Finite Difference Simulation of Biological Cr(VI) Removal in Aquifer Microcosm Reactors

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ABSTRACT

Cr(VI) pollution of the environment results from extensive use of chromate and dichromate in industries. Industries such as metal finishing, petroleum refining, iron and steel industry and leather tanning discharge large amounts of Cr(VI) waste in the environment. Exposure to Cr(VI) causes both acute and chronic illnesses such as skin dermatitis and cancer. Cr(VI) is also known to be toxic to plants, animals and microorganisms. The purpose of this project was to develop an *in situ* bioremediation process to prevent the spread of Cr(VI) in groundwater aquifers. The study was conducted using five port columns. Cr(VI) concentration was measured in the influent, in five equally spaced intermediate ports within the column and in the effluent, to facilitate finite difference modeling of the Cr(VI) concentration profile within the column. Three feed concentrations: 20, 30, and 40 mg/L, were evaluated. Near complete Cr(VI) removal was achieved in less than 4 days in the inoculated reactor with organic carbon source at an initial Cr(VI) concentration of 20 mg/L, whereas only 69.5% of Cr(VI) removal was achieved at 20 mg/L in an inoculated column without organic carbon source after 4 days. Reaction kinetics of Cr(VI) reduction in the anaerobic cultures were non- competitively inhibited with k_m = mg.L^{-1}, K_c = 672.0935 mg.L^{-1}, R_c = 0.0390947 mg.mg^{-1} and C_r = 100 mg.L^{-1}. Cr(VI) reduction across the columns was simulated by a one dimensional *dispersion- reaction* model.

Keywords: microcosm reactor, *in situ*, kinetics, chromium (VI) reduction, organic electron donors.

1. 1 Introduction

Cr(VI) is a highly soluble and mobile toxic metal commonly discharged into the environment from anthropogenic sources. Cr(VI) is well known for its carcinogenic and mutagenic effects in mammals including humans. Due to its toxic effects on human the World Health Organization (WHO) has set the maximum acceptable chromium concentration in drinking water at 0.05 mg/L (Kiilunen, 1994; Lu and Yang, 1995).

Improper disposal of Cr(VI) waste from these industries to the environment is a subject of paramount concern and this problem is even made worse when industrial or mining facilities are closed down and the owners which should be responsible for their waste treatment are difficult to track down. The current method for Cr(VI) remediation in contaminated sites involves the expensive pump/dig – treat method (Nyer, 1992; Watts, 1998).

On the other hand, biological reduction of Cr(VI) to less toxic Cr(III) provides a less costly approach to soil and aquifer remediation. Recent studies have shown that many bacterial strains are capable of reducing Cr(VI) to Cr(III). However, most of these studies were conducted in batch studies (Wang *et al*., 1987; Ohtake *et al.*, 1990 and others). Only recently, studies on *in situ* Cr(VI) bioremediation processes have been conducted at the laboratory level (Molokwane and Chirwa, 2009). The aim of this study is to develop a predictive model to facilitate finite scale up and application in the field. The internal mechanism of Cr(VI) removal are further investigated and internal processes inside the reactor system are evaluated.

The *in situ* biological technologies for converting toxic Cr(VI) to less toxic Cr(III), have the potential to minimize the risk associated chromate contaminated site while also minimizing the cost of the remediation. Pump and treat method on the other hand suffers from high cost associated with energy use, pumping and excavation. This method is also not environmentally friendly since it produces secondary waste due to addition of various chemical reagents used that may be also hazardous to the environment.

2.1 Materials and Methods

2.1.1 Culture and Media

Seven CRBs isolated from dried sludge collected from sand drying beds at the Brits Wastewater Treatment Works (North West) were used in this study. When tested in suspended growth systems, the isolated consortium culture achieved a complete reduction of Cr(VI) under initial concentrations up to 200 mg/L, a much higher concentration compared to the current highest groundwater Cr(VI) concentration at the remediation wells at the study site which is approximately 40 mg/L (≅40 mg/L).

2.1.2 Columns setup

Cores from the actual site were extracted and installed in a laboratory as continuous flow columns as shown in Figure 1 below. The columns were constructed using a PVC glass (60 cm long) of 5 cm internal diameter. Five equally spaced intermediate ports were drilled along each column.

