ICP-MS for an experiment with 0.025 mM influent

Cr(VI) concentration

Segment	Distance	Total Cr mg/L	Cr mg	Sand g	Total Cr µg/g sand
0-1 cm	0.5	0.84	0.0008	0.92	0.91
1-2 cm	1.5	1.33	0.0013	0.95	1.39
2-4 cm	3.0	2.05	0.0020	1.40	1.46
4-6 cm	5.0	2.53	0.0025	1.26	2.01
6-8 cm	7.0	2.53	0.0025	1.00	2.53
8-10 cm	9.0	3.36	0.0034	1.51	2.22
10-12 cm	11.0	3.04	0.0030	1.11	2.74
12-15 cm	13.5	4.00	0.0040	1.87	2.14

TABLE 27. Sand protein data for an experiment with 0.025 mM influent Cr(VI) concentration

Depth range	Replica	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-15 cm
Distance		0.5	1.5	3.0	5.0	7.0	9.0	11.0	13.5
Absorbance	1	0.5036	0.3641	0.5117	0.5923	0.5256	0.5905	0.6741	0.6760
	2	0.4525	0.4583	0.5196	0.5759	0.6380	0.6229	0.6019	0.7738
	3	0.5783	0.5135	0.5465	0.5516	0.4877	0.6363	0.6502	0.7052
Protein	1	25.33	10.00	26.22	35.08	27.75	34.88	44.07	44.27
(mg/L)	2	19.71	20.35	27.09	33.27	40.10	38.44	36.13	55.02
	3	33.54	26.42	30.04	30.60	23.58	39.91	41.44	47.48
Protein before	1	25.33	10.00	26.22	35.08	27.75	34.88	44.07	44.27
dilution (mg/L)	2	19.71	20.35	27.09	33.27	40.10	38.44	36.13	55.02
	3	33.54	26.42	30.04	30.60	23.58	39.91	41.44	47.48
Protein (mg)	1	0.0253	0.0100	0.0262	0.0351	0.0277	0.0349	0.0441	0.0443
	2	0.0197	0.0204	0.0271	0.0333	0.0401	0.0384	0.0361	0.0550
	3	0.0335	0.0264	0.0300	0.0306	0.0236	0.0399	0.0414	0.0475
Sand weight	1	1.46	0.70	1.66	1.85	1.30	1.30	2.07	1.99
(gm)	2	1.13	1.22	1.45	1.65	2.40	2.29	1.70	1.99
	3	1.82	1.61	1.89	1.72	1.77	1.98	1.90	1.88
Sand protein	1	17.35	14.29	15.80	18.96	21.34	26.83	21.29	22.25
(mg/g)	2	17.45	16.68	18.68	20.17	16.71	16.79	21.25	27.65
	3	18.43	16.41	15.90	17.79	13.32	20.16	21.81	25.26
Average		17.74	15.79	16.79	18.97	17.13	21.26	21.45	25.05
S.D.[1]		0.60	1.31	1.64	1.19	4.03	5.11	0.31	2.71
C.I.[2]		0.68	1.48	1.85	1.34	4.56	5.78	0.35	3.06

[1]Standard deviation, [2]Confidence interval (95%)

TABLE 28. Influent and Effluent Cr(VI) absorbance data from an experiment with 0.011 mM influent Cr(VI) concentration

Column Start Time: 7/26/2002 12:15 hrs

Filtered Influent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
8/3/2002 15:30	8.14	0.1404	0.65
8/8/2002 14:45	13.10	0.1345	0.62
8/8/2002 15:00	13.11	0.1277	0.57
8/13/2002 11:30	17.97	0.1242	0.55
8/13/2002 11:45	17.98	0.1321	0.60
8/17/2002 22:30	22.43	0.1184	0.52
8/17/2002 22:45	22.44	0.1458	0.68
8/23/2002 16:15	28.17	0.1388	0.64
8/23/2002 16:30	28.18	0.1379	0.64
8/28/2002 15:30	33.14	0.1302	0.59
8/28/2002 15:45	33.15	0.1186	0.52
8/30/2002 12:15	35.00	0.1250	0.56
8/30/2002 12:45	35.02	0.1364	0.63
9/5/2002 11:30	40.97	0.1300	0.59
9/5/2002 11:45	40.98	0.1275	0.57
9/10/2002 12:00	45.99	0.1043	0.43
9/10/2002 12:15	46.00	0.1424	0.66
9/15/2002 19:30	51.30	0.1416	0.66
9/15/2002 19:45	51.31	0.1400	0.65
9/20/2002 14:30	56.09	0.1152	0.50

Filtered Effluent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
8/4/2002 19:30	9.30	0.0348	0.00
8/5/2002 12:00	9.99	0.0332	0.00
8/6/2002 11:00	10.95	0.0362	0.00
8/7/2002 12:00	11.99	0.0370	0.00
8/8/2002 11:00	12.95	0.0364	0.00
8/9/2002 11:00	13.95	0.0370	0.00

Date & Time	Days	Absorbance	Cr(VI) mg/L
8/10/2002 10:00	14.91	0.0349	0.00
8/11/2002 13:00	16.03	0.0356	0.00
8/12/2002 12:00	16.99	0.0361	0.00
8/13/2002 10:30	17.93	0.0354	0.00
8/14/2002 11:00	18.95	0.0357	0.00
8/15/2002 11:00	19.95	0.0357	0.00
8/16/2002 11:30	20.97	0.0364	0.00
8/17/2002 9:00	21.86	0.0370	0.00
8/18/2002 11:00	22.95	0.0332	0.00
8/19/2002 11:00	23.95	0.0338	0.00
8/20/2002 11:30	24.97	0.0367	0.00
8/21/2002 12:00	25.99	0.0348	0.00
8/22/2002 11:30	26.97	0.0342	0.00
8/23/2002 11:00	27.95	0.0346	0.00
8/24/2002 11:00	28.95	0.0353	0.00
8/25/2002 11:30	29.97	0.0359	0.00
8/26/2002 11:00	30.95	0.0370	0.00
8/27/2002 11:00	31.95	0.0343	0.00
8/28/2002 11:00	32.95	0.0334	0.00
8/29/2002 11:00	33.95	0.0357	0.00
8/30/2002 11:00	34.95	0.0341	0.00
8/31/2002 13:00	36.03	0.0336	0.00
9/2/2002 21:30	38.39	0.0333	0.00
9/3/2002 11:00	38.95	0.0345	0.00
9/4/2002 11:00	39.95	0.0322	0.00
9/5/2002 10:30	40.93	0.0343	0.00
9/6/2002 11:00	41.95	0.0349	0.00
9/7/2002 11:00	42.95	0.0353	0.00
9/8/2002 13:00	44.03	0.0356	0.00
9/9/2002 11:00	44.95	0.0346	0.00
9/10/2002 11:00	45.95	0.0370	0.00
9/11/2002 11:00	46.95	0.0337	0.00
9/12/2002 11:00	47.95	0.0335	0.00
9/13/2002 11:00	48.95	0.0370	0.00
9/14/2002 11:00	49.95	0.0370	0.00
9/15/2002 11:00	50.95	0.0360	0.00
9/16/2002 11:00	51.95	0.0332	0.00
9/17/2002 11:00	52.95	0.0334	0.00
9/18/2002 11:00	53.95	0.0338	0.00
9/19/2002 11:00	54.95	0.0331	0.00
9/20/2002 11:00	55.95	0.0347	0.00

