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Experimental and mathematical modeling studies on Cr(VI) reduction by CRB, SRB and IRB, individually and in combination

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ABSTRACT

Cr(VI) reduction studies were carried out with chromium reducing bacteria (CRB), sulphate reducing bacteria (SRB) and iron reducing bacteria (IRB), individually and in combination. Biokinetic parameters such as maximum specific growth rate (μ_{max}), half saturation constant (K_s), yield coefficient (Y_T) and inhibition coefficient (K_i) for individual cultures were evaluated. A mathematical model was proposed for simulating the chromium reduction, COD utilization and biomass growth, by individual cultures as well as by a combination of two or three different cultures, for different initial Cr(VI), SO_4^{2-} and Fe(III) concentrations. The biokinetic parameters evaluated from one set of experiments for individual cultures were utilized in all the validation studies. The performance of the mathematical model in terms of the dimensionless modified coefficient of efficiency (E) indicated that the proposed model simulates the system behavior very well.

1. Introduction

Hexavalent chromium (Cr(VI)) is present in large quantities in liquid and solid wastes generated by many industries such as electroplating, leather tanning, wood preservation etc. Cr(VI) is a redox sensitive element, which is highly toxic, carcinogenic and mobile compared to Cr(III). Conventional treatment methods for abatement of Cr(VI) pollution involve two steps: reduction of Cr(VI) to Cr(III) using a strong reducing agent, followed by precipitation of Cr(III). This process generates large quantities of hazardous sludge, besides consuming large quantities of costly chemicals. Therefore, last two decades have seen the use of microorganisms for the biotransformation of Cr(VI) to Cr(III).

Many studies on aerobic and anaerobic biotransformation of Cr(VI) to Cr(III) under different environmental conditions have been carried out in the past [1–9]. Two types of enzymatic mechanisms of Cr(VI) reduction by CRB have been proposed by various researchers. The aerobic activity of Cr(VI) reduction is generally associated with a soluble protein fraction, utilizing NADH as electron donor either by necessity or for maximum activity [5]. Effects of pH, temperature, other electron acceptors and waste characteristics on Cr(VI) transformation have been quantified. Philip et al. [1], Komori et al. [6], Chen and Hao [7], Ohtake and Fujii [8], and Jeyasingh and Philip [9], among others, have studied the effect of biomass density, initial Cr(VI) concentration, carbon source, pH, temperature, dissolved oxygen, oxidation–reduction potential (ORP), presence of other oxyanions and other metal cations, on Cr(VI) transformation.

Fe(II) and sulphide can reduce Cr(VI) to Cr(III) via chemical reaction [10,11]. The sulphide can also reduce Fe(III) to Fe(II) and Fe(II) thus generated can reduce chromium much faster than sulphide itself [12]. It is reported that sulphide concentration in the range of 230–550 mg/L as total sulphides, at pH 6.2–8, causes inhibition to SRB [13]. On the other hand, presence of Fe(III) and Cr(VI) can prevent sulphide inhibition.

Biotic chromium reduction by iron reducing bacteria can also occur in both aerobic and anaerobic environments [14–17]. Various organisms can reduce iron by coupling its reduction to the oxidation of hydrogen or organic carbon. *Shewanella* alga strain BrY, an iron reducing bacterium, can transform Cr(VI) to Cr(III) through the microbial reduction of Fe(III) to Fe(II) [16]. Iron promoted reduction of chromate by dissimilatory iron reducing bacteria was extensively studied by Wielinga et al. [17]. There have been reports on bio-barriers packed with zero valent iron for Cr(VI) reduction [18,19]. The reduction of Fe(III) to Fe(II) can be accomplished by stimulation of indigenous dissimilatory metal-reducing bacteria. Microbially produced Fe(II) can chemically react with Cr(VI) to form insoluble Cr(III) [20]. It is well known that sulphide, which

Abbreviations: APHA, American Public Health Association; AWWA, American Water Works Association; CRB, chromium reducing bacteria; SRB, sulphate reducing bacteria; IRB, iron reducing bacteria; Cr(III), trivalent chromium; Cr(VI), hexavalent chromium; Fe(III), ferric iron; Fe(II), ferrous iron; Fe EDTA, ferric monosodium ethylene diamine tetra acetic acid; COD, chemical oxygen demand.

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Nomenclature

Cr_6	hexavalent chromium concentration (mg/L)
K_i	inhibition constant (mg/L)
K_s	half saturation constant (mg/L)
M	biomass concentration (mg/L)
S	residual substrate concentration (mg/L)
Greek letters	
η	mg of Cr reduced/g of substrate utilized
μ_{max}	maximum specific growth rate (1/day)

is produced by sulphate reducing bacteria (SRB) during anaerobic processes, can easily combine with heavy metals to form insoluble metal sulphides [21,22]. Tebo and Obraztsova [23] have reported that SRB may also directly reduce Cr(VI) to Cr(III). Several studies have also shown that abiotic reduction of Cr(VI) by Fe^0 and Fe^{2+} was possible [24,25]. A few studies have examined the reduction of Cr(VI) by microbiological activities in the presence of a strong oxidant such as MnO_2 [26,27]. Guha [27] conducted a series of dynamic column experiments to provide an understanding of Cr(VI) reduction by facultative anaerobic BrYMT in presence of β-MnO_2. Guha [27] also developed a mathematical model, which was calibrated and validated using the data obtained from column experiments.

Many reports on Cr(VI) reduction by different microorganisms such as chromium reducing bacteria (CRB) [1–3,5–6], iron reducing bacteria (IRB) [22,24,27] and sulphate reducing bacteria [21,22,28] in presence of different electron donors [17,23,28] are available. Most of these studies have dealt with only a specific microorganism and one or two inorganic compounds as sources/sinks of electrons. However, in nature, most of the water and wastewater systems contain different inorganic compounds and microbial flora. Thus, a proper understanding of the interaction between different inorganic compounds and microbial flora is essential for the design and appropriate management of any bioremediation system. Also, biokinetic models are available only for individual systems like CRB [4,27,31,33,34], SRB [13,35–37], and IRB [38–42]. To the best of authors' knowledge, no model is available to describe the combined action of CRB, SRB and IRB in wastewaters/contaminated aquifers containing Cr(VI), sulphate and Fe(III). In this work, batch kinetic studies were conducted to evaluate the kinetics of Cr(VI) reduction, in presence of organic matter (OM), Fe(III) and sulphate, by CRB, SRB, and IRB, individually and in combination. Also, an attempt was made to model all the batch systems.

2. Materials and methods

2.1. Experimental procedures

2.1.1. Media for bacterial culture enrichment

The composition of nutrient medium (N1) used for bacterial growth was peptone 10 g, beef extract 2 g, yeast extract 1 g, and sodium chloride 5 g in 1 L of distilled water. The medium (M1) used for Cr(VI) reduction experiments consisted of K_2HPO_4 (0.03 g/L), KH_2PO_4 (0.05 g/L), $MgSO_4{\cdot}7H_2O$ (0.01 g/L), 0.01 g/L NaCl, NH_4Cl (0.03 g/L), NaCl (0.01 g/L), molasses (2 g/L), yeast extract 1 g/L and 1 mL of trace element solution in 1 L of distilled water. Trace element solution consisted of $FeCl_2{\cdot}4H_2O$ (12.2 g/L), $MnCl_2{\cdot}4H_2O$ (4.09 g/L), $CoCl_2{\cdot}6H_2O$ (0.927 g/L), $ZnCl_2$ (0.37 g/L), $CuCl_2$ (0.61 g/L), $NaMoO_4{\cdot}2H_2O$ (0.579 g/L), H_3BO_3 (0.16 g/L), KI (0.148 g/L), $NiCl{\cdot}6H_2O$ (0.067 g/L), and EDTA $Na_2{\cdot}4H_2O$ (6.5 g/L). The pH was maintained at 7 ± 0.2 by using HCl or NaOH. All media were autoclaved at 120 °C and 15 psi for 15 min. Ferric monosodium EDTA and sodium sulphate were used as Fe(III) and sulphate sources, respectively.

2.1.2. Enrichment and cultivation of Cr(VI) reducing bacterial strains

Cr(VI) reducing bacterial consortia were enriched from the soil samples collected from chromium contaminated site located in Ranipet, Tamilnadu, India. Five grams of contaminated soil sample was added to 100 mL of sterile nutrient medium N1 with 10 mg/L of Cr(VI) and incubated in a shaking incubator for 24 h at 32 °C. After 24 h, when significant growth was observed, 1 mL of the supernatant of the slurry was transferred to 100 mL of fresh medium, M1, spiked with 10 mg/L of hexavalent chromium and incubated at 32 °C. This procedure was repeated every 2 days. After three transfers (i.e. 6 days), the chromium concentration in the medium M1 was increased in steps (10, 25, 50, 75 and 100 mg/L) to a higher level until the concentration reached a value of 100 mg/L in the last cycle. Once the enriched consortia were ready, they were streaked on agar slants spiked with Cr(VI), incubated at 32 °C for 24 h and stored at 4 °C until further use.

