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Hexavalent Chromium Reduction by *Bacillus* sp. in a Packed-Bed Bioreactor

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The potential for fixed-film biorectors to reduce Cr(VI) was demonstrated using a Cr(VI)-reducing species, *Bacillus* sp. A bench-scale, packed-bed bioreactor was operated to steady-state conditions under a range of influent Cr(VI) concentrations (10−200 mg/L) and hydraulic detention times (6−24 h, clean bed) with near complete removal of Cr(VI). The steady-state Cr(VI) reduction efficiency was not affected by the influent Cr(VI) concentration or hydraulic detention time. Chromium mass balance analysis revealed that nearly all the Cr(VI) fed to the bioreactor was accounted for in the effluent as Cr(VI) and Cr(III). Total cell mass in the bioreactor decreased with increasing Cr(VI) loading rate, but it stabilized after a loading limit was reached (1016 mg of Cr(VI)/L·day) when operated under 24 h hydraulic detention time. The bioreactor showed strong resilience by recovering from Cr(VI) overloading through reduction in influent Cr(VI) concentration.

Introduction

The hexavalent form of chromium (chromate) is highly toxic and is known to be both carcinogenic and mutagenic to living organisms. Chromate is also teratogenic to mammals including humans (*1*). Higher than allowable concentrations of chromate are detected in groundwater and surface water receiving industrial wastes (*2*). Recent studies have shown that certain species of bacteria are capable of transforming hexavalent chromium, Cr(VI), into the much less toxic and less mobile trivalent form, Cr(III) (*3*−*6*).

Bacteria may protect themselves from toxic substances in the environment by transforming toxic compounds through oxidation, reduction, or methylation into more volatile, less toxic, or readily precipitating forms (*7*−*10*). Enzymatic reduction of Cr(VI) into Cr(III) is believed to be one of the defense mechanisms employed by microorganisms living in Cr(VI)-contaminated environments (*10*, *11*). The reduced Cr(III) may precipitate as chromium hydroxide in neutral pH range.

Virtually all the previous studies on biological reduction of Cr(VI) were conducted in batch cultures (*3*). Recently, Shen and Wang demonstrated Cr(VI) reduction in a two-stage, continuous-flow suspended growth bioreactor system (12). *Escherichia coli* ATCC 33456 cells grown in the first stage, completely mixed reactor were pumped into the second stage, plug-flow reactor to reduce Cr(VI). The *E. coli* cells were grown without Cr(VI) in the first stage to minimize the effect of Cr(VI) toxicity on the cells. The previous work revealed that Cr(VI) reduction in the plug-flow reactor system was limited by the substrate (glucose) concentration along the reactor and the specific Cr(VI) reduction capacity of the cells. Near complete removal of Cr(VI) was observed for influent Cr(VI) concentrations up to 4.37 mg/L at a hydraulic detention time of 5.1 h. It was also demonstrated that Cr(VI) removal efficiency could not be improved by merely increasing the liquid detention time after a certain Cr(VI) loading limit had been reached (*12*).

This study demonstrated the potential of fixed-film bioreactors for Cr(VI) reduction. Much of the previous research on pollutant removal by biofilm processes has been focused on substrate adsorption and substrate utilization (*13*−*15*). This paper is the first to report on Cr(VI) reduction through biological mechanisms in a continuous-flow, biofilm reactor without the need to constantly resupply fresh Cr(VI)-reducing cells.

Materials and Methods

Media. Vogel−Bonner (VB) broth was prepared by dissolving 5.0 g of K_2HPO_4, 3.5 g of $Na(NH_4)HPO_4$, 2.0 g of citric acid ($C_6H_8O_7$), 0.2 g of $MgSO_4$, and 5.0 g of D-glucose in 1.0 L of distilled water. The influent feed to the bioreactor was prepared by dissolving 5.0 g of K_2HPO_4, 3.5 g of $Na(NH_4)HPO_4$, 0.2 g of $MgSO_4$, and 5.0 g of D-glucose in 1.0 L of distilled water. Peptone−yeast−glucose (PYG) agar was prepared by dissolving 10 g of bacto-peptone, 5.0 g of bacto-yeast extract (Difco), 3.0 g D-glucose, and 15 g of bacto-agar (difco) in 1.0 L of distilled water. Nutrient agar was prepared either by dissolving dehydrated nutrient agar (Difco) in 1.0 L of distilled water or by adding 15 g of bacto-agar (DIFCO) into 1.0 L of nutrient broth medium before autoclaving. All media were sterilized by autoclaving for 15 min at 121 °C.