TABLE 29. Aqueous phase cell count as colony forming units (CFUs) in the column

effluent from an experiment with 0.011 mM influent Cr(VI) concentration

Date & Time	Days	CFUs	Dilution Factor	CFU/mL
7/31/2002 11:00	4.95	56	1.00E-03	560000
8/2/2002 11:00	6.95	37	1.00E-03	370000
8/5/2002 12:00	9.99	77	1.00E-03	770000
8/7/2002 12:00	11.99	177	1.00E-03	1770000
8/9/2002 11:00	13.95	122	1.00E-03	1220000
8/12/2002 12:00	16.99	85	1.00E-03	850000
8/14/2002 11:00	18.95	51	1.00E-03	510000
8/16/2002 11:30	20.97	28	1.00E-03	280000
8/19/2002 11:00	23.95	123	1.00E-03	1230000
8/21/2002 12:00	25.99	74	1.00E-03	740000
8/26/2002 11:00	30.95	56	1.00E-03	560000
8/29/2002 11:00	33.95	41	1.00E-03	410000
9/3/2002 11:00	38.95	23	1.00E-03	230000
9/6/2002 11:00	41.95	47	1.00E-03	470000
9/9/2002 11:00	44.95	140	1.00E-03	1400000
9/13/2002 11:00	48.95	57	1.00E-03	570000
9/16/2002 11:00	51.95	9	1.00E-03	90000
9/20/2002 11:00	55.95	59	1.00E-03	590000

TABLE 30. Lactate (L), Acetate (A), Fumarate (F) and Succinate (S) data from IC

chromatograms for an experiment with 0.011 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
7/26/2002 12:15	0.00	889892	0	0	1834741	319	0	0	239
7/30/2002 15:30	4.14	880790	0	0	1817103	316	0	0	237
7/30/2002 15:45	4.15	868970	0	0	1804479	312	0	0	235
8/3/2002 15:15	8.13	812433	0	0	1726819	292	0	0	225
8/3/2002 15:30	8.14	858946	0	0	1785266	309	0	0	233
8/8/2002 14:45	13.10	875317	0	0	1826398	314	0	0	238
8/8/2002 15:00	13.11	849404	0	0	1766614	305	0	0	230
8/13/2002 11:30	17.97	873631	0	0	1915337	330	0	0	248
8/13/2002 11:45	17.98	874493	0	0	1899163	330	0	0	246
8/17/2002 22:30	22.43	874530	0	0	1882320	330	0	0	244
8/17/2002 22:45	22.44	860335	0	0	1871134	325	0	0	242
8/23/2002 16:15	28.17	874595	0	0	1904045	330	0	0	246
8/23/2002 16:30	28.18	862462	0	0	1884787	326	0	0	244
8/28/2002 15:30	33.14	878484	0	0	1906169	332	0	0	247
8/28/2002 15:45	33.15	862815	0	0	1888463	326	0	0	244
8/30/2002 12:15	35.00	910781	0	0	1914389	312	0	0	261
8/30/2002 12:45	35.02	852486	0	0	1808239	292	0	0	247
9/5/2002 11:30	40.97	913559	0	0	1907157	313	0	0	260
9/5/2002 11:45	40.98	872168	0	0	1801083	299	0	0	246
9/10/2002 12:00	45.99	818240	0	740086	829349	281	0	144	113
9/10/2002 12:15	46.00	846847	0	0	1796976	290	0	0	245
9/15/2002 19:30	51.30	876458	0	0	1804073	300	0	0	246
9/15/2002 19:45	51.31	872489	0	0	1820726	299	0	0	249
9/20/2002 14:30	56.09	830564	0	0	1727847	285	0	0	236

Filtered Effluent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
7/27/2002 11:00	0.95			1338912	0			260	0
7/28/2002 11:00	1.95	469540	339844	1215773	0	172	72.1	236	0
7/29/2002 11:00	2.95	533977	318670	1117180	0	195	68	217	0
7/30/2002 11:00	3.95	517943	314410	1126493	0	189	67.2	219	0
7/31/2002 11:00	4.95	501677	294962	1113824	0	183	63.4	216	0
8/1/2002 11:00	5.95	462530	271978	1085998	0	170	58.9	211	0
8/2/2002 11:00	6.95	454572	266930	1067917	0	167	58	208	0
8/3/2002 11:00	7.95	349292	359724	1083707	0	130	76	211	0
8/4/2002 19:30	9.30	352560	327106	1072136	0	131	69.6	208	0
8/5/2002 12:00	9.99	306554	279324	1071457	0	115	60.4	208	0
8/6/2002 11:00	10.95			1071606	0			208	0

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
8/7/2002 12:00	11.99			908848			0	177	0
8/8/2002 11:00	12.95	416780	314407	1013130	0	154	67.2	197	0
8/9/2002 11:00	13.95				0			0	0
8/10/2002 10:00	14.91	515576	290013	1195959	0	199	62.5	205	0
8/11/2002 13:00	16.03			1102533	0			190	0
8/12/2002 12:00	16.99			1214072	0			208	0
8/13/2002 10:30	17.93			1023876	0			176	0
8/14/2002 11:00	18.95			964512	0			166	0
8/15/2002 11:00	19.95			1081449	0			186	0
8/16/2002 11:30	20.97			1242465	0			213	0
8/17/2002 9:00	21.86			1248568	0			214	0
8/18/2002 11:00	22.95			997858	0			172	0
8/19/2002 11:00	23.95			1143384	0			196	0
8/20/2002 11:30	24.97			1100740	0			189	0
8/21/2002 12:00	25.99	438254	385742	1215185	0	170	80.8	208	0
8/22/2002 11:30	26.97	393618	378495	1230624	0	154	79.4	211	0
8/23/2002 11:00	27.95	401093	392624	1202402	0	157	82.1	206	0
8/24/2002 11:00	28.95	419496	364468	1235764	0	164	76.7	212	0
8/25/2002 11:30	29.97			1264713	0			217	0
8/26/2002 11:00	30.95			1217093	0			209	0
8/27/2002 11:00	31.95			1239458	0			212	0
8/28/2002 11:00	32.95			1295557	0			222	0
8/29/2002 11:00	33.95			1309593	0			224	0
8/30/2002 11:00	34.95	662465	283133	1122886	0	229	58.2	219	0
8/31/2002 13:00	36.03			817468	0			159	0
9/2/2002 21:30	38.39			797158	0			155	0
9/3/2002 11:00	38.95								
9/4/2002 11:00	39.95			905093	0			176	0
9/5/2002 10:30	40.93	602038	224713	1000056	0	208	47.1	195	0
9/6/2002 11:00	41.95			958973	0			187	0
9/7/2002 11:00	42.95			1009672	0			197	0
9/8/2002 13:00	44.03			1064738	0			207	0
9/9/2002 11:00	44.95	622033	239749	1102992	0	215	50	215	0
9/10/2002 11:00	45.95			1110905	0			217	0
9/11/2002 11:00	46.95			1089936	0			212	0
9/12/2002 11:00	47.95			1102150	0			215	0
9/13/2002 11:00	48.95			1109562	0			216	0
9/14/2002 11:00	49.95			1109523	0			216	0
9/15/2002 11:00	50.95			1102198	0			215	0
9/16/2002 11:00	51.95			1133686	0			221	0
9/17/2002 11:00	52.95			1125126	0			219	0
9/18/2002 11:00	53.95			1120313	0			218	0
9/19/2002 11:00	54.95			989275	0			193	0
9/20/2002 11:00	55.95			942781	0			183	0

