Ecotoxicology and Environmental Safety 193 (2020) 110327

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Enhanced biogeogenic controls on dichromate speciation in subsoil containment

Sangeetha CJ, Shashidhar T*

Department of Civil Engineering, Indian Institute of Technology Hyderabad, Kandi, Telangana 502285, India

ARTICLE INFO

Keywords:
Biogenic speciation
Bioaugmentation
Nutrient-stressed bacteria
Cr (VI) reactive transport
Subsoil containment

ABSTRACT

In general, lab-based Cr (VI) reduction studies do not often corroborate the prevailing biogeochemical controls for on-site pollution abatement. To promulgate its importance, herein, we investigate the existing biogeogenic parameters of a contaminated site to attenuate the underground Cr (VI) toxicity. This study significantly assesses the speciation of dichromate by biogenic agents that are inherent and self-sustaining to treat the contaminated soil. Herein, a group of bacteria exposed to high concentrations of chromium ($\geq$3500 mg/L) plays a vital role as an enhanced biogeogenic control for the detoxification of toxic Cr (VI). All identified bacteria were screened based on their ability to differentiate from extracellular speciation and harvested in a Cr (VI)-enriched molasses to achieve dichromate concentrations as low as 0.05 mg/L in 168 h. Under low O_2 condition, the bacterial growth rate and doubling time were monitored to establish the half-life period of Cr (VI) for adequate containment treatment. Furthermore, to understand the soil decontamination, Cr (VI) reactive transport was demonstrated to facilitate the contaminant reduction under both saturated and unsaturated groundwater conditions. Herein, Cr (VI) speciation to Cr (III) by the influence of abiogenic factors are unlikely or less probable as studied in existing geogenic conditions. Moreover, the evidence of biogenic reduction of Cr (VI) in microcosm suggests its effectiveness in enhanced detoxification of Cr (VI) up to $\leq$ 0.1 mg/L, within the reaction period of 144 h and 192 h, for saturated and unsaturated flow conditions, respectively.

1. Introduction

Chromium (VI)-soil contamination is a global problem for many years due to its mobility and toxicity (Pantsar-Kallio et al., 2001; WHO, 2003; Taylor et al., 1999; Zhitkovich, 2011). The frequent accumulation of this heavy metal has attracted a great deal of attention in recent years owing to its adverse effects. The unregulated industrial discharges and hazardous landfills are the major sources of heavy metal contaminants that cause severe damage to living standards. These contaminants are environmentally harmful, potentially causing a public health threat when directly exposed to living organisms (Caravanos et al., 2014b). In order to avoid imminent danger, strict regulations are enforced on industries for safe discharge of chromium in wastewaters. Supplementary data 1.1 shows the discharge standards for chromium in various effluents and its tolerance limits as set by National & International organizations. Despite all, the problem appears more permanent and amended regulations for the prevention of contamination seems to have not changed the current pollution level. This represents a global burden (Pure Earth, 2012), as chromium ranks third in most toxic threat among other priority contaminants viz. lead, mercury, arsenic, pesticides, and radionuclides as described in the annual report of World's Worst Pollution Problem (Pure Earth, 2010).

Cr (VI) levels in the environment of developing countries are always incommensurable to developed countries (Caravanos et al., 2014a). For developing countries like India, industrialization plays an economically important role. Persistent contribution to economic development, accompanying the establishment of various industries led to the development of these uncontrollable anthropogenic contaminants (Ingle et al., 2011). Besides, the industrial discharges in developed countries are well-monitored, and an appropriate purification strategy has been proved over developing countries (Ericson et al., 2013). However, in India, industrial activities are not strictly monitored (Caravanos et al., 2014a; Chatham-Stephens et al., 2013), which led to unaccounted contaminated sites. Most reasons behind these actions are limited governmental capacities & incentives to enforce the law against unregulated industrial activities (Dowling et al., 2017).

Chromium in soil mainly occurs in two forms: Hexavalent chromium [Cr (VI)] and trivalent chromium [Cr (III)], of which Cr (VI) is a toxic carcinogen and Cr (III) is a micronutrient. Hexavalent form of chromium is highly soluble and mobile, while the trivalent form is

* Corresponding author.
E-mail address: shashidhar@iith.ac.in (S. T).

https://doi.org/10.1016/j.ecoenv.2020.110327
Received 26 November 2019; Received in revised form 14 January 2020; Accepted 11 February 2020

insoluble (Batool et al., 2012; Kirpnick-Sobol et al., 2006; Long et al., 2013; Saha et al., 2011). The cyto- and genotoxic effects of Cr (VI) are largely studied by the researchers to understand its potential risk of cancer in humans. Cr (VI) generally pose great danger to living cells due to the structural similarity of chromate, sulfate and phosphate, as it gains smooth entry into cytoplasm via anionic transporters (Saha et al., 2011). On the other hand, the trivalent form is considered to be inert due to its d3 electronic configuration and are kinetically slow reactive than toxic Cr (VI). In biological pH, Cr (III) undergoes precipitation to form hydroxides, whereas Cr (VI) remains labile to undergo a reduction in cytoplasm (or) can pass through nuclear membrane by causing adverse effects (Saha et al., 2013). The toxicity of Cr (VI) can affect the susceptible through direct exposure of contaminated soil to human (Soil-human) or by indirect exposure to crops/vegetables through bioaccumulation (Soil-crop-human) (Saha et al., 2013, 2011; Wang et al., 2011).

The degree of Cr (VI) contamination is highly dependent on the origin of soil and the extent of anthropogenic activity surrounding the environment (WHO, 2003). Most high concentrations of Cr (VI) enters the soil through improper waste disposals by tanneries but not limited to industries such as mineral mining, electroplating, inorganic chemicals and steel manufacturing plants (Dhal et al., 2013). These high concentrations of Cr (VI) could be reduced or immobilized to Cr (III) by physicochemical or biological methods (Malaviya and Singh, 2011; Shashidhar et al., 2007a, 2006). In India, leather tanning industries are overseen to be a significant contributor to Cr (VI) contamination by the pollution control bodies and thus, other industries gain very less attention. In a contaminated site at Ranipet, a population of nearly 3,482,000 were at risk due to tannery based discharge alone (Pure Earth, 2007). The risk associated with other industrial discharges for cumulative Cr (VI) burden remains unspecified that attracts the attention of international organizations to initiate Toxic Site Identification Program (TSIP) in low- and middle-income countries (Ericson et al., 2013).

Soil remediation is usually done by in-situ (or) ex-situ physiochemical & biological methods (Ballesteros et al., 2017; Bianco Prevot et al., 2018; Gomes et al., 2013; Khalid et al., 2017; Mulligan et al., 2001; Nicolova et al., 2017; Yuan et al., 2018). In most cases, in-situ remediation is economically viable than ex-situ pump and treatment operations (Gong et al., 2018; Hashim et al., 2011). In addition, the direct biological means of site remediation as proven to be more economical and sustainable as it greatly enables the detoxification of groundwater at the source level (Batool et al., 2015; EPA, 2016; Groudev et al., 2014). These methods employ inherent or foreign microorganisms, most predominantly bacteria, to co-metabolize the metal toxicants while utilizing the organic matter and other available carbon sources (Bansal et al., 2019; Batool et al., 2015; Emenike et al., 2017; Jobby et al., 2018; Lee et al., 2008; Leita et al., 2011; Slater and Atekwana, 2013). The inherent microbial treatment (i.e. Bioaugmentation) of contaminated sites is advantageous in a variety of conditions such as site adaptability, suitability of field operations, complexity of underground processes and minimal waste generation (Azubuike et al., 2016; Chen et al., 2019; Dzionek et al., 2016; Gomes et al., 2013; Liu, 2018; Long et al., 2013; Schindler et al., 2017). In general, the persistence of Cr (VI) depends in no small extent on the physicochemical properties of the site, the media (i.e. the soil, water and air to which the contaminants are bound and released), the nature of the pollutants and the inherent existence of bacteria in a nutrient-stressed environment (Emenike et al., 2016; Stanin, 2005). As for the biogeochemical interactions, the speciation of toxic Cr (VI) to Cr (III) is controlled by factors such as soil pH, organic & H_2O content, multi-elements, porosity, permeability and geomicrobiology (Adriano et al., 2004; Barnie et al., 2018; Lee et al., 2008; Xiao et al., 2012). Therefore, it is essential to consider the site-specific conditions of a contaminated site in order to assess its potential for biogenic detoxification of Cr (VI).