Reagents. Stock Cr(VI) solution (1000 mg/L) was prepared by dissolving 3.75 g of 99.9% K_2CrO_4 in about 250 mL of distilled water and diluting to 1.0 L. Stock Cr(III) solution (1000 mg/L) was prepared by dissolving 5.15 g of 99.5% $CrCl_3·6H_2O$ in distilled water and diluting to 1.0 L. Potassium permanganate solution was prepared by dissolving 4.0 g $KMnO_4$ in 100 mL distilled water. Azide solution was prepared by dissolving 0.5 g of NaN_3 in 100 mL of distilled water. Diphenyl carbazide solution was prepared by dissolving 250 mg of 1,5-diphenyl carbazide in 50 mL of HPLC-grade acetone and stored in a brown bottle. Low-alkalinity copper reagent (12 g of $KNaC_4H_4O_6·4H_2O$, 24 g Na_2CO_3, 4.0 g of $CuSO_4·5H_2O$, and 16 g of $NaHCO_3$ in 1.0 L of distilled water) was prepared according to the procedure of Hodge and Hofreiter (*16*). Arsenomolybdate reagent (25 g of $(NH_4)_6Mo_7O_{24}·4H_2O$, 21 mL of 96% sulfuric acid, and 3.0 g of $Na_2HAsO_4·7H_2O$ in 1.0 L of distilled water) was prepared according to Hodge and Hofreiter (*16*). 1,5 Diphenylcarbazide and $(NH_4)_6Mo_7O_{24}·4H_2O$ were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were purchased from Fisher Scientific Co. (Pittsburgh, PA).

Bacterial Strain. *Bacillus* sp. was originally isolated in our laboratory from chromate-contaminated soil (*17*). Sterile nutrient broth (200 mL) in 500-mL Erlenmeyer flask stoppered with a sterile cotton plug was inoculated by one loop of pure *Bacillus* sp. culture grown on PYG or nutrient agar slants. The flasks were then incubated overnight (approximately 24 h) at 30 °C on a continuous shaker at 300 rpm (Model G4, New Brunswick Scientific Co., Inc., Edison, NJ). The cells were then harvested by centrifugation at 4500*g* and 4 °C for 5 min and washed three times using a 0.85% NaCl solution of 10% volume of the original culture. Harvested cells were used as the seed for the fixed-film bioreactor.

Reactor System. The reactor system was designed to operate under completely mixed and fully aerated conditions. The reactor (Figure 1) consisted of a 12.5 cm long, 2.54 cm

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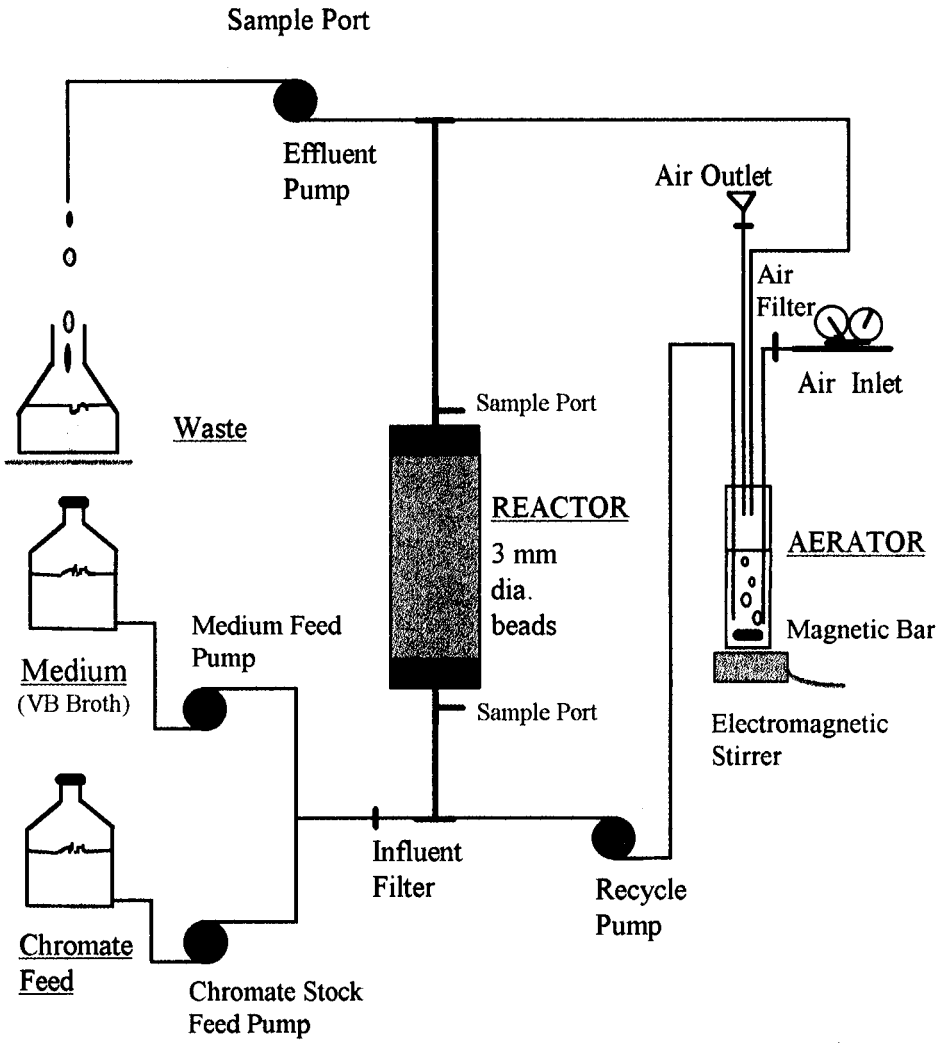