TABLE 31. Total Cr data from ICP-MS for an experiment with 0.011 mM influent

Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
8/3/2002 15:30	8.14	0.65	0.64	0.00
8/8/2002 14:45	13.10	0.62	0.58	0.00
8/8/2002 15:00	13.11	0.57	0.50	0.00
8/13/2002 11:30	17.97	0.55	0.49	0.00
8/13/2002 11:45	17.98	0.60	0.54	0.00
8/17/2002 22:30	22.43	0.52	0.50	0.00
8/17/2002 22:45	22.44	0.68	0.54	0.00
8/23/2002 16:15	28.17	0.64	0.56	0.00
8/23/2002 16:30	28.18	0.64	0.53	0.00
8/28/2002 15:30	33.14	0.59	0.44	0.00
8/28/2002 15:45	33.15	0.52		
8/30/2002 12:15	35.00	0.56	0.54	0.00
8/30/2002 12:45	35.02	0.63	0.51	0.00
9/5/2002 11:30	40.97	0.59	0.52	0.00
9/5/2002 11:45	40.98	0.57		
9/10/2002 12:00	45.99	0.43	0.48	0.05
9/10/2002 12:15	46.00	0.66	0.53	0.00
9/15/2002 19:30	51.30	0.66	0.55	0.00
9/15/2002 19:45	51.31	0.65	0.52	0.00
9/20/2002 14:30	56.09	0.50	0.49	0.00

Filtered Effluent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
8/4/2002 19:30	9.30	0.00	0.03	0.03
8/5/2002 12:00	9.99	0.00	0.12	0.12
8/6/2002 11:00	10.95	0.00	0.14	0.14
8/7/2002 12:00	11.99	0.00	0.14	0.14
8/8/2002 11:00	12.95	0.00	0.16	0.16
8/9/2002 11:00	13.95	0.00	0.17	0.17
8/10/2002 10:00	14.91	0.00	0.17	0.17
8/11/2002 13:00	16.03	0.00	0.16	0.16

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
8/12/2002 12:00	16.99	0.00	0.16	0.16
8/13/2002 10:30	17.93	0.00	0.17	0.17
8/14/2002 11:00	18.95	0.00	0.15	0.15
8/15/2002 11:00	19.95	0.00	0.16	0.16
8/16/2002 11:30	20.97	0.00	0.15	0.15
8/17/2002 9:00	21.86	0.00	0.14	0.14
8/18/2002 11:00	22.95	0.00	0.14	0.14
8/19/2002 11:00	23.95	0.00	0.19	0.19
8/20/2002 11:30	24.97	0.00	0.21	0.21
8/21/2002 12:00	25.99	0.00	0.20	0.20
8/22/2002 11:30	26.97	0.00	0.21	0.21
8/23/2002 11:00	27.95	0.00	0.21	0.21
8/24/2002 11:00	28.95	0.00	0.18	0.18
8/25/2002 11:30	29.97	0.00	0.21	0.21
8/26/2002 11:00	30.95	0.00	0.20	0.20
8/27/2002 11:00	31.95	0.00	0.16	0.16
8/28/2002 11:00	32.95	0.00	0.18	0.18
8/29/2002 11:00	33.95	0.00	0.20	0.20
8/30/2002 11:00	34.95	0.00	0.19	0.19
8/31/2002 13:00	36.03	0.00	0.17	0.17
9/2/2002 21:30	38.39	0.00	0.17	0.17
9/3/2002 11:00	38.95	0.00	0.17	0.17
9/4/2002 11:00	39.95	0.00	0.16	0.16
9/5/2002 10:30	40.93	0.00	0.17	0.17
9/6/2002 11:00	41.95	0.00	0.17	0.17
9/7/2002 11:00	42.95	0.00	0.18	0.18
9/8/2002 13:00	44.03	0.00	0.19	0.19
9/9/2002 11:00	44.95	0.00	0.21	0.21
9/10/2002 11:00	45.95	0.00	0.26	0.26
9/11/2002 11:00	46.95	0.00	0.30	0.30
9/12/2002 11:00	47.95	0.00	0.16	0.16
9/13/2002 11:00	48.95	0.00	0.15	0.15
9/14/2002 11:00	49.95	0.00	0.15	0.15
9/15/2002 11:00	50.95	0.00	0.14	0.14
9/16/2002 11:00	51.95	0.00	0.14	0.14
9/17/2002 11:00	52.95	0.00	0.15	0.15
9/18/2002 11:00	53.95	0.00	0.16	0.16
9/19/2002 11:00	54.95	0.00	0.17	0.17
9/20/2002 11:00	55.95	0.00	0.17	0.17

Unfiltered Effluent Samples

Date & Time	Days	Total Cr mg/L
8/5/2002 12:00	9.99	0.12
8/6/2002 11:00	10.95	0.22
8/8/2002 11:00	12.95	0.21
8/11/2002 13:00	16.03	0.17
8/13/2002 10:30	17.93	0.16
8/15/2002 11:00	19.95	0.18
8/18/2002 11:00	22.95	0.17
8/22/2002 11:30	26.97	0.21
8/25/2002 11:30	29.97	0.18
8/30/2002 11:00	34.95	0.19
9/4/2002 11:00	39.95	0.16
9/8/2002 13:00	44.03	0.21
9/12/2002 11:00	47.95	0.25
9/15/2002 11:00	50.95	0.16
9/19/2002 11:00	54.95	0.19

TABLE 32. Sand total Cr data from ICP-MS for an experiment with 0.011 mM influent

Cr(VI) concentration

Segment	Distance	Total Cr mg/L	Cr mg	Sand g	Total Cr µg/g sand
0-1 cm	0.5	2.60	0.0026	0.51	5.09
1-2 cm	1.5	3.17	0.0032	0.69	4.59
2-4 cm	3.0	3.91	0.0039	0.93	4.21
4-6 cm	5.0	3.10	0.0031	0.72	4.31
6-8 cm	7.0	2.79	0.0028	0.85	3.28
8-10 cm	9.0	2.22	0.0022	0.92	2.42
10-12 cm	11.0	1.77	0.0018	1.00	1.77
12-15 cm	13.5	1.41	0.0014	1.09	1.29

TABLE 33. Sand protein data for an experiment with 0.011 mM influent Cr(VI) concentration

Depth range	Replica	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-15 cm
Distance		0.5	1.5	3.0	5.0	7.0	9.0	11.0	13.5
Absorbance	1	0.6918	0.9090	0.6577	0.5545	0.5923	0.5431	0.5604	0.5608
	2	0.5951	0.8859	0.7604	0.6633	0.5694	0.5738	0.5705	0.5243
	3	0.5730	0.7742	0.7279	0.6311	0.6343	0.5798	0.5290	0.5854
Protein	1	50.13	75.68	46.12	33.98	38.42	32.64	34.67	34.72
(mg/L)	2	38.75	72.96	58.20	46.78	35.73	36.25	35.86	30.42
	3	36.15	59.82	54.38	42.99	43.36	36.95	30.98	37.61
Protein before	1	250.65	378.41	46.12	33.98	38.42	32.64	34.67	34.72
dilution (mg/L)	2	193.76	364.82	58.20	46.78	35.73	36.25	35.86	30.42
	3	180.76	299.12	54.38	42.99	43.36	36.95	30.98	37.61
Protein (mg)	1	0.2506	0.3784	0.0461	0.0340	0.0384	0.0326	0.0347	0.0347
	2	0.1938	0.3648	0.0582	0.0468	0.0357	0.0362	0.0359	0.0304
	3	0.1808	0.2991	0.0544	0.0430	0.0434	0.0370	0.0310	0.0376
Sand weight	1	1.13	1.14	1.63	1.08	2.73	2.23	2.01	1.68
(gm)	2	0.93	1.68	1.69	1.14	1.04	1.46	1.26	2.00
	3	0.91	1.46	1.33	1.39	1.25	1.34	1.60	1.90
Sand protein	1	221.81	331.94	28.29	31.46	1.46	1.23	1.92	20.67
(mg/g)	2	208.35	217.16	34.44	41.03	34.36	24.83	28.46	15.21
	3	198.64	204.88	40.88	30.93	34.69	27.58	19.36	19.80
Average		209.60	251.32	34.54	34.47	23.50	17.88	16.58	18.56
S.D.[1]		11.64	70.09	6.30	5.69	19.09	14.48	13.49	2.93
C.I.[2]		13.17	79.31	7.12	6.43	21.60	16.39	15.26	3.32