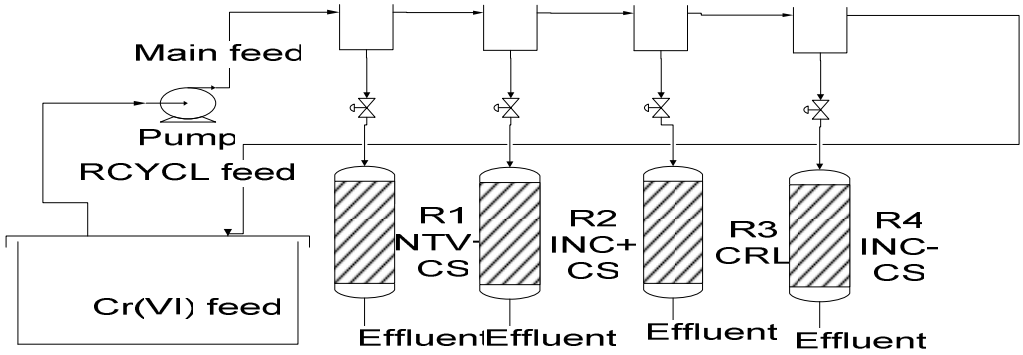


Figure1: Laboratory microcosm reactor set-up

The columns were then tightly packed with media from the target site. The columns were capped on both ends using PVC caps with influent and effluent ports. Prior to capping the

columns on both ends, one of the columns (control) was sterilized by autoclaving for 20 minutes at 121°C. In one of the columns an organic electron donor (sawdust) was amended to represent the heterogeneous carbon source from decaying vegetation.

2.1.3 Columns startup

Primarily, distilled water was fed in each of the columns to observe any leakages in the columns, to maintain a flow rate difference of not more than 5% between the columns and to target the hydraulic retention time (HRT) of 24 hours in each column (for optimum operation of the reactors). Two columns were inoculated with dried sludge culture, and the inoculated cultures in the column were allowed to adhere to soil particles for 24–48 hours prior to contaminant loading. Cr(VI) loading was simulated by gravity feeding as in the case of open aquifers at the site. Microcosm reactors were operated as packed beds at different initial Cr(VI) concentrations of 20, 30, 40 and 50 mg/L respectively. Samples withdrawn from the five equally spaced intermediate ports from each column and also from the effluent port were centrifuged at 6000 rpm for 10 minutes to remove soil particles and then followed by analysis of Cr(VI) as described below.

2.1.4 Microbial diversity analysis

Phylogenetic characterization of cells was performed on individual colonies of bacteria grown both aerobically and anaerobically from soil samples extracted from the microcosm columns at the beginning and at the end of the experiment. LB and PC agar were used for colony development. In preparation for the 16S rRNA sequence identification, the colonies were first classified based on morphology. Genomic DNA was extracted from the pure cultures using a DNeasy tissue kit (QIAGEN Ltd, West Sussex, UK) as per manufacturer's instructions.

The 16S rRNA genes of isolates were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers pA and pH1 (Primer pA corresponds to position 8–27, Primer pH to position 1541–1522 of the 16S gene) (Coenye *et al*., 1999). An internal primer pD was used for sequencing (corresponding to position 519–536 of the 16S gene). The resulting sequences were matched to known bacteria in the GenBank using a basic BLAST search of the National Centre for Biotechnology Information (NCBI, Bethesda, MD).

2.2 Analytical Methods

2.2.1. Analytical methods Cr(VI) and total Cr

Cr(VI) was measured using a UV/vis spectrophotometer (WPA, Light Wave II, Labotech, South Africa). The measurements were carried out at a wavelength of 540 nm (10 mm light path) after digestion of 0.2 mL samples with 1 mL of 1 N H_2SO_4 and reaction with (0.2 mL) of 1,5-diphenyl carbazide (DPC) to produce a purple-pink color (APHA, 2005). Total Cr was measured at a wavelength of 359.9 nm using a Varian AA–1275 Series Flame Atomic Adsorption Spectrophotometer (AAS) (Varian, Palo Alto, CA) equipped with a 3 mA chromium hollow cathode lamp. Before analysis using the AAS, 1 mL samples were acidified with 9 mL of 1N H_2SO_4 to dissolve chromium hydroxide precipitates and to extract adsorbed Cr(VI). Cr(III) was determined as the difference between total Cr and Cr(VI) concentration