Several researchers have developed lab-based biological treatment methods that show a better reduction of Cr (VI) (Hamdan and El-Naas, 2014; Jin et al., 2014; Malaviya and Singh, 2011; Maleki et al., 2015; Nam et al., 2018) with limited knowledge of its reliability concerning field application. In addition, Cr (VI) speciation studies show the exemplary ability of biogenic agents as permeable reactive biobarrier in bioremediation of soil and groundwater (Shashidhar et al., 2007a, 2007b; Thiruvenkatachari et al., 2008). However, their limitations in relating the site-specific properties make in-situ treatment less effective in terms of a detoxification mechanism. Also, the developed batch treatment processes (Pradhan et al., 2017) by single bacterium species (i.e. a single type strain) is unlikely to combat in the field, due to factors such as complex biodiversity, minimal nutrient conditions and biogeochemical interactions. Therefore, this present study evaluates the geogenic characteristics of a heavily contaminated site and demonstrates the role of biogenic control on dichromate speciation via bioaugmentation. The biogenic controls used in this study are primarily screened for exoenzyme Cr (VI) speciation and assessed for *in vitro* biokinetic performance in media containing suitable electron donor. Furthermore, a series of reactive transport experiments are carried out with bio-augmented soils under two different groundwater conditions, to demonstrate the soil-Cr (VI) detoxification.

2. Methodology

2.1. Study area & sampling points

A Cr (VI) contaminated site (16°43′14.20″N, 79°39′5.92″E) in India was identified to evaluate its geogenic characteristics for enhanced natural attenuation. The study area was chosen based on the unregulated industrial activity by a dichromate manufacturing plant, located at Damarcherla in the Nalgonda District (Telangana, India). Supplementary data 1.2 shows Cr (VI) contamination around the industrial site and tons of unattended sludge in dump yards. The identified area is an intensely contaminated site of approximately 40,000 tons of chromium waste (Class A, Schedule 2 Hazardous waste) dumped as landfills. It was reported that the toxicity of Cr (VI) has begun to affect thousands of people, cattle, agricultural lands and other closely related rivers Musi & Krishna (Karnakar Reddy, 2014). Hence, the persistence of contamination is a matter of serious concern as no mitigation measures are planned for the restoration of the contaminated area. As of current situation, the listing of highly polluted sites for clean-up by the administrative & governmental bodies did not account for the present study area (CGWB, 2016; MOEF, 2015). Hence, it is of high importance to examine the stated site and emphasize the need for appropriate mitigation action.

Various type of samples viz. Contaminated soil (S-I) from industrial area, Uncontaminated soil (S-II) from closely associated region, Heap sample from landfill pile (DYH), Industrial sludge (IS) from waste disposal zone, Industrial effluent (IE) from the drain pit zone, Surface water (SW) from the depression closer to sludge disposal zone, Sediment (SED-I) at the entrance to the river and Sediment (SED-II) after the confluence of the River Musi-Krishna, were collected for this study. Supplementary data 1.3 shows the survey area and sampling points mapped by QGIS (An open-source software).

2.2. Soil characterization

Soil characterization is primarily done to evaluate the biogeochemical properties of polluted soil for enhanced speciation of Cr (VI). For this purpose, the contaminated soil (S-I) was collected at a depth of 0–25 cm depth from various points surrounding the industrial zone by grab sampling. In order to demonstrate Cr (VI) reactive transport, uncontaminated soil (S-II) from closely associated region Vadapalle was collected by core cutter at a depth of 0–60 cm and used as a representative soil for microcosm construction. The major parameters such as Soil pH (US EPA SW-846 Method 9045D, 2004), H_2O content

(ASTM D 2216, 2010), Organic content (ASTM 2974, 2014), Bulk density (ASTM D 2937, 2010), Specific gravity (ASTM D 854, 2000) and Soil gradation (ASTM D 422-63, 2007) were estimated according to the standard guidelines of American Society for Testing & Materials (ASTM). The multi-element constituents of all collected samples (Section 2.1) are analyzed using an Inductively Coupled Plasma Mass Spectrometer (ICP MS).

2.3. Soil sorption

The sorption of Cr (VI) and Cr (III) to S-I and S-II was evaluated under a 24 h equilibrium time (Roy, 1991) and their isotherms are established for abiogenic control. Supplementary data 1.4 shows the sorption characteristics of S-I and S-II at temperature 37° C ± 1.75, in pH 7 ± 0.05 and pH 6.4 ± 0.05, respectively. The obtained data were related to Freundlich isotherm (equation (1)) as nonspecific sorption due to soil-surface heterogeneity (Freundlich, 1906),

$$q_e = K_f\, C_{eq}{}^{1/n} \tag{1}$$

Where q_e is the amount of chromium absorbed, C_{eq} is the equilibrium concentration, K_f is the adsorption co-efficient (mL/g), and n is the adsorption intensity. In equation (2), 1/n is the slope and log K_f is the intercept of a straight line from the plot log (q_e) versus log C_{eq} (Supplementary data 1.4). A linear regression method was used to estimate the slope and intercept while determining the variable y based on predictor x.

$$\log(q_e) = log\ K_f + \frac{1}{n}\log C_{eq} \tag{2}$$

2.4. Identification of biogenic agents

Two different bacterial population viz. Total bacteria and Cr (VI) tolerant bacteria from contaminated soil (S-I) were isolated to understand the soil microbial characteristics. In this study, the total bacteria from contaminated soil (S-I) is represented as a background geomicrobial population (Supplementary data 1.5) in order to enhance the soil-biogenic activity. In addition, the bacteria tolerant to Cr (VI) from highly contaminated samples viz. IE and IS were isolated to evaluate their potential for Cr (VI) tolerance and biospeciation ability.

All obtained strains were isolated in two different growth media; namely, oligotrophic (M1) and less fastidious (M2) media, to assess their potential as inherent biogenic agents. Following the isolation, the bacterial colonies are differentiated by their morphological characteristics and evaluated for MIC & biospeciation in M3 media (Supplementary data 1.5). The suitable biogenic controls for soil-Cr (VI) reduction are selected based on its exoenzyme Cr (VI) speciation ability.

2.5. Biogenic reduction of Cr (VI)

The selected biogenic agents are tested under *in vitro* nutritional conditions to understand their biokinetics at various Cr (VI) concentrations. In this study, all batch experiments are carried out in a minimal nutrient media (M4) for adequate Cr (VI) reduction. The M4 media used for biokinetic testing consists of molasses concentrate (2 g/L) supplemented with other elemental nutrients as per the ingredients stated in Handbook of Microbiological Media (Atlas, 2010). The elemental nutrients of M4 media comprised of macronutrients [Na_2HPO_4 (6.8 g/L), KH_2PO_4 (3 g/L), NaCl (0.5 g/L), $MgSO_4$•$C7H_2O$ (0.5 g/L), $CaCl_2$•H_2O (0.01 g/L), $FeCl_3$•$C6H_2O$ (0.001 g/L)]; and micronutrients [Na_2MoO_4•$C2H_2O$ (0.01 g/L), $CuSO_4$•$C5H_2O$ (0.01 g/L), $ZnSO_4$•$C7H_2O$ (0.01 g/L), $CoCl_2$•$C2H_2O$ (0.01 g/L), $MnCl_2$•$C4H_2O$ (0.01 g/L)] adjusted to pH 7.2 ± 0.2.

For batch biogenic reduction, the bacterial strains grown to mid-exponential stage were separately inoculated into reactors containing Cr (VI)-enriched M4 media. Before the start of experiment, an inert gas (N_2) was bubbled at a flow rate of 25 mL/s in reactors and sealed air-tight to achieve anaerobic condition (Initial DO = 0.41–0.65 mg/L). Subsequently, all biogenic reactions were carried out in an orbital shaking incubator at 37 °C ± 1.75. For abiogenic reduction, the control experiments were carried out in the absence of bacterial strains. During the operation, the samples were withdrawn at regular intervals through a syringe samplers. All samples collected from the reactors are centrifuged at 10,000g for 10 min at 4 °C ± 0.5 for adequate phase separation. Then after, the obtained supernatant was passed through a filter membrane of pore size 0.22 μm for the aqueous phase determination of Cr (VI). And the subsequent cell pellet was washed, rinsed and re-suspended in NaH_2PO_4–Na_2HPO_4 buffer (0.2 M; pH 7 ± 0.05) for the bacterial cell mass quantification.