[1]Standard deviation, [2]Confidence interval (95%)

TABLE 34. Influent and Effluent Cr(VI) absorbance data from an experiment with

0.006 mM influent Cr(VI) concentration

Column Start Time: 5/29/2002 12:15 hrs

Filtered Influent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
6/6/2002 19:00	8.28	0.1188	0.52
6/12/2002 17:15	14.21	0.0747	0.25
6/12/2002 17:30	14.22	0.0923	0.36
6/17/2002 15:15	19.13	0.0809	0.29
6/17/2002 15:30	19.14	0.0962	0.38
6/20/2002 18:15	22.25	0.0837	0.30
6/20/2002 18:30	22.26	0.0896	0.34
6/25/2002 15:15	27.13	0.0661	0.20
6/25/2002 15:30	27.14	0.0792	0.28
6/30/2002 19:15	32.29	0.0639	0.18
6/30/2002 19:30	32.30	0.0893	0.34
7/3/2002 12:15	35.00	0.0859	0.32

Filtered Effluent Samples

Date & Time	Days	Absorbance	Cr(VI) mg/L
6/7/2002 18:00	9.24	0.0342	0.00
6/8/2002 10:00	9.91	0.0340	0.00
6/9/2002 10:45	10.94	0.0344	0.00
6/10/2002 11:30	11.97	0.0341	0.00
6/11/2002 12:00	12.99	0.0354	0.00
6/12/2002 12:00	13.99	0.0356	0.00
6/13/2002 12:00	14.99	0.0365	0.00
6/14/2002 11:15	15.96	0.0337	0.00
6/15/2002 11:30	16.97	0.0342	0.00
6/16/2002 12:00	17.99	0.0340	0.00
6/17/2002 11:00	18.95	0.0334	0.00
6/18/2002 11:00	19.95	0.0367	0.00
6/19/2002 11:00	20.95	0.0370	0.00
6/20/2002 11:30	21.97	0.0345	0.00

Date & Time	Days	Absorbance	Cr(VI) mg/L
6/21/2002 11:30	22.97	0.0335	0.00
6/22/2002 12:30	24.01	0.0365	0.00
6/23/2002 8:30	24.84	0.0357	0.00
6/24/2002 11:00	25.95	0.0368	0.00
6/25/2002 11:30	26.97	0.0338	0.00
6/26/2002 12:00	27.99	0.0334	0.00
6/27/2002 12:00	28.99	0.0324	0.00
6/28/2002 11:30	29.97	0.0336	0.00
6/29/2002 11:00	30.95	0.0349	0.00
6/30/2002 7:00	31.78	0.0330	0.00
7/1/2002 10:30	32.93	0.0334	0.00
7/2/2002 11:00	33.95	0.0332	0.00
7/3/2002 11:00	34.95	0.0330	0.00

TABLE 35. Aqueous phase cell count as colony forming units (CFUs) in the column

effluent from an experiment with 0.006 mM influent Cr(VI) concentration

Date & Time	Days	CFUs	Dilution Factor	CFU/mL
5/30/2002 15:00	1.11	46	1.00E-04	4600000
5/31/2002 16:30	2.18	123	1.00E-03	1230000
6/3/2002 14:00	5.07	221	1.00E-03	2210000
6/5/2002 15:30	7.14	89	1.00E-03	890000
6/8/2002 10:00	9.91	256	1.00E-04	25600000
6/10/2002 10:30	11.93	20	1.00E-04	2000000
6/12/2002 16:30	14.18	158	1.00E-03	1580000
6/14/2002 16:00	16.16	132	1.00E-03	1320000
6/17/2002 15:00	19.11	99	1.00E-03	990000
6/19/2002 17:30	21.22	56	1.00E-04	5600000
6/21/2002 11:30	22.97	41	1.00E-04	4100000
6/24/2002 11:00	25.95	106	1.00E-03	1060000
6/26/2002 12:00	27.99	65	1.00E-03	650000
6/28/2002 11:30	29.97	162	1.00E-03	1620000
7/1/2002 10:30	32.93	231	1.00E-03	2310000

TABLE 36. Lactate (L), Acetate (A), Fumarate (F) and Succinate (S) data from IC

chromatograms for an experiment with 0.006 mM influent Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
5/29/2002 12:15	0.00	856492	0	0	1796158	298	0	0	238
5/31/2002 17:45	2.23	828427	0	0	1746230	288	0	0	231
5/31/2002 18:00	2.24	839424	0	0	1780259	292	0	0	236
6/6/2002 18:45	8.27	899023	0	0	1885790	312	0	0	250
6/6/2002 19:00	8.28	857473	0	0	1823086	298	0	0	242
6/12/2002 17:15	14.21	891871	0	0	1898801	294	0	0	255
6/12/2002 17:30	14.22	781288	0	0	1711372	259	0	0	230
6/17/2002 15:15	19.13	858875	0	0	1840564	284	0	0	247
6/17/2002 15:30	19.14	787416	0	0	1720998	261	0	0	231
6/20/2002 18:15	22.25	820438	0	0	1787559	272	0	0	240
6/20/2002 18:30	22.26	804395	0	0	1752332	267	0	0	235
6/25/2002 15:15	27.13	848823	0	0	1836663	281	0	0	247
6/25/2002 15:30	27.14	799305	0	0	1747516	265	0	0	235
6/30/2002 19:15	32.29	881621	0	0	1848739	297	0	0	234
6/30/2002 19:30	32.30	871804	0	0	1828771	294	0	0	232
7/3/2002 12:15	35.00	874095	0	0	1835892	295	0	0	233

Filtered Effluent Samples

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
5/30/2002 11:30	0.97			1187723	0			241	0
5/30/2002 17:45	1.23			1270340	0			258	0
5/31/2002 11:00	1.95	524042	299556	1156866	0	185	66.6	235	0
6/1/2002 10:30	2.93	487902	256385	1020620	0	173	57.7	208	0
6/2/2002 11:30	3.97	511947	236642	1118213	0	181	53.7	227	0
6/3/2002 12:30	5.01	593330	259519	1145601	0	208	58.4	233	0
6/4/2002 12:00	5.99	533498	256847	1091826	0	188	57.8	222	0
6/5/2002 12:00	6.99	517436	284706	1116021	0	183	63.5	227	0
6/6/2002 11:30	7.97	482889	311419	1084752	0	171	69	221	0
6/7/2002 10:30	8.93	535633	289669	1158880	0	189	64.5	235	0
6/7/2002 18:00	9.24			1134865	0			231	0
6/8/2002 10:00	9.91			1167243	0			218	0
6/9/2002 10:45	10.94			1179941	0			221	0
6/10/2002 11:30	11.97	520959	261904	1123956	0	177	63.1	210	0
6/11/2002 12:00	12.99	473521	263228	1161786	0	162	63.4	217	0
6/12/2002 12:00	13.99	412203	286030	1140811	0	143	68.4	214	0
6/13/2002 12:00	14.99	482809	264074	1106974	0	165	63.6	207	0
6/14/2002 11:15	15.96	491740	248121	1114203	0	168	60.1	209	0
6/15/2002 11:30	16.97	457818	281270	1103894	0	157	67.3	207	0