FIGURE 1. Schematic of fixed-film bioreactor system.

internal diameter column of Pyrex glass (Corning Glassware Co., Corning, NY). The reactor column was packed with 2996 spherical Pyrex glass beads of 3.0 mm diameter (Corning) to provide a total external surface area of 2406 cm^2 for attached growth of *Bacillus* sp. The reactor was set up in a 30 °C temperature room and was operated in an up-flow mode with effluent recycle. A recycle ratio of 100:1 was employed to obtain completely mixed flow pattern in the reactor. An aeration chamber (23 cm long and 0.8 cm internal diameter) was installed on the recycle line, and compressed air was continuously sparged in a counter-flow direction to the liquid flow to maintain a near-constant dissolved oxygen level of 2.90 ± 0.75 mg of DO/L in the reactor. The liquid in the aeration chamber was vigorously mixed with the air using a magnetic stirrer. Precalibrated double-headed peristaltic pumps (MasterFlex, Cole-Palmer Inst. Co., Niles, IL) were used to feed phosphate-buffered glucose solution into the reactor. The pumps were calibrated for rates of 1.3, 2.6, and 5.3 mL/h, resulting in clean-bed hydraulic detention times of 24, 12, and 6 h, respectively. Plated samples of the influent feed on PYG agar showed that no viable cells were fed to the reactor. Microfilters of 0.45 μm pore size (Millipore, Bedford, MA) were installed on all feed and air lines to prevent reactor culture contamination.

Reactor Startup. The reactor assembly was first sterilized by autoclaving for 15 min at 134 °C and then assembled under a laminar flow hood (SterilCARD Class II Type A/B3, Model SG 400, The Baker Co., Inc., Sanford, ME). The reactor system was then operated under a feed Cr(VI) concentration of 10 mg/L and a liquid detention time of about 6 h for 2 days without cells to serve as control. No abiotic Cr(VI) removal was found as the effluent Cr(VI) concentration quickly reached the influent level and remained constant. Finally, the reactor was inoculated by introducing 30 mL of overnight grown culture of *Bacillus* sp. After inoculation, the reactor was operated under 24-h hydraulic detention time with VB broth medium for 6 days until apparent growth appeared on glass beads. The reactor was then operated under the phosphate-buffered glucose solution with the lowest influent Cr(VI) concentration (10 mg/L) and the highest hydraulic detention time (24 h).

Analytical Methods. Cr(VI) and total chromium were determined by colorimetric method using a Milton Roy Spectronic 1201 (Milton Roy, Rochester, NY) following the method described in the *Standard Methods for the Examination of Water and Wastewater* (*18*). Samples (1.0 mL) withdrawn from the fixed-film bioreactor effluent sampling ports (Figure 1) were centrifuged at 4500*g* for 5 min to remove suspended cells prior to analysis. Total chromium was determined by measuring Cr(VI) concentration after digestion following the procedure described in ref 18. Effluent Cr(III) for phases I−VI (day 1−day 46) was measured indirectly by taking the difference between the measured total chromium and Cr(VI). After day 46, Cr(III) in the effluent from the bioreactor was measured directly by ion chromatography. Cr(VI) and Cr(III) was separated following the procedure of Shen and Wang using an ion chromatograph (Model DX-300, Dionex Co., Sunnyvale, CA) equipped with an HPIC-CS5 column (Dionex) (*19, 20*). The two separated forms of chromium were detected at 520 nm using a variable wave-

length UV−visible light detector. At least three samples were taken for the determination of chromium during each steady-state operation. Cr(VI), Cr(III), and total Cr concentrations were all reported as milligram Cr per liter. Cr(III) accumulation in pelleted cells was determined by measuring the difference in Cr(III) level in solution before and after washing the cells three times in 0.1 N HCl and distilled water (*11*). The pelleted cells were obtained by centrifugation at 4500*g* for 10 min from an overnight grown culture with a density of about 5×10^4 cells/mL (3.4 mg of cells/mL).

Glucose was measured by Nelson's colorimetric modification of Somogyi's Micro Copper Method (*16*). Samples for glucose determination were prepared by adding 1.0 mL of low-alkalinity copper reagent to 1.0-mL samples and boiling for 1 min. After cooling for 1 min, 2.0 mL of arsenomolybdate reagent was added to the solution. The change of color from orange to dark blue indicated the presence of glucose. The solution was then diluted to 25 mL, and glucose concentration was determined by a spectrophotometer (Milton Roy) at 500 nm.

Viable cells were determined using the pour plate method on peptone−yeast−glucose (PYG) agar as described in Section 9215B of ref 18. Colonies were counted after incubation for 24 h on a colony counter (Quebec Colony Counter Model 3330, American Optical, Co., Buffalo, NY). The colony morphology on the plate was examined periodically under a microscope to ensure culture purity. No contamination was noted throughout the experiments. The total suspended cell concentration was measured as volatile suspended solids (VSS) according to section 2540 of ref 18. Samples for the analysis of suspended cell concentration determined as VSS and viable count were withdrawn from sampling ports at the top and bottom of the reactor column at appropriate intervals.