2.1.3. Enrichment and cultivation of iron reducing bacterial strains

Wastewater was collected from a municipal wastewater treatment plant located in Chennai. The anaerobic sludge was enriched using a medium consisting of 5.0 g ferric ammonium citrate (FAC), 0.5 g K_2HPO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.01 g $CaCl_2{\cdot}2H_2O$ and 1 mL of trace element solution per litre at a pH of 7.0. 10 mL of culture was added to 400 mL of fresh medium every alternate day. Fe(III) concentration was increased in increments of 1000 mg/L every 10–15 days until the final concentration was 5000 mg/L. The reactor (aspirator bottle with a working volume of 400 mL and total volume of 500 mL) was maintained as a source of inoculum for further experiments and media additions were performed only when necessary, to produce sufficient biomass for inoculation.

2.1.4. Enrichment and cultivation of sulphate reducing bacterial strains

Enrichment of SRB was also carried out using wastewater sample collected from a municipal wastewater treatment plant located in Chennai. The growth medium for enrichment of SRB consisted of 1.2 g/L sodium lactate, 3 g/L sodium citrate, 0.1 g/L yeast extract, 4.5 g/L Na_2SO_4, 0.06 g/L $CaCl_2{\cdot}2H_2O$, 1.0 g/L NH_4Cl, 0.5 g/L KH_2PO_4, 2.0 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L $FeSO_4{\cdot}7H_2O$, 0.3 g/L EDTA disodium salt and 1 mL of trace element solution in 1 L of distilled water. The pH of the medium was adjusted to 7 ± 0.2. During enrichment, 10 mL of culture was added to 400 mL of fresh medium every alternate day. Sulphate concentration was increased in increments of 500 mg/L every 10–15 days until the final concentration was 4500 mg/L. Sulphate to COD ratio was increased from 1 to 1.5 (COD to sulphate ratio was decreased from 1 to 0.667) because sulphidogenesis is the dominant process when COD to sulphate ratio is 0.667 as per stoichiometry [43]. The enriched cultures were used in further experiments.

2.2. Analytical procedures

2.2.1. Liquid phase chromium analysis

Diphenyl carbazide method (DPC) [29] was used to determine the Cr(VI) concentration. In this method, 0.5 mL of sample was mixed with 0.2 mL 5N H_2SO_4 and 0.2 mL of DPC (250 mg/50 mL acetone) and the color intensity was measured at 540 nm using UV–vis spectrophotometer (Techcom, UK). Total chromium concentration was analyzed using atomic absorption spectrometer (PerkinElmer, USA). Cr(III) concentration was determined by subtracting Cr(VI) concentration from the total chromium concentration.

2.2.2. Measurement of cell density in liquid phase

The optical density (OD) method was used to measure biomass concentration under aerobic condition. OD was determined by turbidometric measurement in a spectrophotometer at 600 nm and correlated to dry cell weight [4]. Cells were grown overnight, centrifuged, washed three times with physiological saline water, resuspended in saline water, homogenized, and used as stock solution. Different dilutions were made from the above stock solution. A known volume of these solutions was filtered through 0.45 μm filter paper and weight of the dried cells was estimated. Corresponding absorbance was measured at 600 nm using a UV–vis spectrophotometer (Techcom, UK). This information was used for preparing a calibration curve between the dry cell weight and the absorbance. For unknown samples, the absorbance was measured at 600 nm and then it was converted to dry cell weight using absorbance versus dry cell weight calibration curve.

2.2.3. Microbial quantification by protein estimation

In all anaerobic experiments, protein concentration was used to estimate the biomass concentration because metal sulphide and iron precipitates interfere with the optical density. To estimate the cell concentration, a known volume of cell suspension was filtered through 0.45 μm filter paper followed by weighing the dried cell mass retained on the filter paper. Protein standard curve was plotted using bovine serum albumin. Protein contents of different cultures, with known bacterial concentrations (dry weight in mg/L), were determined using Lowry's method [30] and the correlation between protein content of the cells and dry cell weight was established. The modified Lowry's method used was as follows. 2 mL of sample was taken and centrifuged at 8000 rpm for 8 min. The pellets were then resuspended in 2 mL of phosphate buffer (pH 7) and sonicated at 100 Hz at 15 s intervals (15 s on and 15 s off) for 3 min. The solution was centrifuged again at 8000 rpm for 8 min. 2 mL of alkaline copper reagent was added to 0.5 mL of supernatant or diluted supernatant of suitable concentration, incubated for 10 min, followed by addition of 0.2 mL of Folin phenol reagent, and incubated again for 30 min. Reagent blank, containing 0.5 mL of distilled water instead of bacterial suspension, was treated in a similar way. The optical density was measured at 600 nm using a UV–vis spectrophotometer (Techcom, UK) against the reagent blank. Samples with known bacterial concentrations were used for preparing the calibration curve.

2.2.4. Chemical oxygen demand (COD)

COD of liquid samples was estimated using closed reflex method as suggested in standard methods [29]. Closed reflux digestion was conducted in HACH COD digester (model no. 45600, USA).

2.2.5. COD estimation for samples containing ferric EDTA

Since ferric monosodium EDTA is not completely oxidized during normal COD digestion, an extended digestion procedure was followed, in which double strength $K_2Cr_2O_7$ was used (0.2N) and the digestion time was increased to 4 h to ensure complete oxidation of ferric EDTA [44].

2.2.6. Iron

Fe(II) and Fe(III) were analyzed by 1,10 phenanthroline colorimetric method [29]. For the analysis of soluble Fe(II), 0.1 mL of sample was added to 0.8 mL of ammonium acetate buffer (pH 4.8) and 0.2 mL of phenanthroline (1 mg/mL). For Fe(III) analysis, 0.1 mL of sample was treated with 0.2 mL of hydroxylamine hydrochloride for 1 h to reduce Fe(III) to Fe(II) before adding 0.8 mL ammonium acetate (pH 4.8) buffer and 0.2 mL phenanthroline (1 mg/mL). The absorbance was measured at 510 nm using UV–vis spectrophotometer (Techcom, UK) after allowing the color to develop for 30 min.

2.2.7. Sulphate

Sulphate was analyzed by turbidimetric method prescribed in standard methods [29]. Before measuring sulphate in a 2 mL sample, generated sulphide (sample taken from the medium with SRB contains both sulphate and sulphide. This sulphide is referred to as "generated sulphide") was fixed by adding 0.1 mL NaOH (6N) and 0.2 mL zinc acetate (1 M) in a 5 mL centrifuge tube. Following this, the sample was spun for 5 min at 7000 rpm to remove the precipitate and the supernatant was analyzed for sulphate. This pretreatment was essential because, when sample was taken out from reactor, unless otherwise sulphides were precipitated, oxygen would oxidize sulphides to sulphates, resulting in erroneous results during the analysis for sulphates. 8 mL of pretreated sample or a suitable portion diluted to 8 mL was taken in a test tube and to this 2 mL of buffer solution (30 g magnesium chloride, 5 g sodium acetate, 1 g potassium nitrate and 20 mL acetic acid in 500 mL and made up to 1 L) was added. A pinch of barium chloride crystals was also added while stirring and absorption was measured after 5 ± 0.5 min.

2.3. Batch kinetic experiments

Different batch kinetic experiments were performed to evaluate various biokinetic parameters and rate constants. These experiments include (i) Cr(VI) reduction by CRB under aerobic conditions, (ii) Cr(VI) reduction by CRB, IRB, or SRB under anaerobic conditions, (iii) Cr(VI) reduction by a mixture of IRB and CRB, or SRB and CRB under anaerobic conditions, (iv) Cr(VI) reduction by a mixture of CRB, SRB, and IRB under anaerobic conditions, and (v) EDTA degradation studies using CRB, IRB or SRB. These experiments were conducted as follows. Cultures were grown in fresh medium (N1) for 1 day, centrifuged and washed with 0.85% saline solution before using them for kinetic experiments. Predetermined amounts of Cr(VI) and selected electron acceptor (Fe^{3+} or SO_4^{2-}) were added to medium (M1) as per the requirement of each experiment. Molasses which was present in the mineral medium acted as an electron donor in all the experiments. After autoclaving, 10 mg/L of Na_2S was added to remove dissolved oxygen present in the medium. Then the medium was flushed with nitrogen to remove oxygen in the head space of the reactor. Microbial cultures were then added to obtain an initial bacterial concentration between 30 and 40 mg/L in the reaction medium. An initial COD of 3100–3200 mg/L was maintained in all the experiments. Samples were withdrawn at pre-decided time intervals and analyzed for Cr(VI), total chromium, biomass, COD, Fe(II), Fe(III), and sulphate concentrations.

All chemicals used in the present study were analytical reagent (AR) grade supplied by Ranbaxy and Merck Pvt. Ltd. (India). Clean glassware made by 'Borosil' (India) was used for preparation of reagents and measuring volumes. Incubations were carried out at room temperature ($32 \pm 2\,^{\circ}C$) without shaking for anaerobic, and with shaking for aerobic systems. The contents of the aerobic flasks were in contact with the atmosphere since they were closed only with cotton plugs.