Date & Time	Days	L Area	A Area	S Area	F Area	L mg/l	A mg/l	S mg/l	F mg/l
6/16/2002 12:00	17.99	462770	290569	1156334	0	159	69.4	216	0
6/17/2002 11:00	18.95	441077	301209	1147284	0	152	71.7	215	0
6/18/2002 11:00	19.95			1043557	0			196	0
6/19/2002 11:00	20.95			1041404	0			195	0
6/20/2002 11:30	21.97	467936	293772	988258	0	160	70.1	185	0
6/21/2002 11:30	22.97	448534	289114	1133867	0	154	69.1	212	0
6/22/2002 12:30	24.01	448159	260900	946915	0	154	62.9	178	0
6/23/2002 8:30	24.84	491611	190883	1095596	0	168	47.6	205	0
6/24/2002 11:00	25.95			971767	0			182	0
6/25/2002 11:30	26.97	532150	200981	1092061	0	181	49.8	205	0
6/26/2002 12:00	27.99	520646	261222	1099619	0	177	63	206	0
6/27/2002 12:00	28.99	545605	298681	1084828	0	187	65	205	0
6/28/2002 11:30	29.97	557761	270727	1020158	0	191	59.5	193	0
6/29/2002 11:00	30.95	589749	260739	1035826	0	202	57.6	196	0
6/30/2002 7:00	31.78	551695	282989	988107	0	189	61.9	187	0
7/1/2002 10:30	32.93	590113	279135	1022663	0	202	61.2	194	0
7/1/2002 10:30	32.93	73663	44855	267444	0	32.8	15.3	53.2	0
7/2/2002 11:00	33.95	550701	238632	1108884	0	189	53.3	210	0
7/3/2002 11:00	34.95	562456	271500	1017930	0	193	59.7	193	0

TABLE 37. Total Cr data from ICP-MS for an experiment with 0.006 mM influent

Cr(VI) concentration

Filtered Influent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
6/6/2002 19:00	8.28	0.52	0.53	0.01
6/12/2002 17:15	14.21	0.25	0.35	0.10
6/12/2002 17:30	14.22	0.36	0.40	0.05
6/17/2002 15:15	19.13	0.29	0.32	0.04
6/17/2002 15:30	19.14	0.38	0.38	0.00
6/20/2002 18:15	22.25	0.30	0.35	0.04
6/20/2002 18:30	22.26	0.34	0.32	0.00
6/25/2002 15:15	27.13	0.20	0.29	0.09
6/25/2002 15:30	27.14	0.28	0.32	0.05
6/30/2002 19:30	32.30	0.34	0.36	0.02
7/3/2002 12:15	35.00	0.32	0.34	0.02

Filtered Effluent Samples

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
6/8/2002 10:00	9.91	0.00	0.13	0.13
6/9/2002 10:45	10.94	0.00	0.16	0.16
6/10/2002 11:30	11.97	0.00	0.13	0.13
6/11/2002 12:00	12.99	0.00	0.13	0.13
6/12/2002 12:00	13.99	0.00	0.13	0.13
6/13/2002 12:00	14.99	0.00	0.12	0.12
6/14/2002 11:15	15.96	0.00	0.12	0.12
6/15/2002 11:30	16.97	0.00	0.12	0.12
6/16/2002 12:00	17.99	0.00	0.11	0.11
6/17/2002 11:00	18.95	0.00	0.11	0.11
6/18/2002 11:00	19.95	0.00	0.10	0.10
6/19/2002 11:00	20.95	0.00	0.12	0.12
6/20/2002 11:30	21.97	0.00	0.13	0.13
6/21/2002 11:30	22.97	0.00	0.12	0.12
6/22/2002 12:30	24.01	0.00	0.09	0.09
6/23/2002 8:30	24.84	0.00	0.09	0.09
6/24/2002 11:00	25.95	0.00	0.09	0.09

Date & Time	Days	Cr(VI) mg/L	Total Cr mg/L	Cr (III) mg/L
6/25/2002 11:30	26.97	0.00	0.09	0.09
6/26/2002 12:00	27.99	0.00	0.10	0.10
6/27/2002 12:00	28.99	0.00	0.08	0.08
6/28/2002 11:30	29.97	0.00	0.08	0.08
6/29/2002 11:00	30.95	0.00	0.09	0.09
6/30/2002 7:00	31.78	0.00	0.08	0.08
7/1/2002 10:30	32.93	0.00	0.08	0.08
7/2/2002 11:00	33.95	0.00	0.09	0.09
7/3/2002 11:00	34.95	0.00	0.08	0.08

Unfiltered Effluent Samples

Date & Time	Days	Total Cr mg/L
8/5/2002 12:00	9.99	0.12
8/6/2002 11:00	10.95	0.22
8/8/2002 11:00	12.95	0.21
8/11/2002 13:00	16.03	0.17
8/13/2002 10:30	17.93	0.16
8/15/2002 11:00	19.95	0.18
8/18/2002 11:00	22.95	0.17
8/22/2002 11:30	26.97	0.21
8/25/2002 11:30	29.97	0.18
8/30/2002 11:00	34.95	0.19
9/4/2002 11:00	39.95	0.16
9/8/2002 13:00	44.03	0.21
9/12/2002 11:00	47.95	0.25
9/15/2002 11:00	50.95	0.16
9/19/2002 11:00	54.95	0.19

TABLE 38. Sand total Cr data from ICP-MS for an experiment with 0.006 mM influent

Cr(VI) concentration

Segment	Distance	Total Cr mg/L	Cr mg	Sand g	Total Cr µg/g sand
0-1 cm	0.5	6.11	0.0061	0.80	7.63
1-2 cm	1.5	1.73	0.0017	0.73	2.38
2-4 cm	3.0	2.73	0.0027	1.47	1.86
4-6 cm	5.0	1.83	0.0018	1.45	1.26
6-8 cm	7.0	1.43	0.0014	1.30	1.10
8-10 cm	9.0	1.06	0.0011	1.41	0.75
10-12 cm	11.0	0.92	0.0009	1.32	0.70
12-15 cm	13.5	1.26	0.0013	2.52	0.50

TABLE 39. Sand protein data for an experiment with 0.006 mM influent Cr(VI)

concentration

Depth range	Replica	0-1 cm	1-2 cm	2-4 cm	4-6 cm	6-8 cm	8-10 cm	10-12 cm	12-15 cm
Distance		0.5	1.5	3.0	5.0	7.0	9.0	11.0	13.5
Absorbance	1	0.6642	0.4657	0.6577	0.5545	0.5923	0.5431	0.5604	0.5608
	2	0.6018	0.4107	0.7604	0.6633	0.5694	0.5738	0.5705	0.5243
	3	0.7353	0.6778	0.7279	0.6311	0.6343	0.5798	0.5290	0.5854
Protein	1	46.65	21.21	45.82	32.59	37.44	31.13	33.35	33.40
(mg/L)	2	38.65	14.15	58.99	46.54	34.50	35.06	34.64	28.72
	3	55.77	48.40	54.82	42.41	42.82	35.83	29.32	36.55
Protein before	1	46.65	21.21	45.82	32.59	37.44	31.13	33.35	33.40
dilution (mg/L)	2	38.65	14.15	58.99	46.54	34.50	35.06	34.64	28.72
	3	55.77	48.40	54.82	42.41	42.82	35.83	29.32	36.55
Protein (mg)	1	0.0467	0.0212	0.0458	0.0326	0.0374	0.0311	0.0333	0.0334
	2	0.0387	0.0142	0.0590	0.0465	0.0345	0.0351	0.0346	0.0287
	3	0.0558	0.0484	0.0548	0.0424	0.0428	0.0358	0.0293	0.0366
Sand weight	1	1.35	0.63	1.95	2.54	2.73	2.23	2.01	2.04
(gm)	2	0.69	0.42	3.18	2.83	2.39	2.62	2.86	3.04
	3	1.13	0.88	2.26	2.34	2.51	2.13	1.45	2.53
Sand protein	1	34.56	33.66	23.50	12.83	13.71	13.96	16.59	16.37
(mg/g)	2	56.02	33.70	18.55	16.44	14.44	13.38	12.11	9.45
	3	49.35	55.00	24.26	18.12	17.06	16.82	20.22	14.45
Average		46.64	40.79	22.10	15.80	15.07	14.72	16.31	13.42
S.D.[1]		10.98	12.31	3.10	2.71	1.76	1.84	4.06	3.57
C.I.[2]		12.43	13.93	3.51	3.06	1.99	2.08	4.60	4.04