In bacterial growth experiments, the rate of change in concentration of bacteria between lag and stationary phase is given by equation (3),

$$C = C_0\, e^{kt} \tag{3}$$

The above equation is simplified (Zhu et al., 2013) to determine the specific growth rate of bacteria under the influence of various initial concentration of Cr (VI),

$$Specific\ growth\ rate,\ \mu = \frac{ln\ (C/C_0)}{(t - t_0)} \tag{4}$$

Where C_0 is the initial bacterial concentration (Dry cell weight, mg/L), C is the bacterial concentration at time t (Dry cell weight, mg/L), K is the specific growth rate, also known as μ (hr^{-1}), and t_0 & t are time period of exponential phase (hr). Here, the time required to double the bacterial population is given as doubling time T_d (hr), determined by equation (5) (Luo et al., 2016; Reyimu and Özçimen, 2017),

$$T_d = \ln 2/\mu \tag{5}$$

In Cr (VI) reduction experiments, the rate of decrease in Cr (VI) concentration per unit time is given by equation (6),

$$C_t = -\,kt + C_0 \tag{6}$$

Where C_t is the concentration of Cr (VI) after the reaction time t, C_0 is the initial concentration of Cr (VI) at t = 0, and k is the rate constant. The half-life ($t_{1/2}$) is the time required for Cr (VI) concentration to decrease by half compared to its initial concentration C_0 is determined by equation (7),

$$t_{1/2} = C_0/2k \tag{7}$$

2.6. Soil microcosm studies

2.6.1. The soil column

A cylindrical column with a diameter of 10 cm and a length of 50 cm was fabricated for the construction of soil microcosm. A perforated plate having a pore diameter of 0.1 cm with a removable casing on both ends of column were provided to ensure uniform distribution of fluid during reactive transport. Between the perforated plates, a representative soil S-II (particle size less than 4.75 mm) with an average porosity of 0.46% was compacted under different saturated conditions. The chambers above the perforated plates at each end of the column comprises of primary inlet and outlet for the fluid-flow transport. In addition, five other sampling ports were provided at 10, 20, 30, 40, and 50 cm along the entire length of the column to facilitate the collection of pore fluids via typical rhizon pore-water samplers (EIJKELKAMP SOIL & WATER). Supplementary data 1.6 shows the schematics of soil column arrangement for Cr (VI) reactive transport.

2.6.2. Cr (VI) fluid transport in soil microcosm

The fluid transport of Cr (VI) was carried out under two different groundwater flow conditions: i.) Saturated and ii.) Unsaturated flow. During the flow transport experiments, 200 mg/L of Cr (VI) in M4 media was pumped at a constant flow rate of 4.6 and 5 mL/min for

saturated and unsaturated conditions, respectively, using a peristaltic pump (MICLINS INDIA). The fluid flow in the soil is vertical that gradually penetrates along the length of column to reach the collection tank.

Two types of microcosm were used to understand the effects of abiogenic and biogenic controls on reactive transport of Cr (VI). In column-abiogenic experiments, the sterile soil (S-II) with the background multi-element concentration was used for Cr (VI) reactive transport, while in column-biogenic experiments, the soil (S-II) amended with exoenzyme bacteria (Average Inoculum concentration = 67.63 mg/L) was used to reduce the spiked Cr (VI) concentration below the permissible level (0.05 mg/L).

The soil columns were prepared for different levels of saturations with M4 minimal nutrients prior to Cr (VI) fluid transport experiments. In saturated flow condition, the column was thoroughly soaked with minimal nutrients (M4) until it reached a constant flow rate. During the course, the washed-out bacteria were re-pumped back into the saturated column to maintain the active microbial population. While in unsaturated flow condition, the column was partially soaked with bacterial strains and minimal nutrients (M4) at a moisture content ranging from 7% to 10%. Upon continuous pumping of Cr (VI), the reactant fluid infiltrates the soil column and gets collected at pore-water sampler for the determination reduced Cr (VI) concentration.

3. Quantification

3.1. Chromium species quantification

Chromium species were determined by 1, 5-diphenylcarbazide colorimetric method (US EPA Method 7196A, 1992) at a wavelength 540 nm using a UV–Vis spectrophotometer (LAB INDIA ANALYTICAL UV-VIS 3200). The quantitative determination is based on the reaction of diphenylcarbazide with Cr (VI) to produce diphenyl carbozone and Cr (III) ions under acidic condition. The end products of the reaction combine to form a purple colored Cr (III)–diphenyl carbozone complex, which is proportional to the concentration of Cr (VI). In case of determination of Cr (III), $KMnO_4$ was used as an oxidizing agent to convert Cr (III) ions to Cr (VI), while the unreacted $KMnO_4$ was removed by NaN_3. The Cr (VI) ions in test aliquots are further subjected to diphenylcarbazide to quantify the indirect concentration of Cr (III). Supplementary data 1.7 shows the calibration curve for the determination of chromium in the test solutions.

3.2. Bacterial enumeration & biomass quantification

The total number of bacteria was determined by microbial plate count assay on oligotrophic (M1) and less fastidious (M2) media. The culture dishes are designated as TNTC (Too numerous to count, > 300 CFUs), CC (Countable colonies, 30–300 CFUs) and TFTC (Too few to count, < 30) based on viable counts of the bacterial colonies (Colony forming units, CFUs) as given in Supplementary data 1.8. A serially diluted sample (one part of sample in saline) designated as CC was considered to determine CFUs/g. dry cell weight of the sample. The total biomass concentration in test aliquots is determined by correlation method of turbidity and dry cell weight measurements. Supplementary data 1.8 shows the calibration curve for quantification of biomass at a wavelength 575 nm.

3.3. Multi-elements extraction & quantification

All collected samples (section 2.1) were digested in 75 mL pressure-controlled vessel (MarsXpress PFA CEM MARS™) using a microwave digester (CEM MARS™ 6 Microwave Digestion & Extraction System) with different acid matrices under specified conditions (Supplementary data 1.9). The sample mass (or) volume was chosen based on the US EPA SW-846 standards (Method 3052, 2004; Method 3015A, 2007). After digestion, the samples were filtered and diluted in double deionized H_2O to get particle-free samples. The subsequent samples were then analyzed using ICP MS (Aurora M90: BRUKER DALTONICS) equipped with a nebulizer and a spray chamber. Supplementary data 1.9 shows the operating parameters of the instrument. Prior to operation, the instrument was conditioned with multi-element tuning solution at a concentration 5–500 μg/L. For quantitative determination, a three-point calibration was performed using standards in the range 5–50 μg/L, made from a 1000 μg/mL stock solution (INORGANIC VENTURES, Multi-Element Standard). The quality control inspections were performed according to the US EPA SW-846 quality assurance standards (Method 6020B, 2014) to ensure the accuracy of the data.

4. Data analysis

All statistical analysis were carried out at 95% confidence interval, and the assumptions of Normality and Homogeneity of variance were confirmed by Shapiro-Wilk (or) Kolmogorov Smirnov test and Levene's (or) Brown-Forsythe test.

A one way ANOVA followed by Tukey's test was carried out to determine the significant difference in biospeciation ability among different bacterial isolates. Similarly, the significant changes in bacterial growth between the tests groups under different Cr (VI) conditions were statistically evaluated using ANOVA. In case of significant results, a Dunnett's test was carried out to verify the differences between the test and control samples.

The time series plots of bacterial growth were fitted with different nonlinear sigmoidal models, namely, Logistic, Gompertz and Richards (López et al., 2004; Zwietering et al., 1990). Among all three, the S Logistic function Type 1 ($y = a/(1 + e^{-k\ (x-xc)})$) showed best fitting of the experimental data with COD R^2 in the range 0.98–0.99. Similarly, the time series Cr (VI) reduction data was fitted in a linear model ($C_t = -kt + C_0$) with COD R^2 in the range 0.95–0.99. Further, a Wilcoxon test was carried out to evaluate the column Cr (VI) reduction under abiogenic and biogenic controls, while trend analysis was carried out by Mann-Kendall test.

5. Results & discussion

5.1. Soil characteristics

The industrial site in Damarcherala and closely associated region Vadapalle (1.4 Km away from the industrial site) were examined for similar soil characteristics to demonstrate Cr (VI) reactive transport. Supplementary data 1.10 shows the physicochemical properties of S-I from the contaminated industrial site and S-II from uncontaminated region Vadapalle. Table 1 shows the multi-element concentration of samples collected from various locations. The total chromium in samples, viz. S-I, DYH, IS, IE, SW, SED-I and SED-II, describes the degree of chromium contamination with other trace elements. The background multi-element constituents of uncontaminated soil S-II used in Cr (VI) reactive transport are presented as abiogenic controls.

Soil characteristics such as texture, particle size distribution, porosity and permeability (Juarez et al., 2013) significantly influence the biogenic speciation of Cr (VI) by affecting the availabe oxygen, moisture, and nutrients (Chau et al., 2011; Zhou et al., 2002). In this study, the soil was classified as well-graded sand as per the recommendations of ASTM D 2487 and the texture was found to be sandy loam. The particle size distribution (PSD) of S-I and S-II indicate different soil fractions as shown in Supplementary data 1.11. Here, the

Table 1
Multi-element concentration of samples (Mean ± Standard Deviation).