2.4. Mathematical model

2.4.1. Governing equations for microbial growth, COD and Cr(VI) reduction by single strain

A mathematical model was developed for the processes describing Cr(VI) reduction kinetics in batch systems by CRB, SRB and IRB, individually, under different conditions. The mathematical model not only describes the chromium reduction but also the temporal variations of substrate (COD) and biomass concentrations in the system. The model considered the inhibitory effect of Cr(VI) on the microbial growth [31]. A simple Monod's inhibition model was considered in the present study to describe the Cr(VI) inhibition. The

only other possible inhibitor present in the system was sulphide. However, sulphide generated in the system could have reduced either Cr(VI) or Fe(III) as soon as it was formed. Hence inhibition due to sulphide might not have been significant. Also, concentration of generated sulphide in the system at any time was much lower than the reported inhibition concentration (300–550 mg/L of sulphide is necessary to impart sulphide inhibition). Availability of sulphate/iron was unlimiting in the respective systems and therefore this was also not considered in the model. The model equations are:

$$\frac{dM_i}{dt} = M_i\left(\frac{\mu_{\max,i}S}{K_{s,i}+S}\right)\left(\frac{K_{i,i}}{K_{i,i}+Cr_6}\right) \quad \text{for } i = 1, 2, \text{ and } 3 \tag{1}$$

$$\frac{dS}{dt} = M_i\left(\frac{1}{Y_T}\right)\left(\frac{\mu_{\max,i}S}{K_{s,i}+S}\right)\left(\frac{K_{i,i}}{K_{i,i}+Cr_6}\right) \quad \text{for } i = 1, 2, \text{ and } 3 \tag{2}$$

$$\frac{dCr_6}{dt} = M_i(\eta)\left(\frac{1}{Y_T}\right)\left(\frac{\mu_{\max,i}S}{K_{s,i}+S}\right)\left(\frac{K_{i,i}}{K_{i,i}+Cr_6}\right) \quad \text{for } i = 1, 2, \text{ and } 3 \tag{3}$$

where, M_i is biomass concentration in mg/L, subscript i represents the particular bacterial strain (i=1 for CRB; i=2 for SRB; i=3 for IRB), S is concentration of residual substrate (organic matter, OM) in mg/L, Cr_6 is concentration of hexavalent chromium in mg/L, $\mu_{\max,i}$ is the maximum specific growth rate for the bacterial strain i, $K_{i,i}$ is the chromium inhibition constant for bacterial strain i in mg/L, $K_{s,i}$ is the half saturation constant for bacterial strain i in mg/L, η_i is mg of Cr reduced/g of substrate utilized by bacterial strain i, $Y_{T,i}$ is the yield coefficient for bacterial strain i. Eqs. (1)–(3) are valid for kinetics of chromium reduction by each bacterial strain acting independently.

2.4.2. Governing equations for microbial growth, COD and Cr(VI) reduction by more than one bacterial strain

When more than one bacterial strain is present, it may be assumed that availability of substrate as well as chromium for that particular bacterial strain is proportional to the partial concentration of that bacterial strain (ratio of concentration of the particular bacterial strain to the total bacterial concentration). Implicit to this assumption is the absence of the symbiotic/asymbiotic effect of one strain on the other. With this assumption, following governing equations can be formulated for the combined action of more than one bacterial strain:

$$\frac{dM}{dt} = \sum_i \frac{dM_i}{dt} \tag{4}$$

$$\frac{dS}{dt} = \sum_i \frac{dM_i}{dt}\left(\frac{1}{Y_{T,i}}\right) \tag{5}$$

$$\frac{dCr_6}{dt} = \sum_i \frac{dM_i}{dt}\left(\frac{\eta_i}{Y_{T,i}}\right) \tag{6}$$

$$M = \sum_i M_i \tag{7}$$

$$S = \sum_i S_i \tag{8}$$

$$Cr_6 = \sum_i Cr_{6,i} \tag{9}$$

$$S_i = S\left(\frac{M_i}{M}\right) \tag{10}$$

$$Cr_{6,i} = Cr_6\left(\frac{M_i}{M}\right) \tag{11}$$

$$\frac{dM_i}{dt} = \frac{M_i\mu_{\max,i}S\left(\frac{M_i}{M}\right)}{K_{s,i}+S\left(\frac{M_i}{M}\right)}\left(\frac{K_{i,i}}{K_{i,i}+Cr_6\left(\frac{M_i}{M}\right)}\right) \tag{12}$$

2.4.3. Solution of differential equations by Euler finite difference method

Eqs. (4)–(12) were solved by a simple explicit Euler finite difference method (programmed using MATLAB 2006b) as given below to determine the concentrations of biomass, substrate, and Cr(VI) at time $t+\Delta t$, from the known values at time t.

$$M_i(t+\Delta t) = M_i(t) + \frac{M_i(t)\Delta t\mu_{\max,i}S(t)\left(\frac{M_i(t)}{M(t)}\right)K_{i,i}}{\left(K_{s,i}+S(t)\left(\frac{M_i(t)}{M(t)}\right)\right)\left(K_i+Cr_6(t)\left(\frac{M_i(t)}{M(t)}\right)\right)} \tag{13}$$

$$M(t+\Delta t) = \sum_i M_i(t+\Delta t) \tag{14}$$

$$S(t+\Delta t) = \sum_i S(t)\left(\frac{M_i(t)}{M(t)}\right) - (M_i(t+\Delta t) - M_i(t))\left(\frac{1}{Y_{t,i}}\right) \tag{15}$$

$$Cr_6(t+\Delta t) = \sum_i Cr_6(t)\left(\frac{M_i(t)}{M(t)}\right) - (M_i(t+\Delta t) - M_i(t))\left(\frac{\eta_i}{Y_{t,i}}\right) \tag{16}$$

The model equations were also solved using classical fourth-order Runge Kutta method (programmed using MATLAB 2008a). Results obtained using both Runge Kutta and Euler methods matched very well. A very small value of computational time step, Δt, was taken in all simulations, based on grid convergence test.

3. Results and discussion

3.1. Experimental results

3.1.1. Kinetics of chromium reduction by CRB in aerobic conditions

Chromium reduction studies were carried out using CRB under aerobic conditions. The microbial strains used for the study were enriched and isolated from chromium contaminated soil collected from Ranipet, Tamilnadu, India. Concentrations of biomass, COD and Cr(VI) were monitored with respect to time. In these studies, initial concentration of Cr(VI) was varied from 0 to 100 mg/L, whereas the initial COD concentration was kept at 3040 mg/L. Initial biomass concentration in all the experiments was equal to 30 mg/L. Results of these studies are presented in Fig. 1(a)–(c).

It can be observed from Fig. 1(a) that the chromium reduction was almost complete within 70 h when the initial Cr(VI) concentration was less than or equal to 20 mg/L, in aerobic conditions. Although Cr(VI) reduction occurred for higher concentrations also, the reduction was not complete. COD removal also followed a similar trend. The COD removal rate was very fast up to 50 h, for a Cr(VI) concentration of 30 mg/L. There was a decrease in the COD removal rate when Cr(VI) concentrations were high (Fig. 1(b)). This may be due to the inhibitory effect of Cr(VI) on the CRB. Similar results were reported by other researchers also (Philip et al. [1], Gopalan and Veeramani [2], Campos et al. [3]). The inhibition effect is clear from the biomass concentrations in the systems (Fig. 1(c)). The maximum biomass concentration of 900 mg/L was achieved in the system with zero Cr(VI) concentration. The reduction in maximum biomass concentration due to increase in Cr(VI) concentration was marginal up to a Cr(VI) concentration of 30 mg/L. However, the effect was very significant when the Cr(VI) concentration was