[1]Standard deviation, [2]Confidence interval (95%)

TABLE 40. Calibration data for Cr(VI)

Cr(VI)	Absorbance at 540 nm				
mg/L	1	2	3	4	Average
0.00	0.0341	0.0368	0.0345	0.0341	0.0349
0.01	0.0346	0.0355	0.0359	0.0353	0.0353
0.05	0.0403	0.0423	0.0422	0.0420	0.0417
0.10	0.0500	0.0503	0.0506	0.0501	0.0503
0.25	0.0762	0.0754	0.0758	0.0759	0.0758
0.50	0.1160	0.1138	0.1151	0.1136	0.1146
1.00	0.1992	0.1960	0.1949	0.1943	0.1961
2.00	0.3632	0.3628	0.3609	0.3595	0.3616

$y = 0.1635x + 0.0339$

$R^2 = 0.9999$
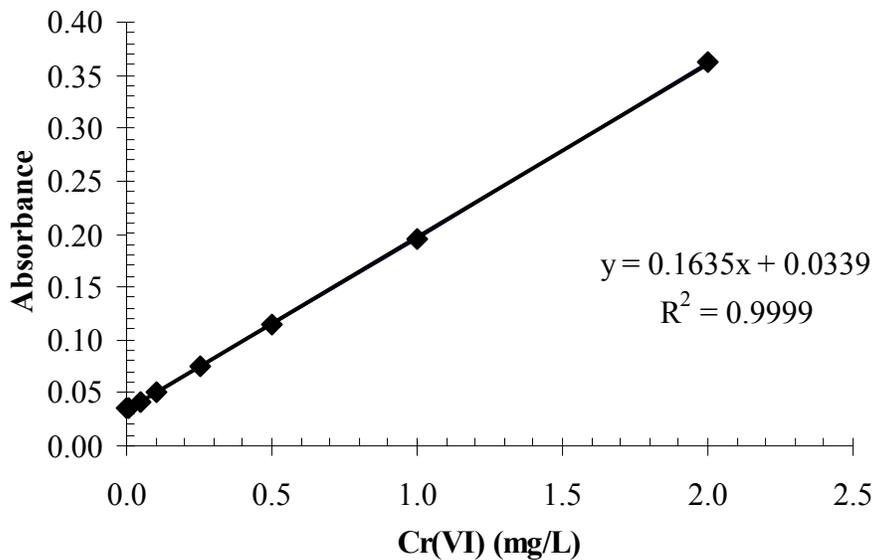
Figure 15. Calibration curve for Cr(VI) measurement

TABLE 41. Calibration data for protein

Protein	Absorbance at 595 nm			
mg/L	1	2	3	Average
0	0.2409	0.2439	0.2403	0.2417
10	0.3169	0.3143	0.3027	0.3113
20	0.4006	0.4292	0.4146	0.4148
30	0.4875	0.4733	0.5007	0.4872
50	0.6298	0.6359	0.6684	0.6447
60	0.6915	0.7175	0.7322	0.7137
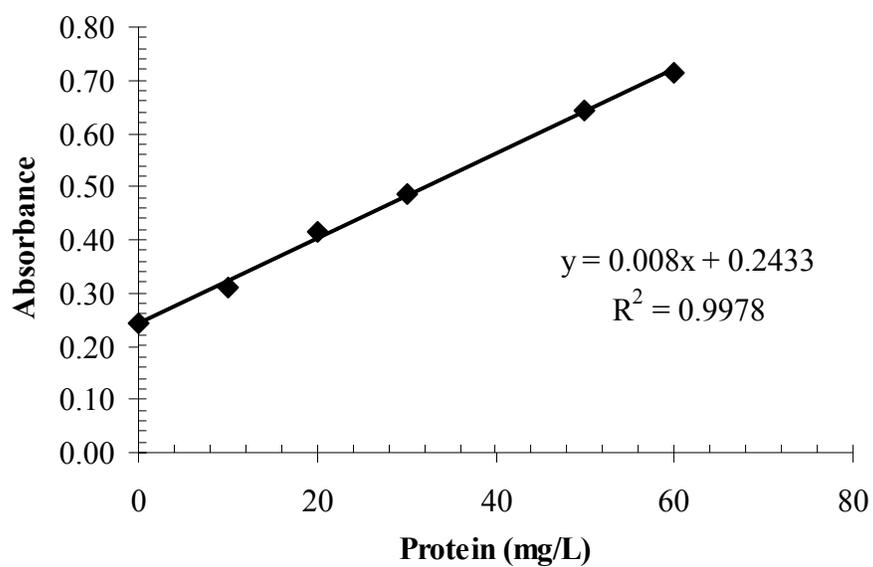
Figure 16. Calibration curve for protein measurement

TABLE 42. Tracer test data

	Date & Time	Time hr	Cr(VI) Absorbance	Cl Area	Fumarate Area	Cr(VI) mg/L	Cl- mg/L	Fumarate mg/L
1	5/1/2002 16:00	1.0	0.0349			0.00		
2	5/2/2002 9:30	18.5	0.0361	82847		0.00	5.55	
3	5/2/2002 11:00	20.0	0.0494	259722		0.21	17.87	
4	5/2/2002 12:00	21.0	0.0718	524685		0.49	36.33	
5	5/2/2002 13:00	22.0	0.1071	912331	91421	0.94	63.33	12.64
6	5/2/2002 14:00	23.0	0.1513	1277615	308265	1.50	88.77	42.49
7	5/2/2002 15:00	24.0	0.1913	1551218	503406	2.00	107.83	69.35
8	5/2/2002 16:00	25.0	0.2155	1849823	666494	2.31	128.62	91.80
9	5/2/2002 17:00	26.0	0.232	1989908	729038	2.52	138.38	100.41
10	5/2/2002 19:00	28.0	0.2452	2109141	863419	2.69	146.69	118.91
11	5/3/2002 10:30	43.5	0.2526	2125202	999661	2.78	147.81	137.66
12	5/3/2002 20:00	53.0	0.2497	2242860	986251	2.74	156.00	135.81
	Breakthrough Solution		0.2659	2033951	1022032	2.95	141.45	140.74

Recipe of SGM

TABLE 43. Components of Simulated Groundwater Media (SGM)