Once steady-state operation conditions were obtained, glass beads were removed from the reactor column by opening the reactor under a laminar flow hood (The Baker Co.). Twelve beads were taken from the center and from two opposite locations on the inside perimeter of the reactor column, from both the top and bottom (2 beads per location) of the reactor. The removed beads were replaced by 12 sterile clean beads while the rest of the beads and the liquid in the reactor were left undisturbed. Six beads were used for the determination of viable attached cell count. Each glass bead sample was washed three times by shaking vigorously for 10−15 min. in tightly closed 10-mL microreaction vessels (Supelco, Inc., Bellefonte, PA) containing 10 mL of sterile VB broth. Samples (1.0 mL) from each microreaction vessel were serially diluted in 30-mL test tubes containing 9.0 mL of 0.85% NaCl solution. The diluted samples of 1.0 mL were then deposited on sterile Petri dishes containing PYG agar for colony counting. The remaining six beads were washed once with gentle shaking for 10 min in 10 mL of distilled water to remove sorbed medium from biofilm surface. The sample beads were dried in an oven for 30 min at 105 °C, cooled to room temperature in a desiccator, and then weighed to determine the dry weight of the glass beads plus biomass. The beads were then washed three times (for 10−15 min each wash) with vigorous shaking, dried again at 105 °C for 30 min, and then weighed. The attached cell mass was computed from the loss in weight of the beads after washing. The standard error using this method was within estimated to be ±5.0% of the mean. At least two samples were taken for analysis of attached biomass throughout the duration of each steady-state operation condition.

Dissolved oxygen was measured using a DO meter (YSI Model-58, Yellow Springs Instruments Co., Inc., Yellow Springs, OH). The DO meter was calibrated in an air of 100% relative humidity before each measurement throughout this experiment. DO measurements were taken only when steady-state operation conditions were obtained.

Results and Discussion

Cr(VI) Reduction. The bioreactor system was operated continuously for a period of 148 days over a range of influent Cr(VI) concentrations (10−500 mg/L) and liquid detention times (6−24 h). The data in Figure 2A show influent and effluent Cr(VI) concentrations throughout the course of this study. Cr(VI) breakthrough was observed in phase VII when the influent Cr(VI) concentration was increased from 200 to 500 mg/L on day 47. Except for phases VII, X, XII, and XV, Cr(VI) reduction was near complete and steady-state operation conditions were obtained (Table 1). The steady-state DO level did not differ significantly between different operating conditions, varying from 1.8 to 3.5 mg/L (Table 1).

The data in Figure 2B show that the pattern of glucose utilization corresponded to Cr(VI) reduction in the reactor. Glucose concentration was reduced from 5.0 g/L in the influent to lower than 1.0 mg/L in the effluent accompanied by near complete removal of Cr(VI) during phases I−VI. Cr(VI) reduction in the reactor decreased with concomitant decrease in glucose utilization when influent Cr(VI) concentration was increased to 500 mg/L at the beginning of phase VII. The rapid decrease in effluent glucose concentration after reducing influent Cr(VI) concentration from 500 to 10 mg/L in phase VIII indicated rapid recovery of metabolic activity in the bioreactor.

Based on the stoichiometric relation for Cr(VI) reduction using glucose as the electron donor:

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

the feed glucose concentration 5 g/L (28 mmol/L) exceeded the theoretical requirement for the reduction of even the highest influent Cr(VI) concentration (500 mg/L or 192 mmol/L). The observed steady-state effluent Cr(III) concentrations approximated influent Cr(VI) concentrations (Table 1), indicating that nearly all the Cr(VI) fed to the bioreactor was reduced to Cr(III) during steady-state operation conditions.

System Response to Increased Influent Cr(VI) Concentration. Figure 2A shows the pattern of system response to the increased influent Cr(VI) concentration. In general effluent Cr(VI) concentration began to increase soon after Cr(VI) influent concentration was increased. This trend was observed whenever the influent Cr(VI) concentration was increased to a higher value in phases I−VI, phase XIII, and phase XIV. For example, the effluent Cr(VI) concentration increased to approximately 6.2 mg/L and then decreased to a stable level of 0.2 ± 0.15 mg/L when influent Cr(VI) concentration was increased from 100 to 150 mg/L during Phase X operation (Figure 2A). Glucose utilization preceded Cr(VI) reduction as the system adjusted to the new loading conditions (Figure 2B). This trend suggests that higher metabolic activity in the bioreactor may result in higher rate of Cr(VI) reduction.