2.3 Model Development

Advection and dispersion are the main modes of transport of Cr(VI) in the groundwater. Retardation may not play any role in defining the fate and transport of Cr(VI) in a long run

2.3.1 Reactor Mass balance

A detailed mathematical model that influence the reduction of Cr(VI) in the fixed bed reactor consist of the following aspects in its mass balance equation:

(i) The advection

(ii) The flux

(iii) The removal rate by suspended cells

(vi)The adsorption rate governed by mass transport and surface reaction

2.3.2 Advection

Describes the transport of the dissolved substance along with bulk fluid flow and is represented as:

$$\frac{-d(CV)}{dt} = Au(C_{in} - C) \quad (1)$$

Where: A= cross sectional area (L^2), u= velocity of the flow (LT^{-1}), C_{in}= Cr(VI) influent concentration, C= effluent Cr(VI) concentration at time, t.

2.3.3 Flux in biofilm

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

$$\frac{d(CV)}{dt} = -D_w \frac{dC}{dx} = -\frac{D_w}{L_w}(C_b - C_s) = -j_c.A_f \quad (2)$$

Where: D_w = dispersion coefficient of Cr(VI) in water (L^2T^{-1}), dC/dx = Cr(VI) concentration gradient ($ML^{-3}.L^{-1}$), L_w = thickness of stagnant layer (L), C_b= bulk liquid Cr(VI) concentration (ML^{-3}), C_s = Cr(VI) surface concentration (ML^{-3}), NB : $C_b >> C_s$, therefore C_s is negligible with respect to bulk concentration.

Under low loading conditions, Cr(VI) removal by the biomass may be limited by the rate at which Cr(VI) disperses into cell layer attached to the soil particles. Therefore the relationship between Cr(VI) reduction rate and dispersion rate may be presented as:

$$j = -j_c.A_f \quad (3)$$

Where: j = mass transport rate (MT^{-1}), j_C = flux rate ($ML^{-2}T^{-1}$) and A_f =biofilm surface area (L^2).

2.3.4 Cr(VI) Removal by suspended cells

The removal of dissolved species of Cr(VI) in a reactor is also governed by kinetics obtained in the suspended system. Therefore the rate of Cr(VI) reduction by suspended biomass under anaerobic condition is presented by modified Michaelis-Menten model associated with Cr(VI) toxicity threshold. The non-competitive inhibition model with Cr(VI) toxicity threshold and cell inactivation is represented as :

$$\frac{-dC}{dt} = \frac{k_m C}{K^{\left(1+\frac{C_r}{C_0}\right)}\left(K_C + C\right)}\left(X_0 - \frac{C_0 - C}{R_c}\right) = -r_C \tag{4}$$

Where: k_m =specific maximum Cr(VI) reduction rate (T^{-1}), C_r = Cr(VI) toxicity threshold, K_c= half saturation coefficient (ML^{-3}), K = constant (ML^{-3}), X_o= initial biomass concentration (ML^{-3}), R_c= reduction capacity of cells (MM^{-1})

2.3.5 Removal by Adsorption

The removal of Cr(VI) in the biofilm by adsorption is represented as:

$$-\frac{dC}{dt} = k_{ad}\left(C_{eq} - C\right) = -q_C \tag{5}$$

Where: k_{ad} = adsorption rate coefficient (T^{-1}), C_{eq}= equilibrium concentration at surface area (ML^{-3})

Therefore the overall mass balance of the packed-bed reactor is represented as:

$$-\frac{d(CV)}{dt} = Au(C_{in} - C) - r_C \Delta V - j_C . A_f - q_C \Delta V \tag{6}$$

Where: C= effluent Cr(VI) concentration (ML^{-3}), V= volume of the reactor (L^3), C_{in} = influent Cr(VI) concentration (ML^{-3}),Q= influent flow rate (L^3T^{-1}), A= cross sectional area (L^2), u= velocity of the flow (LT^{-1}), q_c= the rate of Cr(VI) removal by adsorption ($ML^{-3}T^{-1}$)