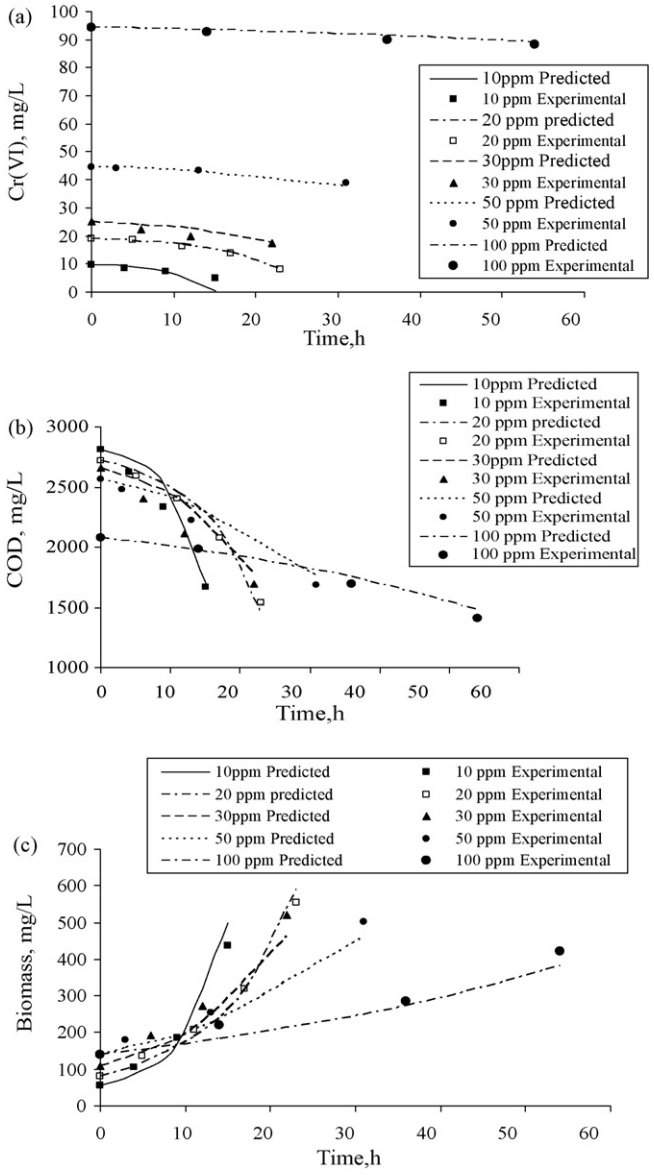

Fig. 1. (a) Experimental and model predicted Cr(VI) reduction by CRB under aerobic conditions for different initial Cr(VI) concentration. (b) Experimental and model predicted COD consumption by CRB under aerobic conditions for different initial Cr(VI) concentrations. (c) Experimental and model predicted growth of CRB under aerobic conditions for different initial Cr(VI) concentrations.

more than 50 mg/L. There was correlation between rate of biomass production and rate of COD consumption in all the experiments. Although the COD consumption rate was low initially, there was a steep decrease in COD after the starting of the log phase of bacterial growth. Once the growth reached stationary phase, specific growth rate was zero. The COD removal rate during this phase was lower than that during the log growth phase although it did not become zero. This was because of the substrate utilization for maintenance. Though Cr(VI) reduction was not complete for initial Cr(VI) concentrations greater than 20 mg/L, all experiments were conducted for higher initial Cr(VI) concentrations in order to determine the Cr(VI) inhibition constant.

3.1.2. Kinetics of chromium reduction by CRB in anaerobic conditions

Chromium reduction studies were carried out using CRB under anaerobic conditions, keeping all other experimental conditions

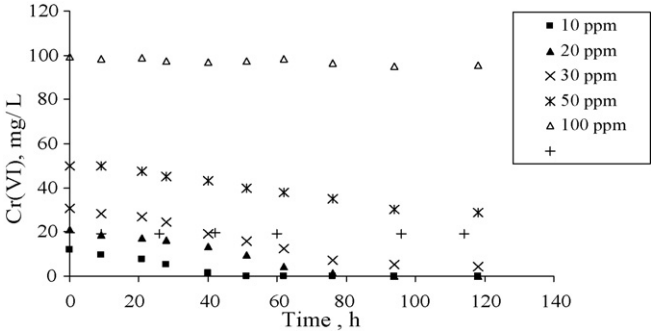

Fig. 2. Kinetics of Cr(VI) reduction by CRB for different initial Cr(VI) concentrations under anaerobic conditions.

same as in aerobic system. Results for Cr(VI) reduction from these experiments are presented in Fig. 2. It can be observed that the rate of Cr(VI) reduction was faster under anaerobic conditions for low concentrations of Cr(VI) (Fig. 2). However, the reduction rate decreased significantly for high concentrations of Cr(VI). COD removal efficiency was less in anaerobic conditions as compared to removal efficiency in aerobic conditions. The inhibition effect was more in aerobic system as compared to anaerobic system (results not shown). Maximum biomass concentration achieved in the system, with zero Cr(VI) concentration, was 300 mg/L, which was much lower than that achieved in the aerobic system. Also, there was practically no growth when the Cr(VI) concentration in the system was 100 mg/L.

In aerobic conditions, oxygen molecule competes with Cr(VI) to accept electron by uncompetitive inhibition, and thermodynamically oxygen reduction generates more energy to cells rather than chromium reduction. Hence, chromium reduction becomes less pronounced in aerobic process. For a given initial concentration of Cr(VI), higher concentrations of Cr(VI) prevail for a longer time in aerobic condition as compared to those in anaerobic condition. These high concentrations of Cr(VI) decrease the specific growth rate of CRB. As a result, a pronounced inhibition is usually observed in aerobic condition compared to that in anaerobic condition [5], for relatively low concentrations of Cr(VI). On the other hand, the biomass yield is much less in anaerobic condition as compared to aerobic condition. For a high initial Cr(VI) concentration, anaerobic system shows more inhibition because specific chromium concentration (Cr(VI) loading, mg of Cr(VI) per mg of biomass) is significantly higher in anaerobic condition. The overall COD reduction was also less in anaerobic system as the biomass concentration in such system was much lower than aerobic systems.

3.1.3. Kinetics of chromium reduction by SRB

Chromium reduction studies were carried out using SRB under strict anaerobic conditions. In these studies, initial concentration of Cr(VI) was varied from 0 to 50 mg/L, whereas the COD concentration was kept constant at 3072 mg/L. Initial biomass concentration in all the experiments was equal to 28 mg/L. The effect of sulphate concentration on the microbes was also studied by varying the sulphate concentration from 0 to 2000 mg/L (Fig. 3(bi)). However, in all Cr(VI) reduction studies, sulphate concentration was kept at 1000 mg/L. Results of these studies are presented in Fig. 3(a)–(c).

It can be seen from Fig. 3(a) that almost complete reduction of chromium occurred when the initial Cr(VI) concentrations were less than 20 mg/L. The COD reduction efficiency was almost the same irrespective of sulphate concentration (up to 1000 mg/L), when Cr(VI) was absent in the system (Fig. 3(bi)). However, the presence of Cr(VI) affected the COD removal efficiency of the SRB significantly (Fig. 3(bii)). The rate of sulphate reduction remained

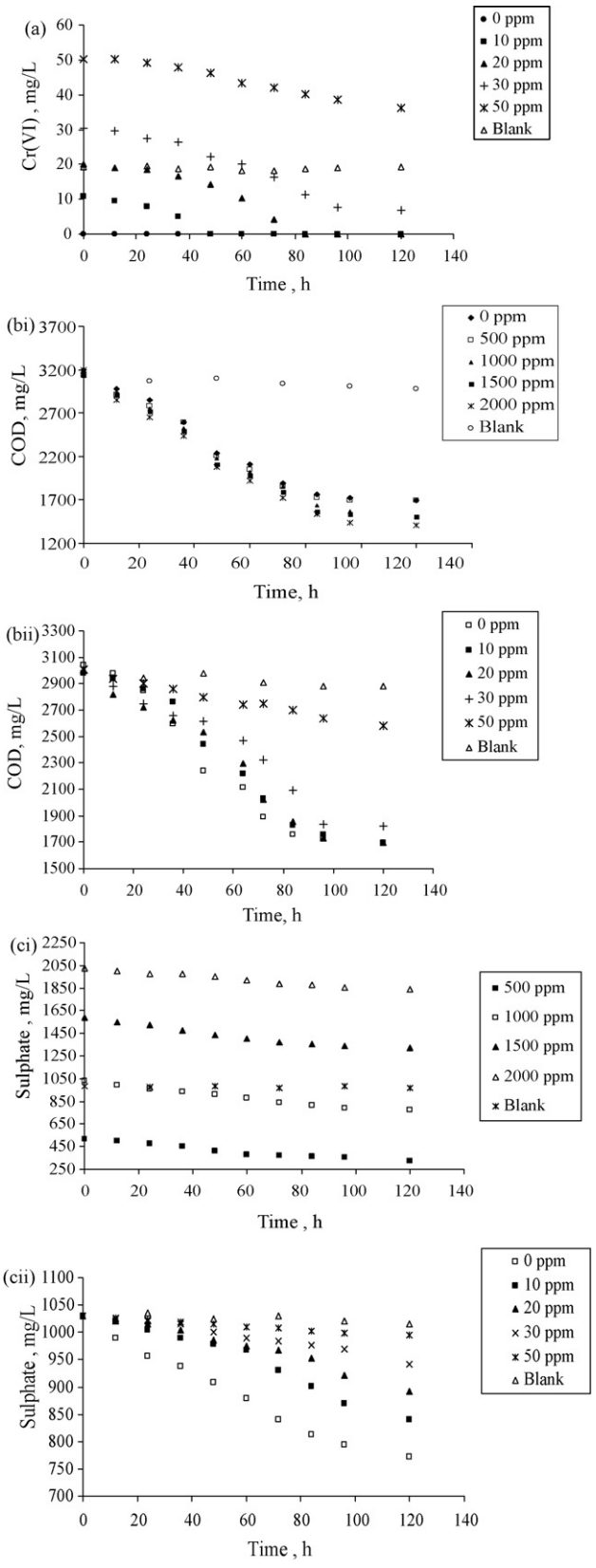

Fig. 3. (a) Kinetics of Cr(VI) reduction by SRB for different initial Cr(VI) concentrations under anaerobic conditions. (bi) Kinetics of COD reduction by SRB for different initial sulphate concentrations under anaerobic conditions. (bii) Kinetics of COD reduction by SRB for different initial Cr(VI) concentrations under anaerobic conditions. (ci) Kinetics of sulphate reduction by SRB for different initial sulphate concentrations under anaerobic conditions. (cii) Kinetics of sulphate reduction by SRB for different initial Cr(VI) concentrations under anaerobic conditions.

constant irrespective of sulphate concentration in the absence of Cr(VI) (Fig. 3(ci)). However, the rate of sulphate reduction reduced significantly as the Cr(VI) concentration in the system was increased (Fig. 3(cii)). These results indicate that SRB is highly sensitive to Cr(VI).