[c]Element	S-I mg/Kg	S-II mg/Kg	DYH mg/Kg	IS mg/Kg	IE mg/L	SW mg/L	SED-I mg/Kg	SED-II mg/Kg
^{53}Cr	[a]175.17 ± 8.25	[b]0.87 ± 0.08	[a]751.94 ± 35.5	[a]16403.45 ± 53.4	[a]1875.14 ± 5.50	[a]187.51 ± 5.16	[a]16.25 ± 5.49	[a]18.30 ± 1.0
^{60} Ni	0.022 ± 0.003	[b]0.31 ± 0.002	1.56 ± 0.22	1.11 ± 0.12	1.16 ± 0.15	1.16 ± 0.01	0.31 ± 0.12	0.94 ± 0.08
^{63}Cu	0.60 ± 0.019	[b]0.020 ± 0.001	0.13 ± 0.01	3.15 ± 0.19	9.39 ± 0.76	0.93 ± 0.008	0.02 ± 0.011	0.67 ± 0.11
^{66}Zn	0.33 ± 0.032	[b]0.16 ± 0.033	1.25 ± 0.33	bdl	29.48 ± 2.26	2.94 ± 0.003	0.11 ± 0.029	0.11 ± 0.03
^{95}Mo	0.09 ± 0.012	[b]0.041 ± 0.009	0.33 ± 0.09	bdl	bdl	bdl	0.002 ± 0.0003	0.28 ± 0.03
^{111}Cd	0.06 ± 0.007	[b]0.016 ± 0.001	0.01 ± 0.004	bdl	bdl	0.001 ± 0.0001	bdl	bdl
^{44}Ca	12.05 ± 1.2	[b]6.98 ± 0.52	28.85 ± 2.02	21.87 ± 0.50	1.2 ± 0.09	0.2 ± 0.0001	0.002 ± 0.0003	0.001 ± 0.0001
^{56}Fe	1.80 ± 0.09	[b]0.44 ± 0.02	2.18 ± 0.23	0.44 ± 0.09	0.5 ± 0.05	0.5 ± 0.036	2.1 ± 0.31	2.91 ± 0.26
^{23}Na	143.65 ± 12.98	[b]75.88 ± 20.65	289.2 ± 45.7	964 ± 1.50	221.67 ± 9.19	109.67 ± 9.2	12.09 ± 4.32	18.25 ± 3.29
^{24}Mg	90.5 ± 9.23	[b]9.64 ± 1.63	57.43 ± 2.63	47.45 ± 2.70	136.8 ± 1.53	76.88 ± 5.01	4.31 ± 0.90	5.19 ± 2.70
^{39}K	12.87 ± 1.7	[b]9.64 ± 1.63	102.6 ± 12.67	145.9 ± 0.1	16.02 ± 1.09	6.02 ± 1.29	1.50 ± 0.27	1.20 ± 0.02
^{5}Mn	1.5 ± 0.2	[b]1.33 ± 0.001	5.53 ± 1.45	1.33 ± 0.002	0.21 ± 0.01	0.1 ± 0.001	0.32 ± 0.02	1.02 ± 0.01
^{27}Al	0.02 ± 0.0012	[b]0.10 ± 0.0137	2.50 ± 0.37	0.01 ± 0.005	0.01 ± 0.001	0.02 ± 0.001	0.05 ± 0.001	0.002 ± 0.001
^{9} Be	0.001 ± 0.002	[b]0.01 ± 0.001	0.08 ± 0.01	bdl	bdl	bdl	0.001 ± 0.0001	0.003 ± 0.001
^{47}Ti	0.57 ± 0.02	[b]3.48 ± 0.12	2.8 ± 0.92	bdl	bdl	bdl	0.0002 ± 0.0001	0.0001 ±
^{9}Co	0.058 ± 0.02	[b]0.13 ± 0.008	0.36 ± 0.08	13.29 ± 0.03	bdl	0.001 ± 0.0003	0.030 ± 0.002	0.01 ± 0.002
^{115}In	0.0007 ± 0.0002	[b]0.007 ± 0.001	0.03 ± 0.001	bdl	bdl	bdl	bdl	bdl
^{118}Sn	0.0128 ± 0.003	[b]0.013 ± 0.001	0.06 ± 0.001	bdl	bdl	bdl	0.071 ± 0.02	0.05 ± 0.03
^{121}Sb	0.011 ± 0.0013	[b]0.011 ± 0.001	0.05 ± 0.01	bdl	bdl	bdl	bdl	bdl
^{137}Ba	1.382 ± 0.09	[b]1.050 ± 0.34	6.91 ± 0.34	0.07 ± 0.005	31.11 ± 0.512	3.11 ± 0.05	2.03 ± 0.34	9.12 ± 0.73
^{140}Ce	0.0029 ± 0.0001	[b]0.039 ± 0.004	0.14 ± 0.01	bdl	bdl	bdl	0.02 ± 0.01	0.01 ± 0.004
^{205}Tl	0.0047 ± 0.0001	[b]0.008 ± 0.0002	0.04 ± 0.004	bdl	nd	bdl	bdl	0.007 ± 0.0001
^{208} Pb	0.0457 ± 0.002	[b]0.043 ± 0.009	0.22 ± 0.049	bdl	bdl	0.01 ± 0.004	0.69 ± 0.04	0.99 ± 0.006
^{232}Th	0.012 ± 0.003	[b]0.010 ± 0.001	0.05 ± 0.012	bdl	bdl	bdl	0.05 ± 0.01	0.35 ± 0.021

The data represents a triplicate analysis of homogenized grab samples.
[a] Denotes the level of chromium contamination i.e. chromium concentration exceeding the desirable limit as per ISI-IS: 2296–1982 and US EPA guidelines.
[b] Denotes the background multi-elemental concentration of uncontaminated soil (S-II) used for microcosm construction; bdl denotes below detection level.
[c] Denotes multi-elements based on relative isotopic abundance as determined by ICP MS.

different soil fractions indicate different microhabitat for soil microbes that varies in surface properties and associated organics for contaminant sorption and microbial enzymatic activity (Hemkemeyer et al., 2018). Besides, fine-grained fractions of clay and fine silts indicate a considerable interaction with diverse microbial communities than larger coarser particles (Sessitsch et al., 2001; Zhang et al., 2007). In this study, the soil texture and PSD are suggestive of porosity of 45.5%- 48.2% with parameters such as bulk density (1.35 g/cm^3 - 1.42 g/cm^3) and particle density (2.61 g/cm^3). Here, soil porosity indicate adequate pore-space to retain the biogenic agents for enhanced speciation of Cr (VI). These factors are certainly a major constrain in which the inherent bacteria encounters spatial distribution.

In general, pH neutral is referred as optimal pH for soil microbial activities (Reible and Lanczos, 2006), as most geomicrobes are quite susceptible to rapid changes in soil pH. In this study, the pH of the collected soils (S-I & S-II) was found to be weakly acidic to neutral (6.9 ± 0.05 to 7.1 ± 0.05) which appears to be a favorable condition for biogenic speciation of Cr (VI). Also, soil pH determines the solubility and speciation of Cr (VI) based on its interaction with charged colloids. This condition indicates the availability of Cr (VI) as CrO_4^{2-} (at pH greater than 6.4) and $Cr_2O_7^{2-}$ (at pH between 6 and 4). As the acidity increases, Cr (VI) tends to dissociate as $HCrO_4^-$ (pH below 6.4) and H_2CrO_4 (pH below 1) (Dhal et al., 2013; Richard and Bourg, 1991; Stanin, 2005; Wang, 2017). These forms of Cr (VI) contaminants have high mobility and are very unstable compared to Cr (III) in soil environments.

The organic and H_2O content of the collected soils (S-I & S-II) was found to be in the range 1.25 ± 0.65 to 3.4 ± 0.89% and 12.8 ± 0.85 to 14.5 ± 0.51%. The observed values of S-I indicates a low organic condition for enhanced speciation of Cr (VI). Although the implied condition appears less suitable, the reduction of high concentrations of Cr (VI) to Cr (III) is still favorable under weakly acidic pH with adequate minerals and soil microflora (Arnfalk et al., 1996; Eary and Rai, 1991; Stollenwerk and Grove, 1985). However, the process specifies a substantial amount of external biogenic agents and organic nutrients for a successful containment treatment.