Cr(VI) reduction in the reactor recovered completely when the influent Cr(VI) concentration was reduced from 500 to 10 mg/L in phase VIII (Figure 2A and Table 1). The initial decrease in effluent Cr(VI) concentration, soon after the influent Cr(VI) concentration was lowered to 10 mg/L, was possibly due to the dilution effect since glucose was not utilized in the reactor for a period of 3.5 days after the reduction in the influent Cr(VI). The elevated glucose concentration in the effluent indicated that biological activity was inhibited by Cr(VI) toxicity. However, biological activity recovered as evidenced by a rapid decrease in glucose concentration from 5000 mg/L to approximately 1.0 mg/L, 5 days into phase VIII. During the period of system recovery (phase VIII), the number of viable suspended cells increased from 2.0×10^1 to 1.1×10^{10} cells, and viable attached cells increased from 4.0×10^4 to 6.6×10^7 cells in the reactor

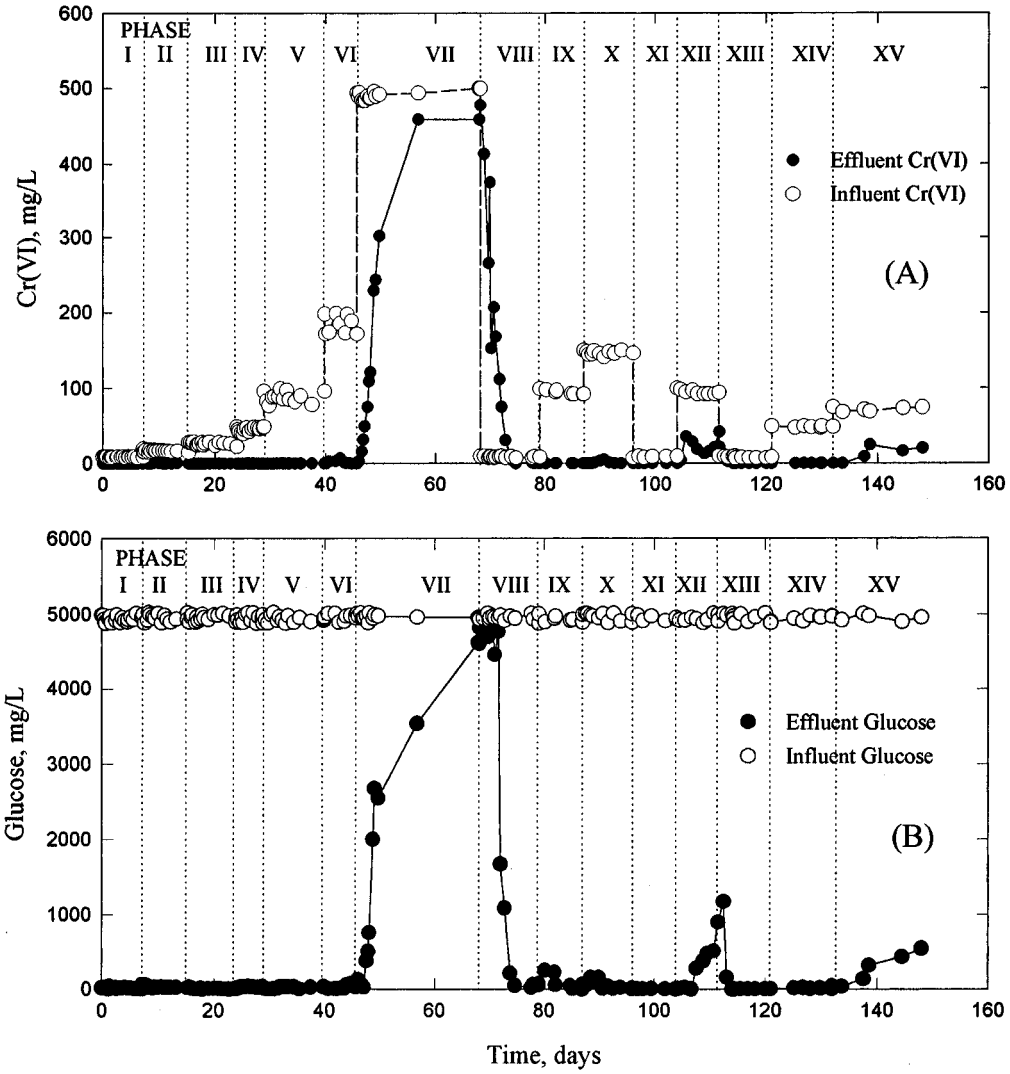

FIGURE 2. Influent and effluent (A) Cr(VI) concentration and (B) glucose concentration from the fixed-film bioreactor.