	Stock	Name	Formula	Mol. Wt.	Conc. In SGM		Conc. in Stock	Mol. Wt. of Anion	Conc. of Anion	
					mg/L	mM	mg/L		mM	mg/L
100 mL Stock A	A	Sodium Metasilicate	Na$_2$SiO$_3$-9H$_2$O	284.05	455	1.602	4550	76.09	1.602	121.8833
	A	Sodium Carbonate	Na$_2$CO$_3$	105.98	160	1.510	1600	60	1.510	90.583129
	A	Sodium Sulfate	Na$_2$SO$_4$	142.04	6	0.042	60	96.06	0.042	4.0577302
	A	Sodium Hydroxide	NaOH	39.99	290	7.252	2900	17	7.252	123.28082
5 mL Stock B	B	Potassium Chloride	KCl	74.55	26.5	0.355	5300	35.45	0.355	12.601274
	B	Calcium Chloride dihydrate	CaCl$_2$-2H$_2$O	146.98	8	0.054	1600	35.45	0.109	3.8590284
	B	Magnesium Hydroxide	Mg(OH)$_2$	58.31	33.5	0.575	6700	17	1.149	19.533528
	B	Phosphoric Acid	H$_3$PO$_4$	97.97	1960	20.006	392000	94.97	20.006	1899.9816
10 mL Stock C	C	Sodium Nitrate	NaNO$_3$	85	137.1	1.613	13710	62.01	1.613	100.01848
0.1 mL each Trace element	Tr-1	Lithium Chloride *	LiCl	42.39	0.021	4.95E-04	210	35.45	4.954E-04	0.0175619
	Tr-2	Cupric Sulfate *	CuSO$_4$-5H$_2$O	249.61	0.08	3.20E-04	800	96.06	3.205E-04	0.0307872
	Tr-3	Zinc Sulfate *	ZnSO$_4$-7H$_2$O	287.44	0.106	3.69E-04	1060	100.06	3.688E-04	0.0368994
	Tr-4	Boric Acid *	H$_3$BO$_4$	77.81	0.6	7.71E-03	6000	74.81	7.711E-03	0.5768667
	Tr-5	Aluminium Sulfate *	Al$_2$(SO$_4$)$_3$-18H$_2$O	666.14	0.123	1.85E-04	1230	96.06	5.539E-04	0.0532112
	Tr-6	Nickel Chloride *	NiCl$_2$-6H$_2$O	237.6	0.11	4.63E-04	1100	35.45	9.259E-04	0.0328241
	Tr-7	Cobalt Sulfate *	CoSO$_4$-7H$_2$O	280.99	0.109	3.88E-04	1090	96.06	3.879E-04	0.037263
	Tr-9	Manganese Chloride **	MnCl$_2$-4H$_2$O	197.84	0.629	3.18E-03	6290	35.45	6.359E-03	0.225415
	Tr-10	Pottasium Bromide **	KBr	119	0.03	2.52E-04	300	79.9	2.521E-04	0.0201429
	Tr-10	Potassium Iodide **	KI	166	0.03	1.81E-04	300	126.9	1.807E-04	0.0229337
	Tr-12	Ferrous Sulfate	FeSO$_4$-7H$_2$O	277.91	0.3	1.08E-03	3000	96.06	1.079E-03	0.1036954
		* Solvent 0.0074M H$_3$PO$_4$ ** Solvent 0.074M H$_3$PO$_4$								

Appendix D.

Miscellaneous

1. Replicate Column Experiment at 0.036 mM (1.85 mg/L)

Figure 17 shows results from two columns that were run in parallel for an average influent Cr(VI) concentration of 0.036 mM (1.85 mg/L). It can be seen from the Figure that influent and effluent Cr(VI) concentrations of the two replica matched very nicely. However, the breakthrough time of these replicate runs did not match the trend
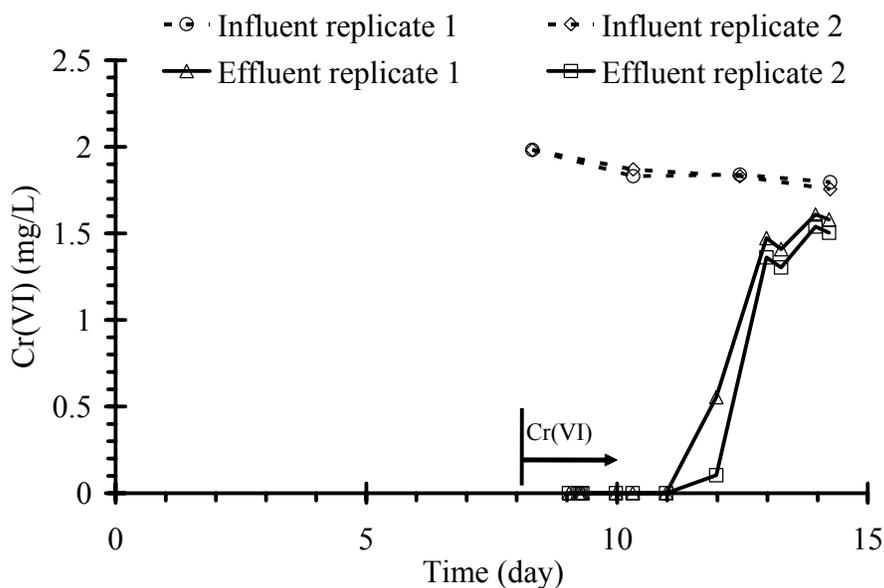
Figure 17. Replicate column run at 0.036mM (1.85 mg/L) influent Cr(VI) concentration.

observed with the other experimental runs at various influent Cr(VI) concentrations and they did not match with a triplicate run at 0.037 mM Cr(VI). It is possible that the MR-1

strain that was taken from the -80$^\text{o}$C freezer was mislabeled with other strains or consortia yielding different growth characteristics and subsequent Cr(VI) reduction rates. Since the breakthrough time from the data in Figure 17 occurs at approximately 4 pore volumes, which is less than the breakthrough time observed for the feed concentration of 1.94 mg/L and does not make sense based on what we know about Cr(VI) reduction kinetics. Consequently, it was decided not to incorporate these results (Figure 17) into the discussion in chapter 2.

2. Note to Figure 3 and 4

Stoichiometrically, 1 mM of fumarate produces 1 mM of succinate. But it can be seen in Figure 3b and 4b there is a slight deviation from this stoichiometry. Succinate concentration was found to be 10% - 20% lower than the theoretically expected value prior to Cr(VI) breakthrough. Succinate is also an intermediate of the Tricarboxylic Acid (TCA) Cycle. Intermediates of the TCA cycle are utilized in other metabolic pathways and cellular activities. It is possible that a small portion of succinate that was produced from influent fumarate was used in other metabolic pathways or cellular activities. It might be of interest to investigate further on the fumarate metabolism of MR-1.