5.2. Soil sorption

Sorption plays a crucial role in fixing chemical constituents and other elements. Table 2 shows the sorption parameters of S-I and S-II to determine the soil's ability to retain the contaminant Cr (VI) and micronutrient Cr (III). In this study, the sorption phenomena is described well by Freundlich isotherm in which the linear regression nearly approached unity. The obtained data were evaluated, and the correlation between the model and the dependent variable is statistically significant at $p < 0.05$. The derived values indicate that the retention of Cr (III) to S-I and S-II is quite significant compared to Cr (VI). Here, the

Table 2
Soil Sorption parameters derived by Freundlich isotherm.

Sorption	Freundlich Equation: $\log(q_e) = \log K_f + \frac{1}{n}\log C_{eq}$	Pearson's R	Adjacent R	COD R^2	K_f, mL/g	1/n
S-I - Cr (VI)	y = 0.746x-1.5422	0.994	0.986	0.988	0.028	0.746
S-II - Cr (VI)	y = 0.827x-1.7922	0.994	0.987	0.989	0.016	0.827
S-I - Cr (III)	y = 0.401x+0.3266	0.992	0.982	0.983	2.119	0.401
S-II - Cr (III)	y = 0.413x+0.2666	0.988	0.973	0.983	1.847	0.413

Table 3
Inherent bacterial Isolates and their characteristics.

Sample	(i). Isolates on Cr (VI) enriched M1 and M2 media		(ii). Cr tolerance of isolates & its exoenzyme speciation ability		
	No. of Isolates based on colony morphology	[a] Bacterial population CFUs/g. dry weight of sample	[c]Cr (VI) Concentration, mg/L	[b] Bacterial population CFUs/g. dry weight of sample	Preeminent exoenzyme Cr (VI) speciation ability
S-I^{M1}	5	5.8×10^6	400	5.2×10^5	–
S-I^{M2}	12	5.1×10^7	250	5.6×10^6	–
IS^{M1}	6	2.1×10^5	3500	4.6×10^3	+
IS^{M2}	8	1.5×10^5	3500	3.1×10^3	+
IE^{M1}	4	4.6×10^5	1500	2.9×10^4	–
IE^{M2}	5	3.6×10^5	900	5.4×10^4	–

^{M1} and ^{M2} denotes the type of media enriched with 150 mg/L Cr (VI).
[a] Denotes bacterial population on media enriched with 150 mg/L Cr (VI).
[b] Denotes bacterial population on media enriched with maximum tolerance concentration.
[c] Denotes maximum Cr (VI) concentration at which the isolates can survive.

Cr (VI) is presumed to be mobile due to its high solubility whereas Cr (III) is likely to appear as inert precipitates in slightly acidic to neutral pH. This characteristic nature of S-I and S-II is critical in containment treatment for adequate availability of Cr (VI) and to retain the Cr (III) in soil environment (Choppala et al., 2018, 2013).

5.3. Inherent biogenic agents

5.3.1. Geomicroflora

In this study, the geomicrobial population of contaminated soil S-I is observed as 4.5×10^6 CFU/g and 5.3×10^7 CFU/g, in M1 and M2 media, respectively. Supplementary data 1.12 shows the Cr (VI)-tolerant colonies isolated from S-I that differ in their morphological characteristics. These isolated strains are primarily associated with medium to a finer fraction of contaminated soil.

5.3.2. Isolates and chromium tolerance

Table 3 (i) shows the total count of isolates from different samples when grown on chromium enriched media. Here, the morphological variants grown in oligotrophic media appeared less in contrast with variants grown in less fastidious media. This shows the ability of isolates to survive in oligotrophic condition and facilitate the selection of nutrient-stressed bacteria for soil biogenic experiments (Olsen and Bakken, 1987; Reasoner and Geldreich, 1985). Besides, the Cr (VI)-tolerant population (CFUs) in samples appeared in decreasing order: S–I isolates > IE isolates > IS isolates, due to different background level of Cr (Kamaludeen et al., 2003).

Table 3 (ii) shows the chromium tolerance level of isolates and their characteristics when exposed to high *in vitro* concentration of Cr (VI). The isolates from IE and IS showed high-level tolerance compared to the geomicrobial population as a result of their prolonged exposure to Cr contaminant, by indicating their potential to survive under severe chromium stress. Here, the stress-strain response induced by chromate resistance gene tends to combat their survival in field condition (Emenike et al., 2017; Kamaludeen et al., 2003). In this study, the bacterial isolates from highly contaminated samples are ideal biogenic agents because of their tendency to survive under the influence of contaminant (Batool et al., 2014; Emenike et al., 2016).

5.3.3. Biospeciation ability of isolates

The ability of bacteria in reduction of Cr (VI) is explained by the biospeciation test conducted on different group of isolates. Fig. 1 shows the stacked data of cell cytoplasmic, intracellular and extracellular enzymatic reduction of Cr (VI) by different group of isolates at various time scales. A recent study demonstrated by Li et al. (2019) described alike speciation of Cr (VI) in which one or two possible events such as sorption, membrane-bound and metabolic mechanism (Fernández et al., 2018; Jobby et al., 2018; Kamaludeen et al., 2003) are likely to participate in reduction of toxic Cr (VI). In this study, the mechanism of Cr (VI) reduction is illustrated to differentiate the predominance of exo-enzymatic speciation as shown by Chen et al. (2012). On statistical assessment, a significant difference in the outcome of cell-bound [$F(2, 21) = 11.14$, $p = 0.0005$], intracellularly [$F(2, 21) = 8.879$, $p = 0.001$] and extracellularly [$F(2, 21) = 11.56$, $p = 0.0004$] reduced Cr concentrations were observed between each test groups as determined by one way ANOVA. In addition, a Tukey post hoc test revealed that exo-enzymatically reduced Cr (III) concentrations by IS isolates (Mean, M = 57.43, Standard deviation, SD = 36.15) significantly varied on comparison with IE isolates (M = 26.52, SD = 18.23) at $p = 0.038$ and S-I isolates (M = 1.334, SD = 0.7007) at $p = 0.0002$. And no statistically significant difference is observed in exo-enzymatically reduced Cr (III) concentrations between S-I and IE isolates ($p = 0.12$).

The bacterial detoxification of Cr (VI) is known to undergo one or various mechanisms such as biosorption, bioaccumulation, and biospeciation to reduce the contaminant concentration (Fernández et al., 2018). In this study, the bacterial Cr (VI) uptake remains speculative (Kamaludeen et al., 2003) but can be suggestive of biospeciation due to the formation of impermeable Cr (III) precipitates (Supplementary data 1.13). Here, the bio-reduced Cr (III) precipitates is presumably expected to form more stable organo-Cr (III) complexes, as demonstrated by Gong et al. (2018). These stable forms of Cr (III) are not easily reoxidized to Cr (VI), which makes bioaugmentation more stable than other chemically mediated remediation.

Although the reduction of Cr (VI) by different isolates seemed agreeable, harnessing an entire group of isolates for complete detoxification may not be effective in field-scale applications. In some instances, the bioaccumulation tendency of isolates can cause cells to accumulate the unstable oxidative toxic intermediates (i.e. Cr (V) & Cr (IV)) which can lead to severe cell death. Although some cells try to combat these effects by altered metabolic pathways and efflux mechanisms (Fernández et al., 2018; Jobby et al., 2018; Kamaludeen et al., 2003), the detoxification rate seems affected due to reduced uptake of Cr (VI). Other cells involving biosorption can also lead to indistinguishable fate due to blockage of active sites for Cr (VI) uptake (Kamaludeen et al., 2003). Therefore, the exoenzyme isolates are preferred as desirable biogenic controls for bioaugmentation to reduce high concentrations of Cr (VI) in soil.