TABLE 1. Steady-State Performance of the Fixed-Film Bioreactor

phase	days	liquid detention time (h)	influent Cr(VI) (mg/L)	Cr(VI) loading rate[a] (mg/L·day)	effluent Cr(VI) (mg/L)	effluent Cr(III) (mg/L)	DO (mg/L)	av Cr(VI) removal (%)
I	1−6	24	9.70 ± 0.40	203 ± 8	0.005 ± 0.00	9.6 ± 0.14[b]	3.3	99.9 ± 0.1
II	6−16	24	18.94 ± 1.04	396 ± 21	0.07 ± 0.01	18.8 ± 0.02[b]	2.7	97.6 ± 2.3
III	16−24	24	28.20 ± 1.50	590 ± 31	0.10 ± 0.06	28.1 ± 0.30[b]	2.5	99.8 ± 0.1
IV	24−27	24	48.50 ± 1.50	1016 ± 31	0.45 ± 0.13	48.0 ± 0.10[b]	2.6	99.8 ± 0.1
V	27−41	24	99.87 ± 0.71	2092 ± 15	0.50 ± 0.12	99.3 ± 0.75[b]	2.8	99.8 ± 0.2
VI	41−47	24	197.50 ± 2.50	4138 ± 52	0.41 ± 0.24	196.0 ± 3.65[b]	2.1	99.8 ± 0.2
VII[c]	47−69	24	496.00 ± 3.80	10392 ± 80			3.4	
VIII	69−79	24	9.80 ± 0.20	205 ± 4	0.01 ± 0.50	9.5 ± 0.55	1.8	94.9 ± 5.0
IX	79−87	12	99.88 ± 0.06	4185 ± 3	0.21 ± 0.09	98.0 ± 0.74	3.2	99.8 ± 0.2
X[1]	87−96	12	149.99 ± 0.03	6285 ± 0[d]	0.47 ± 0.00[d]		3.5	99.7 ± 0.0[d]
XI	96−104	12	9.75 ± 0.20	409 ± 8	0.07 ± 0.03	9.7 ± 0.10	3.1	99.3 ± 0.6
XII[c]	105−112	6	99.90 ± 0.04	8372 ± 3			3.3	
XIII	112−121	6	9.89 ± 0.10	828 ± 8	0.03 ± 0.01	8.9 ± 0.50	3.0	99.8 ± 0.2
XIV	121−132	6	49.60 ± 0.39	4157 ± 33	0.17 ± 0.03	48.2 ± 1.60	2.9	99.7 ± 0.2
XV[c]	133−148	6	72.05 ± 0.60	6038 ± 50			3.4	

[a] Based on empty bed volume. [b] Cr(III) measured indirectly by the digestion method (*29*). [c] Steady-state operation not obtained. [d] Value taken at the end of the phase.

(Table 2). The rapid increase in both viable suspended and viable attached cells, coupled with the rapid decrease in glucose concentration, demonstrated system resilience to Cr(VI) overloading. System resilience was also observed during phases XIII and XI at liquid detention times of 6 and 12 h, respectively.

Effect of Hydraulic Detention Time. The Cr(VI) reduction efficiency of the bioreactor was not affected by the hydraulic detention time varying from 6 to 24 h under the same Cr(VI) loading rate. The data in Table 1 show that steady-state Cr(VI) removal efficiencies remained virtually the same at 99.8, 99.8, and 99.7% for phases VI, IX, and XIV, respectively,

TABLE 2. Steady-State Biomass Distribution in Bioreactor

phase	Cr(VI) loading (mg/L)	total suspended cells in reactor (mg of VSS)	viable suspended cell count[a] (no. in reactor)	total attached cells in reactor (mg of VSS)	viable attached cell count[a] (no. in reactor)	effluent VSS (mg/L)	mean cell residence time (days)
I	0.25 ± 0.01	148 ± 14	1.0×10^{10}	983 ± 88	5.0×10^{7}	2200 ± 150	9.31
II	0.49 ± 0.03	139 ± 45	3.0×10^{7}	919 ± 90	6.0×10^{7}	2066 ± 76	9.28
III	0.73 ± 0.04	120 ± 15	5.0×10^{6}	817 ± 34	6.0×10^{8}	1766 ± 65	9.52
IV	1.18 ± 0.09	133 ± 6	4.0×10^{6}	507 ± 33	7.0×10^{8}	1784 ± 50	5.86
V	2.47 ± 0.13	99 ± 10	1.0×10^{6}	665 ± 20	7.0×10^{7}	1977 ± 55	9.41
VI	4.95 ± 0.22	76 ± 6	3.0×10^{4}	744 ± 40	3.0×10^{6}	1472 ± 35	13.15
VII[b]	12.90 ± 0.25	15 ± 4	2.0×10^{1}	645 ± 30	4.0×10^{4}	133 ± 21	
VIII	0.25 ± 0.01	149 ± 17	1.1×10^{10}	1010 ± 78	6.6×10^{7}	2230 ± 120	9.55
IX	5.08 ± 0.10	134 ± 15	7.0×10^{5}	1219 ± 74	7.0×10^{8}	2468 ± 160	6.15
X[b]	7.70 ± 0.16	13	3.0×10^{3}	1081	3.0×10^{6}	1992	
XI	0.50 ± 0.02	166 ± 20	1.0×10^{9}	1522 ± 95	6.0×10^{8}	193 ± 25	4.58
XII[b]	10.20 ± 0.2	8 ± 4	3.0×10^{2}	581 ± 35	5.0×10^{4}		
XIII	1.04 ± 0.02	160 ± 60	2.0×10^{8}	1001 ± 101	1.0×10^{8}	520 ± 30	2.21
XIV	4.96 ± 0.48	35 ± 12	1.0×10^{7}	728 ± 58	1.0×10^{6}	200 ± 20	6.64
XV[b]	7.92 ± 0.68	14 ± 6	8.0×10^{4}	620 ± 44	3.0×10^{5}	119 ± 15	13.80