3. Extension of Figure 5

The straight line in Figure 5 was fitted with 3 experimental data points. If this relationship is to be extended, it may not be linear anymore. Based on the kinetics of dual enzyme kinetic model and model predictions at higher feed Cr(VI) concentration, an extended plot of Figure 5 is presented in Figure 18. Experimental points are shown in
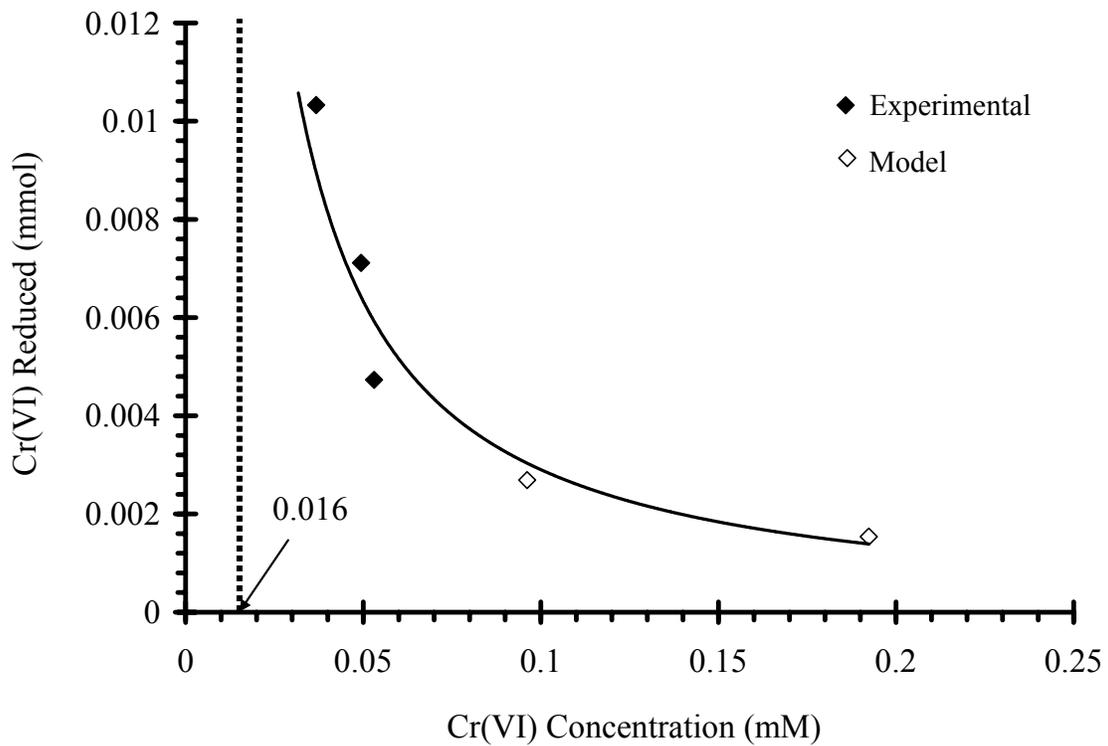
Figure 18. Cr(VI) reduced prior to breakthrough as a function of influent Cr(VI) concentration

filled diamonds. Two points were extrapolated using model simulations at 5 mg/L (0.096 mM) and 10 mg/L (0.192 mM) and are shown as open diamonds. The dotted line was drawn at 0.016 mM Cr(VI), which was calculated from the product of column residence time and stable enzyme activity rate. The stable enzyme activity rate (0.0007 mM/hr) was calculated from the observed residual Cr(VI) reduction after its breakthrough. The mass of Cr(VI) reduced prior to breakthrough is asymptotic to this dotted line at low feed concentrations (less than 0.016 mM).

4. Model Run for Various Influent Cr(VI) Concentrations

Figure 19 and 20 present results of model prediction from a column experiment with an influent Cr(VI) concentration of 0.037 mM (1.94 mg/L). The model predicted an earlier breakthrough of both Cr(VI) and fumarate (about two pore volumes) relative to the experimental data. It can be seen that the predicted effluent Cr(VI) concentration is about 50% higher than the observed experimental data.
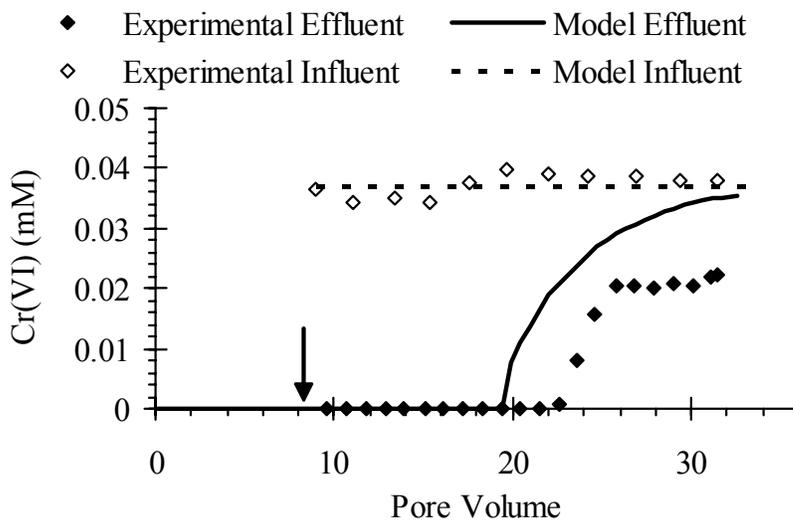
Figure 19. Cr(VI) breakthrough at the column outlet for an influent Cr(VI) concentration of 0.037 mM. Arrow represents the time when Cr(VI) was introduced to the feed.

The difference between the model prediction and experimental observation is not uncommon in literature (Munoz and Irarrazaval, 1998; Phanikumar et al., 2002). The modeling of biodegradation in a flow-through system is very complex and always a
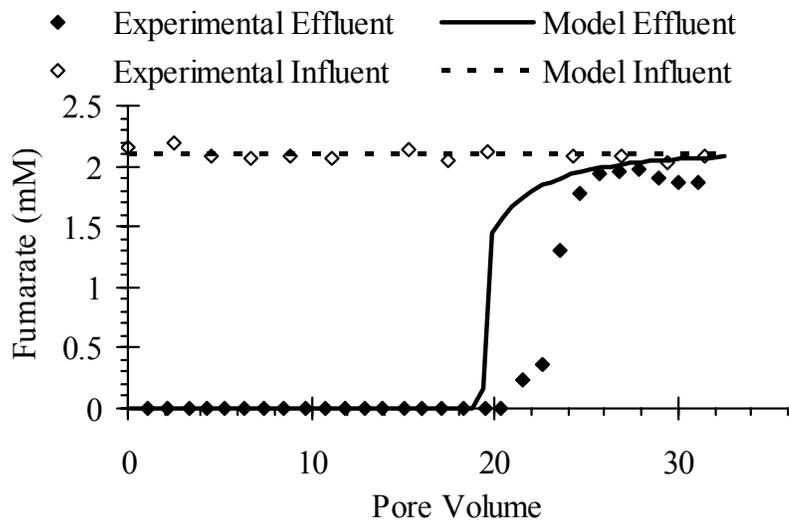
Figure 20. Fumarate breakthrough at the column outlet for an influent Cr(VI) concentration of 0.037 mM.

challenging problem. Uncertainty arises in many different areas, including, assumptions of mathematical formulations, parameter estimation, and numerical accuracy. Rate constants for Cr(VI) reduction and microbial growth kinetic parameters (specific growth rate, yield and saturation constant) were obtained from batch reactor experiments and were used directly in the model. However, it is reported in literature that the rate of biodegradation in a flow-through system is significantly lower than that of estimated in aqueous batch reactors (Kelly et al., 1996; Park et el, 2001). This may be due to the mass-transport limitations in the continuous flow systems resulting from reactive heterogeneity with the porous media. Therefore, direct application of batch reactor kinetic coefficients into a model may not yield accurate prediction in a continuous flow

system. Further, effects of Cr(VI) toxicity on MR-1 growth is poorly understood and expressed in the model. Batch reactor experiments with MR-1 showed MR-1 stopped growing in the presence of Cr(VI) although they reduced Cr(VI) at the same time. When Cr(VI) was below the detection limit they resumed growth. In a continuous flow system, this phenomenon is dynamic, i.e., reduction and growth are taking place simultaneously although it may not be at the same site. Accurate quantification of Cr(VI) toxicity may help to improve the model prediction.

The model was also used to simulate another experiment with 0.011 mM Cr(VI) in the influent. This experiment didn't experience any Cr(VI) or fumarate breakthrough for more than 60 pore volumes. The model simulation predicted no breakthrough for this experiment.

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