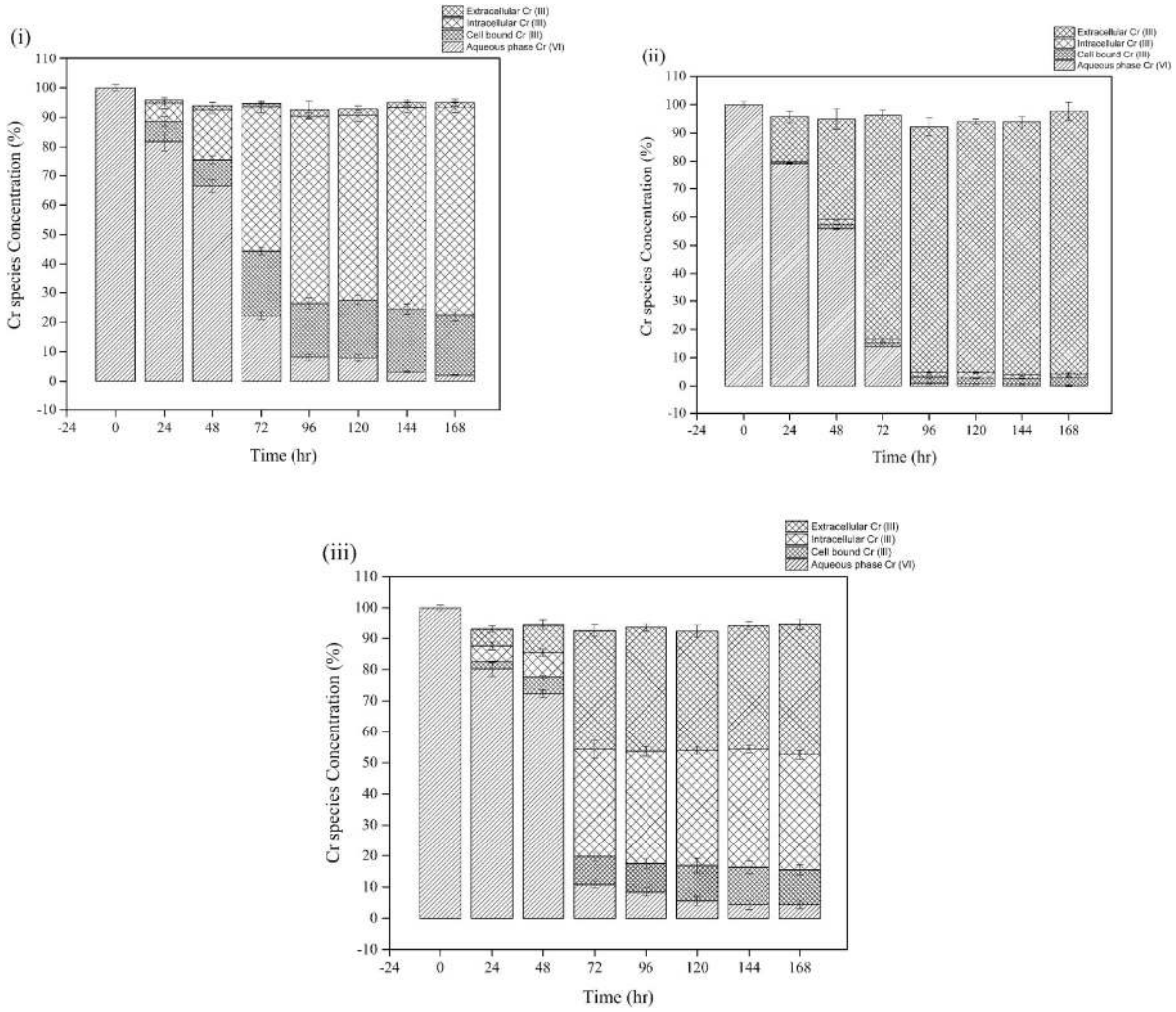

Fig. 1. Biospeciation of Cr (VI) to Cr (III) by a different group of bacterial isolates over a period of time: (i). Cr speciation ability of S-I isolates, (ii) Cr speciation ability of IS isolates and (iii) Cr speciation ability of IE isolates: Error bars depicts the standard error of the mean (n = 3).

5.4. Biokinetic parameters for enhanced biogenic speciation

The growth curve of IS bacterial isolates was studied under *in vitro* condition to determine the specific growth rate to reduce Cr (VI) concentration to 0.05 mg/L within the stipulated length of time. Fig. 2 shows the bacterial growth profile under various initial concentrations of Cr (VI) in a chemically defined nutrient media (M4) and concurrent Cr (VI) reduction kinetics. In Fig. 2 (i) and (ii), the bacterial isolates grown with and without Cr (VI) concentrations exhibited a statistically significant growth at $p < 0.05$. The significant difference in growth pattern ($p < 0.001$) is mainly induced by the initial concentrations of Cr (VI) as a result of metal-mineral-microbial interaction as described by He et al. (2019) and Zhang et al. (2019). The control experiments in the absence of IS isolates showed poor Cr (VI) reduction of about 3.5% and its outcome compared to biogenic Cr (VI) reduction is significant at $p < 0.0001$. In this study, all biogenic Cr (VI) reduction experiments are conducted under anaerobic condition in which Cr (VI) acts as terminal electron acceptor and carbon in M4 media acts as electron donor (Shen and Wang, 1993; Wang et al., 1989). The type of oxygen demand (i.e. aerobic/anaerobic) during the process does not always assure higher Cr (VI) reduction rates since the reaction is mostly dependent on the type of inoculum (bacterial species), its density and available electron acceptors & donors (Wang and Xiao, 1995; Zeng et al., 2019).

Table 4 shows the bacterial growth parameters, Cr (VI) reduction rate (k), and its half-life period. In this study, growth rate of bacteria is affected by a high initial concentration of Cr (VI) concentration ($p < 0.05$) but does not lower the overall reduction except for a delay in the reaction rate. It was found that an increase in concentration of bacteria (inoculum density) contributes to a higher Cr (VI) reduction rate, while higher specific growth rate (μ) is observed at much lower bacterial concentration. This effect of initial inoculum density is also observed by He et al. (2011), Song et al. (2009) and Wang & Xiao. (1995) which is contrary to the study shown by Zhu et al. (2008). In some cases, the increase in inoculum density does not necessarily increase the reduction rate as the reaction kinetics collectively depend on available carbon sources, O_2 and Cr (VI) uptake mechanism (Kamaludeen et al., 2003; Pattanapipitpaisal et al., 2001; Pal and Paul, 2004).

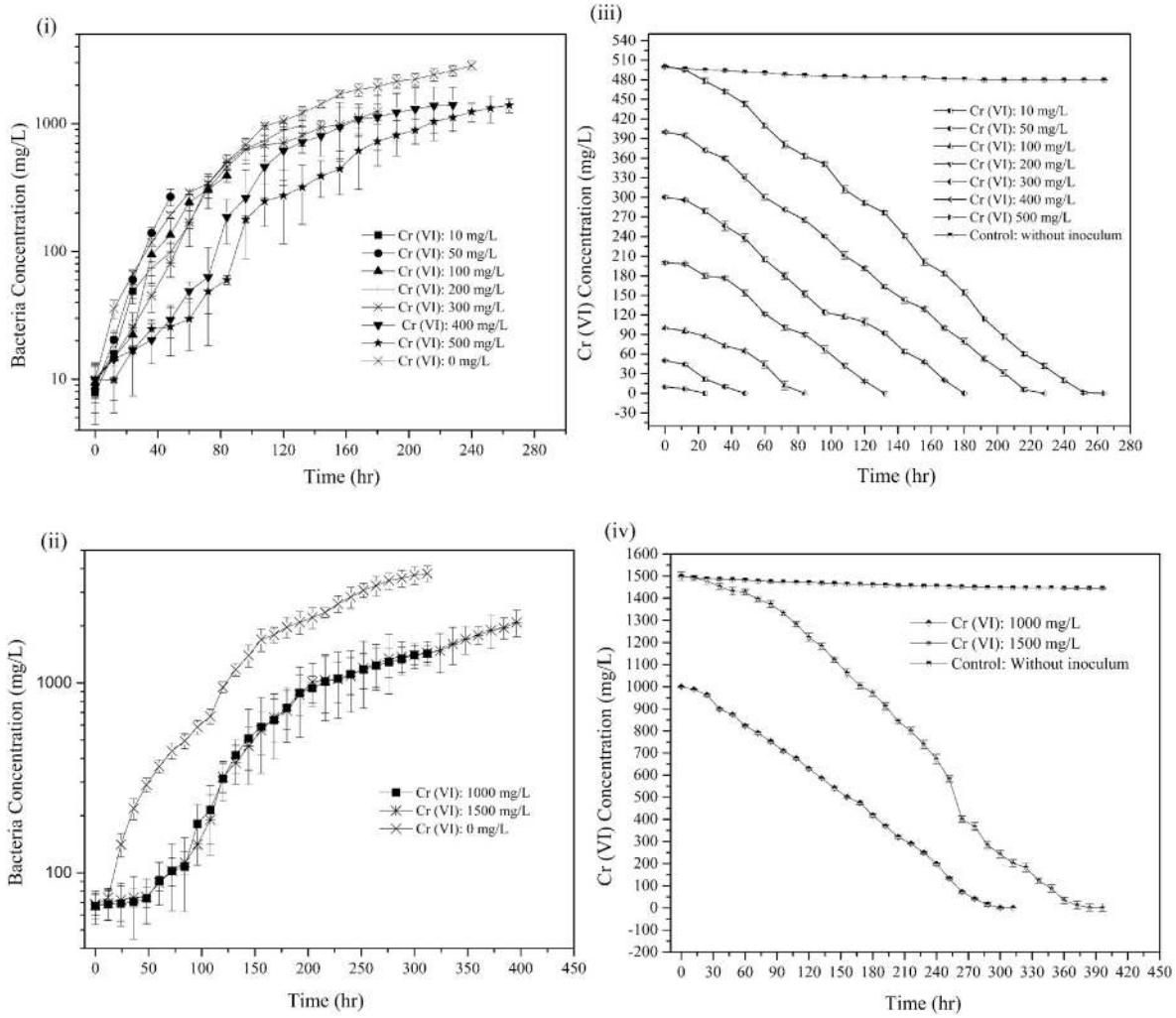

Fig. 2. Bacterial growth characteristics and concurrent Cr (VI) reduction kinetics: (i) and (ii) depicts the growth curve of IS bacterial isolates initiated at an average inoculum concentration of 9.24 mg/L and 67.67 mg/L, respectively; (iii) and (iv) depicts its concurrent Cr (VI) reduction for conditions shown in (i) and (ii), respectively. Error bars depicts the standard error of the mean (n = 3).