[a] Value taken at the end of the phase. [b] Steady-state operation not obtained.

under a Cr(VI) loading rate of about 5 mg/day. Similarly, near complete Cr(VI) reduction was obtained for all three liquid detention times under the same influent Cr(VI) concentrations (10, 50, and 100 mg/L, Table 1) whenever steady-state operation conditions were obtained. These observations suggest that a lower hydraulic detention time may be used to effectively treat Cr(VI)-containing wastes.

Biological Activity in the Bioreactor. Higher influent Cr(VI) concentrations inhibited biological activity in the bioreactor as evidenced by the decreases in total suspended cells, total attached biomass, viable suspended cells, and viable attached cells in the reactor (Table 2). However, the number of viable suspended cells in the reactor dropped more rapidly than the number of viable attached cells. Extremely low viable suspended cell concentrations were observed at very high influent Cr(VI) concentration (2.0×10^{1} viable cells at 500 mg of Cr(VI)/L in phase VI) while the number of viable attached cells was higher (4.0×10^{4} cells in the reactor).

As expressed in VSS, most cells in the reactor were attached to the surface of the glass beads. Total cell mass in the reactor decreased with increasing Cr(VI) loading rate, but it stabilized at rates higher than 1016 mg of Cr(VI)/L (empty bed volume)·day under 24-h hydraulic detention time (Table 2). The mass of attached cells was always higher than the mass of suspended cells in the reactor throughout the course of this study. The decrease in suspended cell concentration after increasing Cr(VI) loading rate was much greater than the decrease in attached mass in the reactor. Attached biomass in the reactor decreased from 983 mg at a low Cr(VI) loading rate of 203 mg of Cr(VI)/L·day to 744 mg at 4138 mg of Cr(VI)/L·day (Table 2). The higher attached biomass in the reactor suggests that more Cr(VI) may be reduced by the attached biomass than by the suspended biomass. Additionally, the lower rate of decrease in attached cells indicated that attached cells were less susceptible to Cr(VI) toxicity than suspended cells in the bioreactor.

The mean cell residence time shown in Table 2 was calculated by dividing total cell mass (mg of VSS) retained in the bioreactor system by the rate of biomass loss through carry-over (mg of VSS/day) during steady-state operation conditions. The data in Table 2 show that the mean cell residence time in the reactor decreased with increasing hydraulic detention time and that the mean cell residence time based on VSS in the reactor was approximately 10 times the hydraulic detention time. The mean cell residence time remained almost constant during the first three phases of operation.

Cr(VI) Mass Balance. The fate of Cr(VI) in the bioreactor system was further analyzed by conducting mass balance on chromium. Figure 3 shows the relationship between cumulative influent Cr(VI) and cumulative effluent Cr(III) and total chromium. The near-perfect balance between the influent Cr(VI) and effluent total chromium indicates that nearly all the Cr(VI) fed to the reactor was accounted for in the effluent in the forms of Cr(VI) and Cr(III). Thus, the amount of Cr(VI) and Cr(III) retained in the bioreactor was negligible.

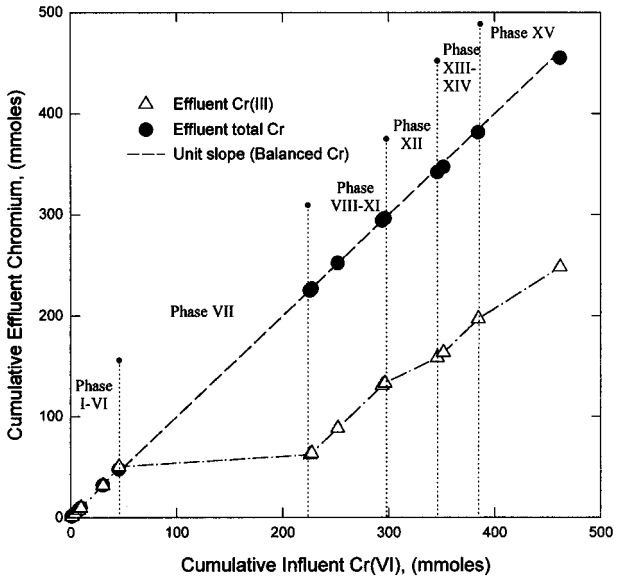

FIGURE 3. Relationship between cumulative influent Cr(VI) and cumulative effluent Cr(III) total chromium.