2.4 Results and Discussion

2.4.1 Model Simulation and Parameter Optimization

The batch studies were conducted under anaerobic condition to determine the kinetic parameters for the reduction of Cr(VI). The kinetic parameters were obtained by performing a nonlinear curve fitting data using a Computer Program for the Identification and Simulation of Aquatic Systems (AQUASIM 2.0). In the sterile packed column model for saturated packed column with dispersion was adapted from AQUASIM 2.0. This model was used in combination with Cr(VI) kinetic parameters adapted from the anaerobic batch studies.

2.4.2 Parameter Optimization

The model was initially simulated using batch kinetic parameters and physical parameters computed in (Table 1) below. Nonbatch physical parameters include the dispersion coefficient, D_w, porosity, θ , the influent flow rate, Q, and the cross sectional area, A. For each kinetic parameter, a search was carried out through a range of values which were initialized by guessed values and values from batch studies. To ascertain that the optimized parameters obtained using the mathematical model were dependable, upper and lower constrains were set for each parameter to allow the omission of invalid parameter values (Table1). Whenever optimization converged at or very close to a constraint, the constrain was relaxed until the constraint no longer forced the model. The process was repeated until uniformly fit values were obtained.

2.4.3 Cr(VI) Removal kinetics in reactor columns

The optimum values of kinetic parameters summarized in Table1 above illustrates that in the carbon source reactor the dispersion coefficient is much higher than that observed in the non-carbon source reactor .This indicates that the rate at which the contaminant disperses into the cell layer attached to the aquatic media influence the removal of Cr(VI) by biomass. It also observed in the table that the cell death rate is faster in the non-carbon source reactor compared to the carbon source reactor.

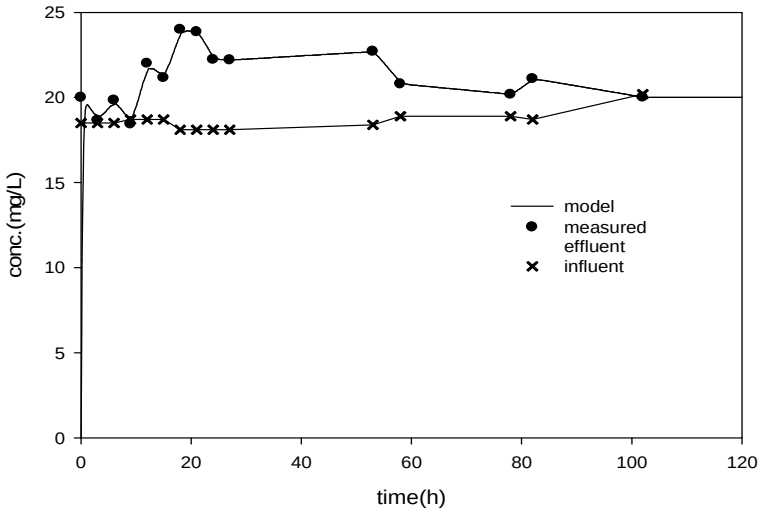
Table1: Optimum kinetic parameters values for the carbon and non-carbon source reactor at initial Cr(VI) concentration of 20 $mg.L^{-1}$

Symbol	Description	Constrains [lower, upper]	+CS column optimized value	-CS column optimized value
$C(mg.L^{-1})$	State variable	-----	1×10^{-6}	1×10^{-6}
$K(mg.L^{-1})$	constant	-----	0.5	0.5
$mu\ (h^{-1})$	Specific biomass growth rate	[0,10000]	0.02654	0.19245
$K_d(h^{-1})$	Cell death rate coefficient	[0,10000]	0.001274	0.003911
$k_m(h^{-1})$	Specific reduction rate	[0,0.02]	0.0050493	0.0126122
$K_c(mg.L^{-1})$	Half saturation coefficient	[0,15]	11.27203	13.79377
$R_c(mg.L^{-1})$	Cell deactivation coefficient	[0,0.5]	0.048144	0.010179
$Q_{in}(L.h^{-1})$	Inflow rate	-----	0.001380	0.001380
$A(m^2)$	Cross sectional area	-----	0.0007853	0.0007853
$D(m^2.h^{-1})$	Dispersion coefficient	[0,100]	95.41819	11.708087