5.5. Cr (VI) reduction in soil microcosm

Fig. 3 shows the influence of abiogenic and biogenic control of Cr (VI) along the depth of a soil column under saturated and unsaturated flow conditions. The inlet concentration of Cr (VI) to the soil column was about 200 mg/L, operated at a continuous flow rate of 5 mL/min (for saturated flow) and 4.6 mL/min (for unsaturated flow) with abiogenic (sterile soil S-II) and biogenic (soil S-II amended with bacterial inoculum) controls. The transport of the Cr (VI) stream to the soil column was stopped when the system reached a steady-state condition.

In Fig. 3 (i) and (ii), the abiogenic experiments showed a slight reduction of Cr (VI) of about 6.9 ± 0.88% (for saturated flow) and 6.06 ± 0.615% (for unsaturated flow), within 24 h of reaction period, after which the system almost reached a steady-state with no further significant decrease in outlet concentration of Cr (VI) at a depth 50 cm of the soil column. Here, the minimal decrease of Cr (VI) at an initial stage before the column breakthrough, is attributed to soil sorption (described in section 2.2) and other elemental interaction (Choppala et al., 2013; Kamaludeen et al., 2003), with no statistically significant difference ($Z = 1.643$, $p = 0.10$) observed in Cr (VI) reduction between two different flow conditions. On the other hand, the biogenic experiments (Fig. 3 (iii) and (iv)) showed a substantial reduction of Cr (VI) of about 99.99 ± 0.4% (for saturated flow) and 99.93 ± 0.75% (for unsaturated flow) at a depth 50 cm of the soil column, within 144 h and 192 h of reaction period, respectively. A significant difference ($Z = 3.020$, $p < 0.003$) was observed between saturated and unsaturated conditioned flows during the reduction of Cr (VI). Here, a maximal decrease in Cr (VI) concentration is attributed to biogenic control which together includes sorption by soil-bacterial extracellular polymeric substance (EPS) (Gadd, 2004; Kantar et al., 2011; Yang et al., 2013) & enzymatic reduction (Fernández et al., 2018).

At first, the inlet concentration of Cr (VI) in biogenic experiments (Fig. 3 (iii) and (iv)) undergoes an initial breakthrough within the reaction period of 24 h (for saturated column) and 36 h (for unsaturated column), with significant Cr (VI) reduction of about 62.64 ± 1.52% and 58.87 ± 1.95%, respectively. The breakthrough at this point is due to interactions of soil-bacterial EPS that causes sorption of Cr (VI), while at this stage the bacterial growth is not prominent to

Table 4
Bacterial growth rates and Cr (VI) reduction rate constants in chemically defined minimal nutrient media M4.

Initial Cr (VI) Concentration, mg/L	S logistic growth model fit equation	[d]R^2, COD	Exponential growth rate equation	Specific growth rate, μ, hr^{-1}	[a]R^2, COD	Doubling, T_d, hr	Cr (VI) reduction rate equation	Cr (VI) reduction Rate constant, k	[b]R^2, COD	[c]Pearson's r	Half-life of Cr (VI), $t_{1/2}$, hr	Time taken to reduce Cr (VI), below 0.05 mg/L, hr
10	$y = 48.85/(1+e^{-0.076(x-21.71)})$[e]	nv	$y = 7.36696e^{0.0758x}$	0.0758	0.982	8.48	$y = 10.667–0.417\times$	0.417	0.950	−0.975	11.991	24
50	$y = 515.23/(1+e^{-0.089(x-47.12)})$	0.999	$y = 11.443e^{0.0667x}$	0.0667	0.988	9.64	$y = 52.055–1.116\times$	1.116	0.973	−0.987	22.402	48
100	$y = 469.63/(1+e^{-0.066(x-60.49)})$	0.994	$y = 9.9883e^{0.0484x}$	0.0484	0.951	13.28	$y = 111.93–1.248\times$	1.248	0.943	−0.971	40.065	84
200	$Y = 1056.99/(1+e^{-0.052(x-88.31)})$	0.997	$y = 24.408e^{0.0324x}$	0.0324	0.966	19.84	$y = 218.99–1.615\times$	1.615	0.984	−0.992	61.92	132
300	$y = 1235.89/(1+e^{-0.035(x-106.14)})$	0.980	$y = 20.548e^{0.0272x}$	0.0272	0.883	23.63	$y = 310.19–1.724\times$	1.724	0.992	−0.996	87.007	180
400	$y = 1412.82/(1+e^{-0.036(x-134.04)})$	0.995	$y = 14.32e^{0.0242x}$	0.0242	0.910	26.57	$y = 415.46–1.868\times$	1.868	0.998	−0.999	107.067	228
500	$y = 1636.31/(1+e^{-0.024(x-192.75)})$	0.997	$y = 19.255e^{0.0184x}$	0.0184	0.901	34.94	$y = 532.23–2.099\times$	2.099	0.994	−0.997	119.105	264
1000	$y = 1462.74/(1+e^{-0.022(x-178.90)})$	0.996	$y = 60.371e^{0.0121x}$	0.0121	0.904	53.14	$y = 1036.52–3.483\times$	3.483	0.997	−0.999	143.555	312
1500	$y = 2070.37/(1+e^{-0.015(x-231.73)})$	0.983	$y = 79.678e^{0.0096x}$	0.0096	0.888	66.97	$y = 1684.69–4.465\times$	4.465	0.977	−0.989	167.974	396

Mean values are considered for determination of growth rate and rate constants.
nv denotes not validated.
c Denotes Pearson's correlation coefficient for Cr (VI) reduction rates.
[a] Denotes Statistical fit value for linear correlation of specific growth rate.
[b] Denotes Statistical fit value for linear correlation of Cr (VI) reduction data.
[d] Goodness of fit for nonlinear sigmoidal logistic function with experimental growth data.
[e] Data do not contain enough information for fit analysis.Data do not contain enough information for fit analysis.

enzymatically reduce the concentration of Cr (VI). Eventually, after an acclimatization period of about 72 h (for saturated column) and 60 h (for unsaturated column), the effect of bacterial growth becomes significant in reactive transport of Cr (VI) to achieve desired concentration ≤ 0.1 mg/L.

In saturated flow (Fig. 3 (iii)), a maximal decrease in Cr (VI) concentration by 0.03 ± 0.001 mg/L was achieved at the outlet after 144 h, while in unsaturated flow (Fig. 3 (iv)), a maximal decrease in Cr (VI) concentration by 0.1 ± 0.004 mg/L was achieved after 192 h. This shows the effect of moisture dynamics on biogeochemical reduction rates ($p \leq 0.003$) through which the distribution of bacteria is largely affected (Yan et al., 2017; Maggi and Porporato, 2007; Karthick et al., 2019). It is also noted that a uniform saturated flow promotes an adequate distribution of bacteria which implies a faster biogenic reduction in a saturated column compared to unsaturated column (Yan et al., 2017). Therefore, it is clear that the level of liquid saturation in the soil column affects bacterial activity in the Cr (VI) reactive transport to control and mitigate the toxicity of chromium in the underground environment.

For a biogenic reaction period of 144 h under saturated condition, the observed decrease in Cr (VI) concentration at a depth: 10–20 cm is 20.32 ± 2.71%; 20–30 cm is 37.74 ± 3.67%; 30–40 cm is 61.74 ± 6.48% and 40–50 cm is 99.91 ± 0.01, while for a biogenic reaction period of 192 h under unsaturated condition, the observed decrease in Cr (VI) concentration at a depth: 10–20 cm is 40.79 ± 1.83%; 20–30 cm is 58.60 ± 3.32; 30–40 cm is 81.27 ± 2.46 and 40–50 cm is 98.31 ± 1.20, which is statistically significant at $p < 0.05$. Here, the gradual decrease in Cr (VI) concentrations along the depth of the soil column is explained by continuous fluid-flow and stress-strain response which causes the bacteria to be contained at the lower region of the soil column. That is why a significant decrease in concentration of Cr (VI) is observed in the lower region (at a depth of 30–50 cm) of the soil column than the upper region (at a depth of 10–30 cm).