3.1.4. Kinetics of chromium reduction by IRB

Studies for chromium reduction by IRB were conducted under anaerobic conditions using Fe(III). In these studies, initial concentration of Cr(VI) was varied from 0 to 50 mg/L, whereas the COD concentration was kept constant at 3040 mg/L. Initial biomass concentration in all the experiments was equal to 30 mg/L, and Fe(III) concentration was varied from 0 to 1600 mg/L. The kinetics of chromium reduction by IRB in presence of 800 mg/L of Fe(III) is presented in Fig. 4(a). Growth of IRB and COD reduction were maximum when Fe(III) concentration was 1600 mg/L (Fig. 4(c) and (bi)). It can be observed from Fig. 4(a) that the Cr(VI) reduction was complete within 70 h for initial concentrations of chromium up to 20 mg/L. Cr(VI) reduction occurred for a high initial Cr(VI) concentration (50 mg/L) also. However, the residual concentration of Cr(VI) was significant in such a case. This could be due to the inhibition effect of Cr(VI) on the IRB. Inhibition effect can also be seen from the COD removal efficiency (Fig. 4(bii)), biomass growth (Fig. 4(c)), and Fe(II) generation (Fig. 4(d)).

Fe(III) was supplied to the system as ferric EDTA. Apart from the external carbon source, EDTA also contributes to the COD of the system. In order to assess the effect of excess COD on Cr(VI) reduction, microbial growth kinetic studies were conducted using different microorganisms and EDTA as the sole carbon source. Experimental results indicated that EDTA utilization and the microbial growth were insignificant. This shows that additional COD contributed by EDTA did not have any effect on Cr(VI) reduction.

3.1.5. Kinetics of chromium reduction by a consortium of CRB and SRB

Chromium reduction studies were carried out using a consortium of CRB and SRB under anaerobic conditions. Concentrations of biomass, COD, sulphate and Cr(VI) were monitored with respect to time. In these studies, initial concentration of Cr(VI) was varied from 0 to 50 mg/L, whereas the COD and sulphate concentration was kept constant at 3104 and 1000 mg/L, respectively. Initial biomass concentration in all the experiments was equal to 40 mg/L.

It can be seen from Fig. 5(a) that the Cr(VI) reduction was almost complete within 35 h when the concentration was less than 20 mg/L, with an initial sulphate concentration of 1000 mg/L in the system. Cr(VI) reduction was complete at lower sulphate concentrations also, though it took relatively longer time to achieve this (results not shown). COD removal also followed a similar trend (Fig. 5(b)). At higher Cr(VI) concentrations, the rate of COD removal decreased due to the inhibitory effect of Cr(VI) on CRB and SRB, as observed in the experiments for individual cultures. The inhibition effect is also clear from the biomass concentrations in the systems (Fig. 5(c)). Maximum biomass concentration of 400 mg/L was achieved in the system with zero Cr(VI) concentration and a sulphate concentration of 1000 mg/L.

Second part of this batch study was conducted with initial biomass of 40 mg/L, sulphate concentration was varied from 0 to 2000 mg/L, Chromium concentration and COD was kept constant at 20 and 3104 mg/L, respectively (results not shown).

The observation from second part of the study indicates that rate of chromium reduction increased with an increase in sulphate concentration (results not shown). The rate of sulphate reduction was very low irrespective of sulphate concentration (results not shown).

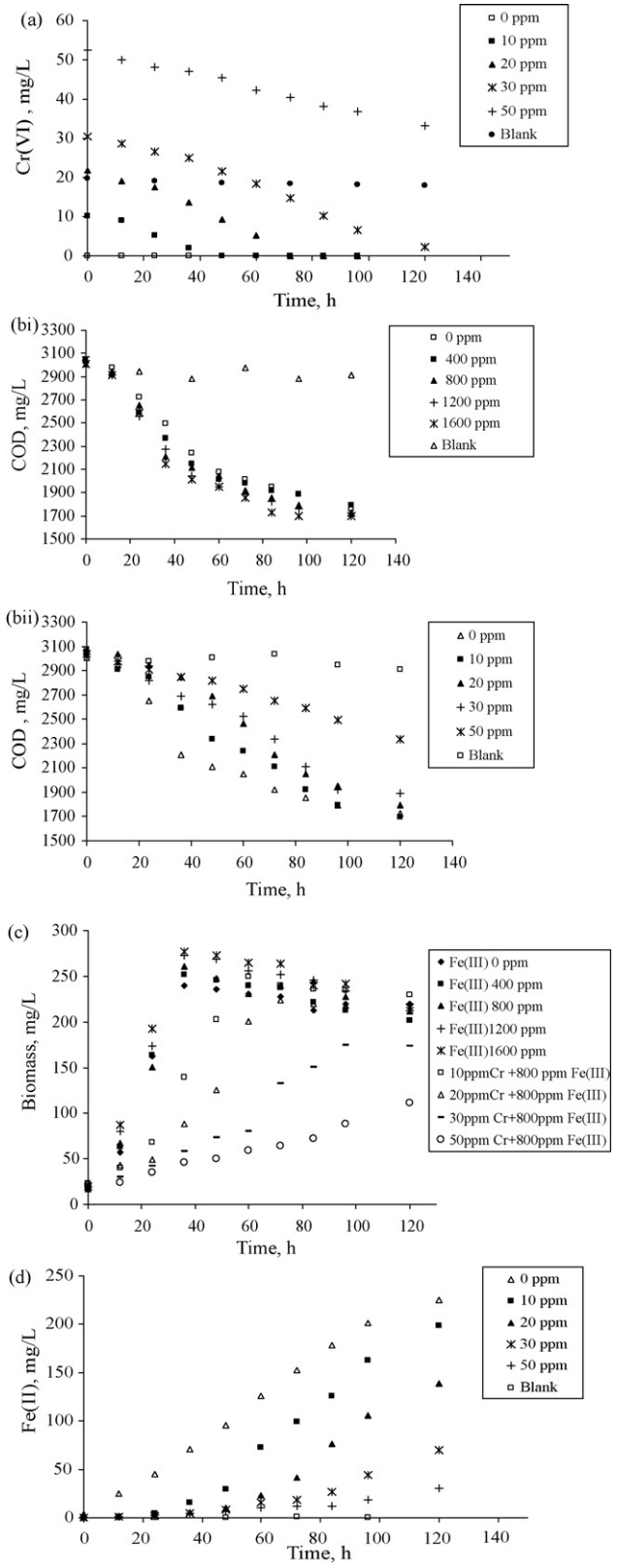

Fig. 4. (a) Kinetics of Cr(VI) reduction by IRB for different initial Cr(VI) concentrations under anaerobic conditions (initial Fe(III) concentration = 800 mg/L). (bi) Kinetics of COD reduction by IRB for different initial iron concentrations under anaerobic conditions (initial Cr(VI) concentration = 20 mg/L). (bii) Kinetics of COD reduction by IRB for different initial Cr(VI) concentrations under anaerobic conditions (initial Fe(III) concentration = 800 mg/L). (c) Growth curve of IRB with different initial Cr(VI) and Fe(III) concentrations under anaerobic conditions. (di) Kinetics of Fe(II) generation by IRB for different initial Cr(VI) concentrations under anaerobic conditions (initial Fe(III) concentration = 800 mg/L).