----- represent constant values

Figure 2 (A-B), shows the results of effluent simulation in a sterile control, carbon and non-carbon source reactor at an initial Cr(VI) concentration of 20 mg/L .The figures illustrates that the model best fit the experimental data with kinetic parameter optimized. It is also observed from Figure 2 (B) that after long operation period adsorption sites on the aquifer media particles are saturated. This implies that after a long run the removal of Cr(VI) in the column reactors is no longer influence by the adsorption process. The only possible mechanism responsible for Cr(VI) removal in the reactors is the reduction process governed by anaerobic batch kinetics as described in the table above.

(A)



(B)

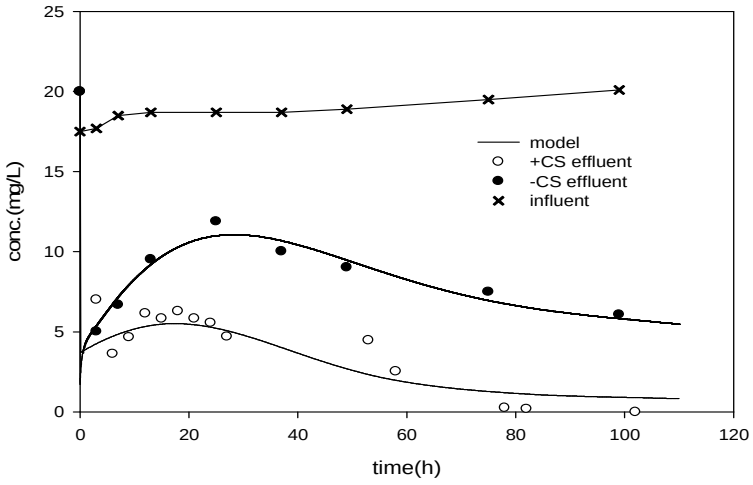


Figure 1: (A) Simulation of sterile control at 20 mg/L (B) Simulation of effluent at 20 mg/L with carbon source and with no carbon source.

Microbial diversity

A microbial shift was observed in the column environment different .The results in Table1 below illustrates that among all the bacteria species initially inoculated in the column reactors the *Bacillus thuringiensis* and the *Bacillus cereus* remained persistent in all inoculated columns after four weeks of operation.

Table1: Characterization remaining bacterial isolates after operation

Reactor	Blast ID	% ID
R1	*Enterococcus faecium*	99
	Bacillus anthracis	99
R2	*Enterococcus faecium*	99
	Bacillus thuringiensis+ Bacillus cereus	99

2.5 Conclusion

Microcosm studies showed that the inoculated soil column effectively reduced Cr(VI) from the contaminated site. This study shows that the *dispersion-reaction* model may be used to optimize kinetic parameters in a biological system and to predict output variables for a range of operation conditions using optimum parameter values. The majority of the soil column parameters were consistent with values found in literature derived from single species (pure) cultures. The model developed here is suitable for simulation of Cr(VI) reduction under a range of Cr(VI) loadings. This model may be easily modified for application in engineering biological system for treating Cr(VI) contaminated.

References

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

Coenye T., Falsen E., Vancanneyt M., Hoste B., Govan J.R.W., Kersters K., Vandamme P., (1999). Classification of Alcaligenes faecalis-like isolates from the environment and human clinical samples as Ralstonia gilardii sp. nov. International Journal of Systematic Bacteriology 49, 405–413.

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

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

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

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

Nyer E.K. (1992). "Treatment methods for inorganic compounds". *Groundwater treatment technology*, Van Nostrand Reinhold, New York, N.Y.,218.

Ohtake H., Fujii E., Toda K. (1990). Reduction of toxic chromate in an industrial effluent by use of a chromate-reducing strain of *Enterobacter cloacae*. *Environmental Technology Letters*, 11 (43), 663-668.

Wang P., Mori T., Komori K., Sasatsu M., Toda K., Ohtake H. (1989). Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. *Applied and Environmental Microbiology*, 55 (7), 1665-1669.

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