The data in Figure 3 also revealed that Cr(VI) was completely transformed to Cr(III) during the early stage of this study (phases I−VI). Cr(VI) breakthrough occurred in phase VII when the bioreactor was overloaded with Cr(VI). Near-complete transformation of Cr(VI) to Cr(III) was also observed in phases VIII, IX, XI, XIII, and XIV as indicated by the approximately unit slopes of the cumulative effluent Cr(III) versus cumulative influent Cr(VI) curves during these periods of operation. The observed Cr(VI) versus total chromium balance also demonstrates that, after Cr(VI) was transformed to Cr(III), the reduced Cr(III) remained in the liquid phase. In addition to the mass balance on chromium for the reactor, batch experiments show that less than 4.0% of Cr(III) was retained in the pelleted cells after 24 h (0.33 ± 0.02 mg of Cr(III)/L in pelleted cells; 8.99 ± 0.22 mg of Cr(III)/L in supernatant; 9.18 ± 0.34 mg of Cr(III)/L in culture medium). Similar results were obtained by Shen and

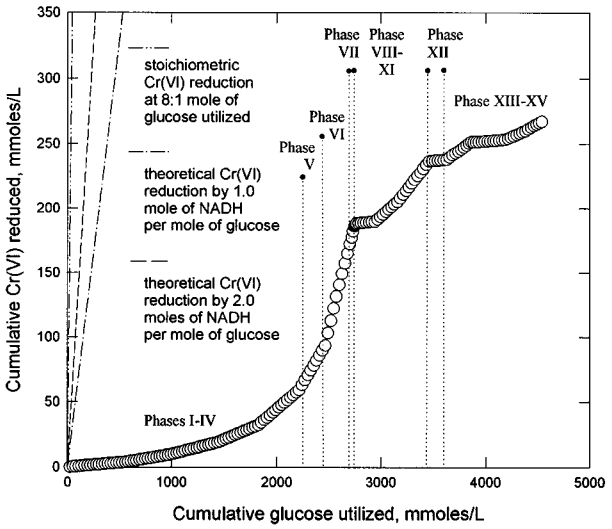

FIGURE 4. Relationship between Cr(VI) reduced and glucose utilized in the reactor.

Wang (1993) with batch cultures of *Escherichia coli* ATCC 33456 in which approximately 2.0% of Cr(III) transformed from Cr(VI) remained in the cell pellets (*19*).

Glucose Utilization in the Bioreactor. Glucose was used as the substrate for cell growth in the reactor. Figure 4 shows the relationship between Cr(VI) reduced and glucose utilized in the reactor. Based on the stoichiometric reaction discussed earlier, the observed Cr(VI) reduction per mole of glucose utilized was less than the theoretical values. Glucose catabolism to the final products, CO_2 and H_2O, can be done through a variety of pathways with reactions catalyzed by NADH (*21*). The amount of NADH mobilized for any pathway depends on the energy requirements of the reactions involved. The most commonly known pathway of the breakdown of hexose sugars involves the formation of pyruvate through glycolysis and the release of CO_2 and H_2O in the *tricarboxylic cycle* with at least two molecules of NADH released for each glucose molecule oxidized (*21*−*23*). *Bacillus* sp. cells may produce a soluble reductase that complexes with Cr(VI) in an enzymatic reaction in which hexavalent chromium receives electrons to form Cr(III) (*17*). Cr(VI) reduction is known to involve a soluble reductase requiring the presence of NADH or NAD(P)H for maximum activity (*24*). The reduction of chromate (CrO_4^{2-}) may be coupled with the mobilization of NADH in the following reaction:

$$CrO_4^{2-} + 6.5H^+ + 1.5NADH \rightarrow Cr^{3+} + 1.5NAD^- + 4H_2O$$

$$\Delta G^{\circ\prime} = -87 \text{ kJ/electrons transferred}$$

(calculated from the reported standard electrode potential ($E^{\circ\prime}$)) (*25*, *26*). The energy required for the reduction of chromate is lower than the energy released in the complete oxidation of glucose ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$, $\Delta G^{\circ\prime} = -121$ kJ/electrons *transferred*). Thus, the enzyme-mediated Cr(VI) reduction, involving the deprotonation of NADH, may result in a flow of electrons from NADH to CrO_4^{2-}. The electrons are mobilized either for necessity (*9*) or to enhance respiratory activity (*24*).

The lower values of the observed Cr(VI) reduction in Figure 4 suggest that a significant proportion of mobilized NADH is committed to other energy requiring cellular processes (*23*). However, the rate of Cr(VI) reduction per mole of glucose utilized approached the theoretical slope for 1 mol of NADH mobilized during the highest Cr(VI) loading with steady-state performance (phase VI). This demonstrated the potential of the bioreactor for Cr(VI) reduction and revealed that the reactor was underloaded with Cr(VI) for most of the experimental period under 24-h hydraulic detention time. Additionally, the data in Figure 4 revealed that Cr(VI) reduction in the reactor was not limited by glucose utilization. Glucose utilization at high influent Cr(VI) concentrations (phases VII, X, XII, and XV) was greatly reduced, suggesting that Cr(VI) toxicity could be the only factor affecting Cr(VI) reduction in the reactor. The low values of total and viable biomass at high Cr(VI) loadings illustrate further the inhibition of cell propagation by Cr(VI) in the bioreactor system.

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