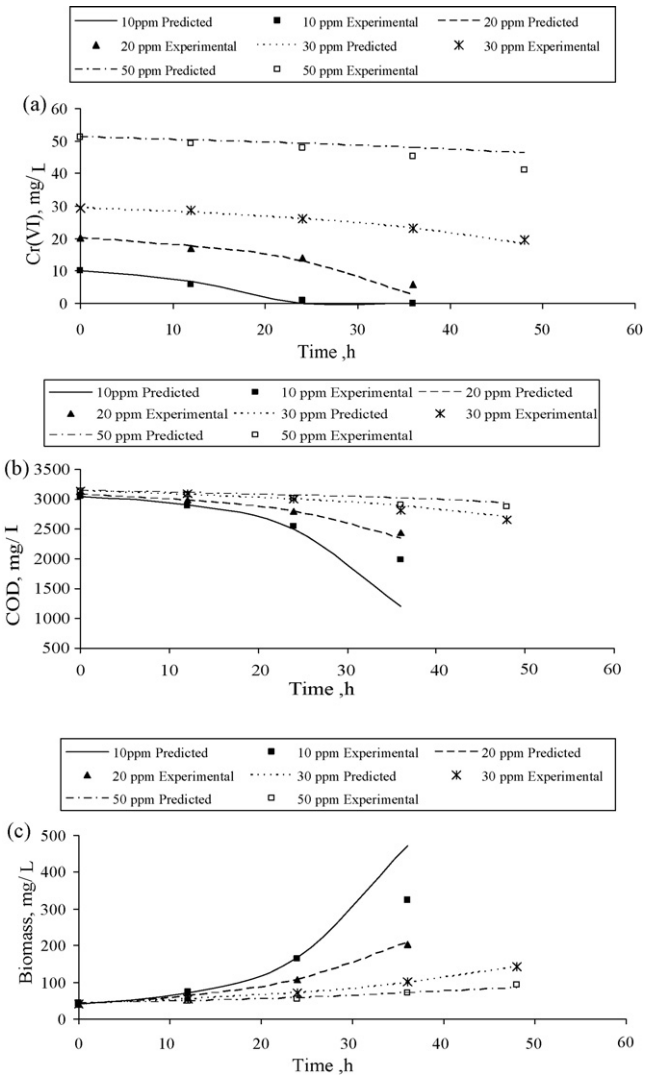

Fig. 5. (a) Experimental and model predicted Cr(VI) reduction by CRB and SRB under anaerobic conditions for different initial Cr(VI) concentrations (initial sulphate concentration = 1000 mg/L). (b) Experimental and model predicted COD consumption by CRB and SRB under anaerobic conditions for different initial Cr(VI) concentrations (initial sulphate concentration = 1000 mg/L). (c) Experimental and model predicted growth of CRB and SRB under anaerobic conditions for different initial Cr(VI) concentrations (initial sulphate concentration = 1000 mg/L).

3.1.6. Kinetics of chromium reduction by a consortium of CRB and IRB

Chromium reduction studies were carried out using a consortium of CRB and IRB under anaerobic conditions. Concentrations of biomass, COD, Fe(II), Fe(III) and Cr(VI) were monitored with respect to time. In these studies, initial concentration of Cr(VI) was varied from 0 to 50 mg/L, whereas the COD concentration and iron concentration was kept constant at 3072 and 800 mg/L, respectively. Initial biomass concentration in all the experiments was equal to 39 mg/L. It was found that chromium reduction was complete within 25 h when the concentration was less than 10 mg/L (Fig. 6(a)), with an initial Fe(III) concentration of 800 mg/L in the system. Cr(VI) reduction was complete at lower Fe(III) concentrations also though it took relatively longer time to achieve this. As in the case of individual cultures, the rate of COD removal decreased for higher Cr(VI) concentrations (Fig. 6(b)) due to inhibition effect. The inhibition effect was also clear from the biomass concentrations (Fig. 6(c)). As expected, Fe(II) generation was very high when Cr(VI) was not present in the system and it significantly reduced when Cr(VI)

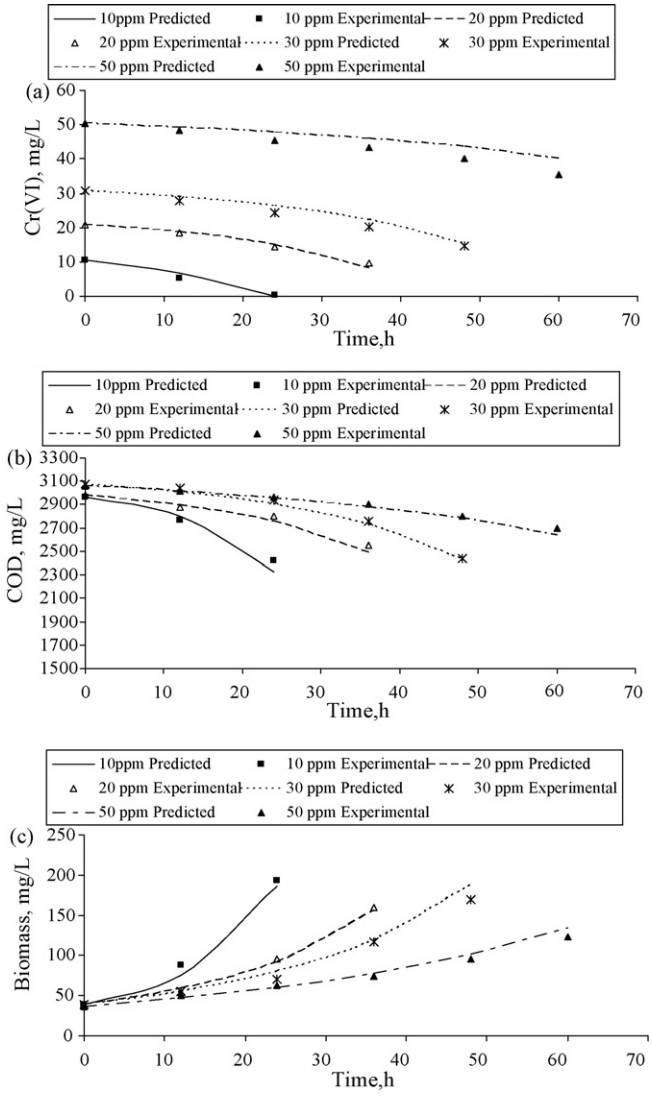

Fig. 6. (a) Experimental and model predicted Cr(VI) reduction by CRB and IRB under anaerobic conditions for different initial Cr(VI) concentrations (Initial Fe(III) concentration = 800 mg/L). (b) Experimental and model predicted COD consumption by CRB and IRB under anaerobic conditions for different initial Cr(VI) concentrations (initial Fe(III) concentration = 800 mg/L). (c) Experimental and model predicted growth of CRB and IRB under anaerobic conditions for different initial Cr(VI) concentrations (initial Fe(III) concentration = 800 mg/L).

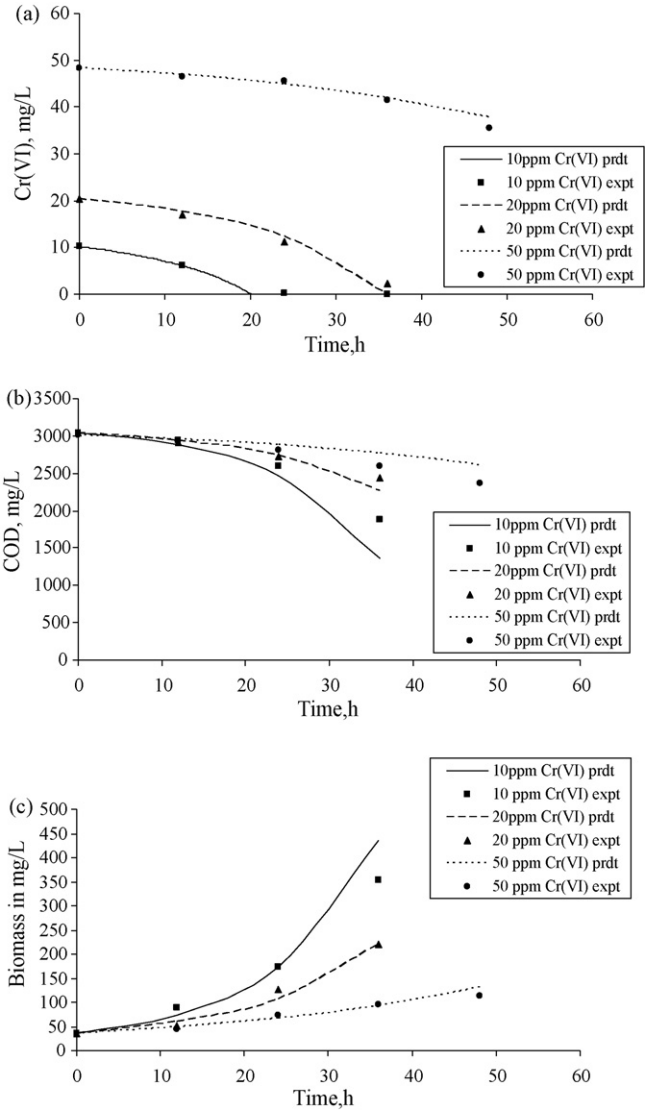

Fig. 7. (a) Experimental and model predicted Cr(VI) reduction by CRB, SRB and IRB under anaerobic conditions for different initial Cr(VI) concentrations (initial sulphate concentration = 500 mg/L, initial Fe(III) concentration = 400 mg/L). (b) Experimental and model predicted COD consumption by CRB, SRB and IRB under anaerobic conditions for different initial Cr(VI) concentrations (initial sulphate concentration = 500 mg/L, initial Fe(III) concentration = 400 mg/L). (c) Experimental and model predicted growth of CRB, SRB and IRB under anaerobic conditions for different initial Cr(VI) concentrations (initial sulphate concentration = 500 mg/L, initial Fe(III) concentration = 400 mg/L).

concentration in the system was high. Second part of this batch study was conducted by keeping the initial biomass as 39 mg/L, Chromium and COD concentration of 20 and 3072 mg/L, respectively, where as iron concentration was varied from 0 to 1600 mg/L (results not shown here). The observation from second part of the study indicates that rate of chromium reduction increased with an increase in iron concentration (results not shown).

3.1.7. Kinetics of chromium reduction by a consortium of CRB, SRB and IRB

Chromium reduction studies were carried out using a consortium of CRB, SRB and IRB under anaerobic conditions. Concentrations of biomass, COD, Fe(II), Fe(III), sulphate and Cr(VI) were monitored with respect to time. In these studies, initial concentration of Cr(VI) was varied from 0 to 50 mg/L, whereas the COD concentration was kept constant at 3040 mg/L. Initial biomass concentration in all the experiments was equal to 37 mg/L. Sulphate and Fe(III) concentrations were maintained at 500 and 400 mg/L, respectively. Experiments were also conducted with sulphate and Fe(III) concentrations of 1000 and 800 mg/L, respectively. Results for Cr(VI) reduction (Fig. 7(a)), COD removal (Fig. 7(b)) and biomass growth (Fig. 7(c)) were very similar to those observed in cases of individual cultures and combinations of two cultures.

It may be concluded from the above experimental results that CRB is very sensitive to Cr(VI) under aerobic conditions whereas SRB is the most sensitive species under anaerobic conditions (Table 2). This is evident from the respective inhibition constants. Cr(VI) reduction rates as well as specific Cr(VI) reduction rates (Cr(VI) reduction rates normalized with biomass concentration) indicated that Cr(VI) reduction was faster and almost complete when all the three microbial species (CRB, SRB and IRB) and all the electron acceptors (Fe(III) and sulphate) were present together in the medium under anaerobic conditions as compared to Cr(VI) reduction by individual species under either aerobic or anaerobic conditions. Bacterial strains such as SRB and IRB produce reduced

Table 1
Details of data sets used for calibration and validation of models.

	Calibration data	Validation data
CRB (aerobic)	Cr (20 ppm)	Cr (10 ppm, 30 ppm, 50 ppm, 100 ppm)
CRB (anaerobic)	Cr (20 ppm)	Cr (10 ppm, 30 ppm, 50 ppm)
SRB	Cr (20 ppm), sulphate (1000 ppm)	Cr (10 ppm, 30 ppm, 50 ppm), sulphate (1000 ppm)
IRB	Cr (20 ppm), Fe(III)(800 ppm)	Cr (10 ppm, 30 ppm, 50 ppm), Fe(III) (800 ppm)
CRB + SRB	No calibration	All sets of experiments
CRB + IRB	No calibration	All sets of experiments
CRB + SRB + IRB	No calibration	All sets of experiments

Numbers in the bracket indicate the initial concentrations.

Table 2
Biokinetic parameters for CRB, SRB and IRB.

Biokinetic parameters	CRB aerobic	CRB anaerobic	SRB anaerobic	IRB anaerobic
μ_{max} (1/h)	0.351	0.0888	0.1056	0.0867
μ_{max} (1/day)	8.424	2.1312	2.5344	2.0794
K_s	120	80	180	220.45
K_i	5.49	7.68	7.12	7.42
η	0.0087	0.0198	0.0281	0.0355
Y_T	0.4011	0.2215	0.2417	0.2662

Table 3a
Comparison of biokinetic parameters of CRB with previous studies.

S. no.	CRB	Condition	μ_{max} (1/h)	K_s (mg/L)	K_i as Cr(VI) in mg/L	Y_T	η	Reference
1	*E. coli* ATCC 33456	Aerobic, glycerol, casein hydrolysate, sodium citrate	0.37–1.15	–	–	–	–	[4]
2	*Shewanella alga* (BrYMT) ATCC 55627	Anaerobic, tryptic soy broth	.311–.346	–	2.83–2.94	–	–	[27]
3	Mixed culture	Aerobic, molasses	0.5846	3835	11.46	0.2615	–	[31]
4	*Arthrobacter rhombi-RE* (MTCC7048),	Aerobic, molasses	0.039	190	3.8	0.377		[33]
5	*Arthrobacter rhombi-RE* (MTCC7048),	Anaerobic, molasses	0.0095	710	8.77	0.13		[33]
6	Mixed culture	Facultative anaerobic, molasses	0.3	40	3.05	0.263	0.3	[34]
7	Mixed culture	Molasses, anaerobic	0.0888	80	7.68	0.2215	0.0198	Present study
8	Mixed culture	Molasses, aerobic	.351	120	5.49	0.4011	0.0087	Present study

chemical species such as sulphide and Fe(II). In turn, these reductants reduce Cr(VI) to Cr(III) [10,11,21–25]. This chemical reduction is much faster as compared to biological reduction. Also, there is no necessity for further addition of any electron acceptor in biological systems as the process is cyclic for Fe(III) and sulphate. It is interesting to note that CRB, SRB and IRB are natural flora in groundwater systems and their synergetic action enhances the Cr(VI) reduction significantly.

3.2. Mathematical model

3.2.1. Calibration and validation for chromium reduction by single consortium

The proposed mathematical model for chromium reduction under different conditions using a single consortium (CRB/IRB/SRB) is calibrated and validated using the experimental data for the corresponding consortium. For example, experimental data for chromium reduction by CRB under aerobic conditions with an initial chromium concentration of 20 mg/L was used for calibration and obtaining the biokinetic parameters for that culture. The same biokinetic constants were used to predict (validation) the chromium reduction, COD consumption and microbial growth in other experiments with different initial chromium concentrations. Table 1 shows the data sets used for calibration and validation purposes. The biokinetic parameters are summarized in Table 2.

Kinetic parameters for Monod-type equations often have linear correlations between them [38]. It was shown by Liu and Zachara [38] that the biokinetic parameters depend on the initial conditions in the experiment and they suggested ways to find the experimental conditions for which the biokinetic parameters do not show

Table 3b
Comparison of biokinetic parameters of SRB with previous studies.

S. no.	SRB	Substrate	μ_{max} (1/h)	K_s (mg/L)	Y_T	K_i as Cr(VI) in mg/L	η	Reference
1	*Desulfotomaculum acetoxidans*	Acetate	0.025	100	–	–	–	[13]
2	*Desulfonema magnum*	Acetate	0.007167	120	–	–	–	[13]
3	*Desulfococcus multivorans*	Ethanol	0.005833	70	–	–	–	[13]
4	*Desulfobulbus propionicus*	Propionate	0.045833	50	–	–	–	[13]
5	Mixed culture	Acetate/peptone	0.065	125 g/m^3	0.572	–	–	[35]
6	Mixed culture	Ethanol	0.0594	18.1 mM	1.32 (mg protein/mol of ethanol)	–	–	[36]
7	Mixed culture containing *Desulfovibrio* sp., *Desulfotomaculum* sp. and others at 31 °C	Acetic acid	0.00858	5.7	0.062 (mg cells/mg acetic acid)	–	–	[37]
8	Mixed culture	Molasses	.1056	180	.2417	7.12	0.0281	Present study

Table 3c
Comparison of biokinetic parameters of IRB with previous studies.

S. no.	IRB	Substrate	μ_{max} (1/h)	K_s	Y_T	K_i as Cr(VI) in mg/L	η	Reference
1	*Shewanella putrefaciens* CN32	Lactate	.19	25 mM	1.28×10^8 (cells/mol of mM of Fe(III))	–	–	[38]
2	S. *putrefaciens* CN32	Lactate	0.029	0.52 mg/L	–	–	–	[39]
3	*G. sulfurreducens*	Acetate	0.1 ± 0.01	0.010 ± 0.001 mM	3.8 (mg dw/mol acetate)	–	–	[40]
4	*Shewanella putrefaciens* CN32	Lactate, citrate	0.32/h	29 mM	5.24×10^9 (cells/mmol of Fe(III))	–	–	[41]
5	Shewanella. *oneidensis* MR-1	Lactate	0.47	13.2 mg/L	19.1 (g dry cell/mol lactate)	–	–	[42]
6	S. *oneidensis* MR-1	Acetate	0.28	12.3 mg/L	16.8 (g dry cell/mol acetate)	–	–	[42]
7	Mixed culture	Molasses	0.0867	220.45 mg/L	0.2662	7.42	0.0355	Present study

significant variation. This issue was not addressed in the present study while determining the kinetic parameters. The objective of the present study was to maximize the chromium reduction, and the biokinetic parameters were determined for experimental conditions corresponding to the optimal environmental conditions (such as substrate concentration and initial biomass concentration) which result in maximum Cr(VI) reduction. Such data will be useful for developing management models. Biokinetic constants obtained in the present study are compared with those reported in the literature in Tables 3a–3c. It is clear from Tables 3a–3c that most of the values for biokinetic parameters obtained in the present study were comparable to those reported earlier, except the maximum specific growth rate of SRB. The culture used in the study was a mixed culture containing SRB. The cultures have not been characterized purely as sulphate reducing bacteria by any of advanced techniques like 16S rRNA sequencing. This could be the reason for higher specific growth rate for SRB in the present study.

Fig. 1(a)–(c) show the comparison between the experimental results and the model fitted results from the calibration and validation study for chromium reduction by CRB under aerobic condition. As suggested by Kohne et al. [32], the performance of the proposed mathematical model was statistically evaluated using the dimensionless modified coefficient of efficiency, *E*.

$$E = 1 - \frac{\sum_{i=1}^{N} [|E(t_i) - O(t_i)|]}{\sum_{i=1}^{N} |O(t_i) - \bar{O}|} \quad (17)$$

where $E(t_i)$ is the numerically simulated value of a variable at time t_i, $O(t_i)$ is the observed value of the same variable at time t_i, and $\bar{O}$ is the mean value of the observed variable. *E* varies between $-\infty$ to 1.0, the higher values indicating better model prediction. Kohne et al. [32] suggest that a positive value of *E* represents an "acceptable" simulation whereas $E > 0.5$ represents a "good" simulation. *E* equal to one indicates a "perfect" simulation. Values of *E* for all the simulations carried out in this study are presented in Table 4. The *E* value for chromium reduction by CRB ranges from 0.53 to 0.93, for different initial chromium concentrations. The *E* values for all the validation studies (CRB/SRB/IRB) are greater than 0.5, indicating a good performance of the proposed mathematical model. It can be observed from Fig. 1(a)–(c), as well as from the *E* values, that the proposed model predicts the chromium reduction by CRB very well for higher concentrations (more than 30 mg/L). However, the model performance for lower concentrations was not as good. In the model, it was assumed that the inhibition can be represented through one single value of K_i, using Monod's inhibition model. This may not represent the inhibition correctly for lower Cr(VI) concentrations. Satisfactory results were obtained in the initial stages even in cases of low initial Cr(VI) concentrations, but the performance deteriorated with time as the Cr(VI) reduction progressed. During the later stages, microbial growth predicted by the proposed model was higher than the observed growth, indicating that the inhibition model did not represent the mechanism appropriately. It may be noted here that Guha [27] used a double substrate model, rather than an inhibition model, for chromium reduction by a single culture. Cr(VI) concentrations in the study by Guha [27] were low. Finally, experimental and analytical errors could also contribute to the mismatch.

3.2.2. Validation of model for chromium reduction by a combination of consortiums

3.2.2.1. CRB and SRB. Biokinetic parameters obtained from batch studies for single consortium are used for the validation of the mathematical model for chromium reduction, COD consumption, and biomass growth by a consortium of CRB and SRB. It may be noted that though sulphate reduction during the process was monitored, no attempt was made to model this. It was assumed that sulphate was unlimiting and the inhibition effect due to sulphate

Table 4
Modified coefficients of efficiency (*E*) obtained while evaluating model performances.

Cr(VI) concentration	10 mg/L			20 mg/L			30 mg/L			50 mg/L			100 mg/L		
	Biomass	COD	Cr(VI)	Biomass	COD	Cr(VI)	Biomass	COD	Cr(VI)	Biomass	COD	Cr(VI)	Biomass	COD	Cr(VI)
CRB aerobic	0.7722	0.7644	0.7584	0.8845	0.9763	0.9990	0.7206	0.6294	0.5301	0.8074	0.7885	0.8096	0.8902	0.8616	0.708
CRB anaerobic	0.8848	0.7971	0.7485	0.9047	0.8365	0.8890	0.7976	0.7017	0.6545	0.7129	0.8196	0.5307	[a]	[a]	[a]
SRB anaerobic	0.8172	0.8345	0.8329	0.8514	0.6564	0.8995	0.9751	0.6785	0.893	0.7902	0.8311	0.7889	[b]	[b]	[b]
IRB anaerobic	0.7794	0.5734	0.7641	0.8491	0.7655	0.6824	0.7362	0.6863	0.7596	0.8177	0.5779	0.5840	[b]	[b]	[b]
CRB + SRB anaerobic	0.5996	0.4052	0.8745	0.9326	0.8613	0.7187	0.9669	0.8416	0.8521	0.8217	0.4373	0.4405	[b]	[b]	[b]
CRB + IRB anaerobic	0.8830	0.7882	0.8424	0.9170	0.7742	0.8485	0.8581	0.8985	0.7506	0.8091	0.8325	0.4371	[b]	[b]	[b]
CRB + SRB + IRB (Fe 400 ppm, sulphate 500 ppm) anaerobic	0.7449	0.5322	0.9779	0.8833	0.7325	0.8354	[b]	[b]	[b]	0.7625	0.5300	0.7938	[b]	[b]	[b]
CRB + SRB + IRB (Fe 800 ppm, sulphate 1000 ppm) anaerobic	0.7740	0.4232	0.9081	0.7988	0.7978	0.7165	[b]	[b]	[b]	0.8458	0.5168	0.6071	[b]	[b]	[b]

[a] No growth was observed during experiments.
[b] Experiments were not conducted.

was not very significant. Model predicted and experimental data for chromium reduction, COD consumption and biomass growth for different initial chromium concentrations are presented in Fig. 5(a), (b) and (c), respectively. Values of dimensionless modified coefficients of efficiency, E are presented in Table 4. As in the case of single consortium, the performance of the model was better for higher concentrations of Cr(VI) as compared to lower concentrations. It can be clearly observed from Fig. 5(b), and (c) that the performance of the model, in predicting the COD consumption and biomass growth, deteriorated especially after all the Cr(VI) in the system was reduced. This may be because the bacterial culture which is acclimatized to reducing Cr(VI) behaves differently when Cr(VI) availability is limited. The proposed model does not consider the limiting effect of Cr(VI) availability.

3.2.2.2. CRB and IRB. Biokinetic parameters obtained from batch studies for single consortium are used for the validation of the mathematical model for chromium reduction, COD consumption, and biomass growth by a consortium of CRB and IRB. Model predicted and experimental data for chromium reduction, COD consumption and biomass growth for different initial chromium concentrations are presented in Fig. 6(a), (b) and (c), respectively. Values of the dimensionless modified coefficients of efficiency, E are presented in Tables 3a–3c. It can be observed from these figures, as well as from the E values (>0.7 except for Cr(VI) reduction for 50 mg/L), that the proposed model was able to predict the experimental results satisfactorily.

3.2.2.3. CRB, SRB and IRB. Biokinetic parameters obtained from batch studies for single consortium are used for the validation of the mathematical model for chromium reduction, COD consumption, and biomass growth by a consortium of CRB, SRB and IRB. Model predicted and experimental data for chromium reduction, COD consumption and biomass growth for different initial chromium concentrations are presented in Fig. 7(a), (b) and (c), respectively. The dimensionless modified coefficients of efficiency, E are presented in Table 4. The E values for the above validation studies are greater than 0.5, indicating a good performance of the proposed mathematical model. The performance of the model was similar to that in the case of single cultures and mixture of two cultures. From these results it is clear that, based on the kinetic parameters for single culture, it is possible to develop a model for simultaneous action of three different cultures (CRB, SRB and IRB), having distinct characteristics, with respect to chromium reduction, substrate utilization and biomass growth.

4. Conclusion

Different bacterial cultures, CRB, SRB and IRB, were enriched and isolated from contaminated soils. These cultures were used for Cr(VI) reduction studies, individually and in combination. Studies were conducted with different initial Cr(VI), SO_4^{2-} and Fe(III) concentrations. Biokinetic parameters such as μ_{max}, K_s, Y_T and K_i for individual cultures were evaluated. A mathematical model was proposed for simulating the chromium reduction, COD utilization and biomass growth, for individual cultures as well as for a combination of two or three different cultures, for different initial Cr(VI), SO_4^{2-} and Fe(III) concentrations. Biokinetic parameters evaluated from one set of experiments for individual cultures were utilized in all the validation studies. Performance of the mathematical model in terms of the dimensionless modified coefficient of efficiency (E) indicated that the proposed model simulated the system behavior very well, especially for higher Cr(VI) concentrations. However, the model performance for lower concentrations was not as good. Monod's inhibition model used in this study might not have represented the inhibition correctly for lower Cr(VI) concentrations.

Different types of microorganisms are generally present in contaminated aquifers. It is important to understand the interaction between various microorganisms under different environmental conditions with respect to chromium reduction. Based on the kinetic studies for single culture, it was possible to develop a model for simultaneous action of three different cultures (CRB, SRB and IRB), having distinct characteristics, with respect to chromium reduction. The proposed mathematical model will be very helpful in the development of a management model for in-situ bioremediation of chromium contaminated aquifers using either reactive zones or bio-barriers. The proposed mathematical model will be useful as a reaction module in any mathematical model, based on the solution of advection–dispersion-reaction equations, for in-situ bioremediation of contaminated aquifers.

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