Supplementary data 1.14 shows Mann-Kendell trend analysis for Cr (VI) reduction data along the depth of soil column. The columns with biogenic control showed a significant monotonic decreasing trend of Cr (VI) concentration over time (Negative Kendall tau, $p < 0.05$) when compared to the columns with abiogenic controls which exhibited no trend ($p \geq 0.08$). In an overall, while comparing the effects of the abiogenic and biogenic controls, it was noted that the downward trend in Cr (VI) concentration is attributed to biogenic controls, which are significant at $p \leq 0.003$ during the reactive transport.

6. Conclusion

In this study, we analyzed the biogeogenic characteristics of an intensely contaminated site and demonstrated the possibility of enhanced natural attenuation by bioaugmentation. The described biogeogenic conditions specified the favorable conditions for biogenic speciation of the Cr (VI) to control their underground toxicity. In addition, the effectiveness of biogenic control over the abiogenic control proves the significance of inherent bacteria in soil containment treatment. Furthermore, on biokinetic assessment, it was understood that, under stipulated conditions of inoculum concentration and its doubling time, the high levels of Cr (VI) could be reduced to a desirable legal limit stated by Indian Standard IS 10500 guidelines. This study significantly considered the increased inoculum concentration and additional minimal nutrient requirements for exercising enhanced biogenic speciation. Both abiogenic and biogenic controls incorporated in soils helps to understand its positive influence in attenuating the toxic effects of Cr (VI) under different conditions of fluid flow. Despite the variable level of fluid saturation (Saturated & unsaturated soil column), the biogenic controls are successfully incorporated in subsoil containment to reduce the Cr (VI) concentration below 0.1 mg/L.

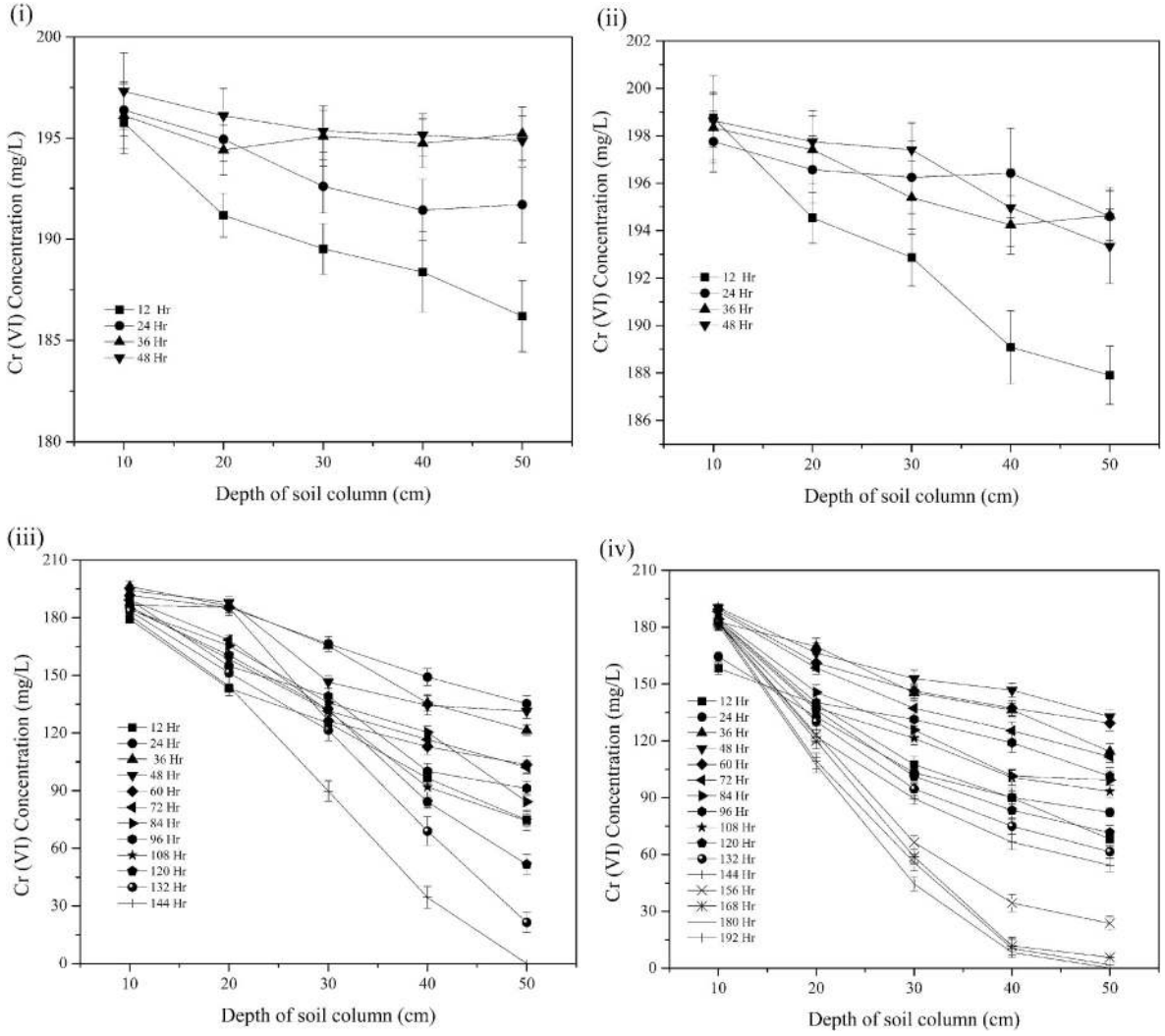

Fig. 3. Influence of abiogenic and biogenic controls on Cr (VI) concentration along the depth of soil column at different time scale: (i) Influence of abiogenic control on Cr (VI) transport under saturated flow condition; (ii) Influence of abiogenic control on Cr (VI) transport under unsaturated flow condition; (iii) Influence of biogenic control on Cr (VI) reductive transport under saturated condition; and (iv) Influence of biogenic control on Cr (VI) reductive transport under unsaturated condition. Error bars depicts the standard error of the mean (n = 3).

Declaration of competing interest

None.

CRediT authorship contribution statement

Sangeetha CJ: Conceptualization, Methodology, Investigation, Writing - original draft, Visualization, Formal analysis. **Shashidhar T:** Project administration, Conceptualization, Methodology, Funding acquisition, Supervision, Investigation, Writing - review & editing, Visualization, Formal analysis, Data curation.

Acknowledgement:

The authors gratefully acknowledge The Department of Science and Technology (DST, Government of India) and The Ministry of Human Resources Development (MHRD, Government of India) for their support. This work was funded by Science and Engineering Research Board (SERB-DST) for fast project titled “Design of Permeable Bio-Barriers to Contain Cr (VI) in a Contaminated Aquifers” with Grant No. DST SR/FTP/ETA- 116/2010.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.110327.

References

Adriano, D.C., Wenzel, W.W., Vangronsveld, J., Bolan, N.S., 2004. Role of assisted natural remediation in environmental cleanup. Geoderma 122, 121–142. https://doi.org/10.1016/j.geoderma.2004.01.003.

Arnfalk, P., Wasay, S.A., Tokunaga, S., 1996. A comparative study of Cd, Cr(III), Cr(VI), Hg, and Pb uptake by minerals and soil materials. Water, Air, Soil Pollut. 87, 131–148. https://doi.org/10.1007/BF00696833.

Atlas, R.M., 2010. Handbook of Microbiological Media. ASM Press.

ASTM D422-63(2007)e1, 2007. Standard Test Method for Particle-Size Analysis of Soils. ASTM International, West Conshohocken, PA. https://doi.org/10.1520/D0422-63R07E01.

ASTM D2216-10, 2010. Standard Test Methods for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass. ASTM International, West Conshohocken, PA. https://doi.org/10.1520/D2216-10.

ASTM D2974-14, 2014. Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils. ASTM International, West Conshohocken, PA. https://doi.org/10.1520/D2974-14.

ASTM D2937-10, 2010. Standard Test Method for Density of Soil in Place by the Drive-Cylinder Method. ASTM International, West Conshohocken, PA. https://doi.org/10.1520/D2937-10.

ASTM D854-00, 2000. Standard Test Methods for Specific Gravity of Soil Solids by Water

Pycnometer. ASTM International, West Conshohocken, PA. https://doi.org/10.1520/D0854-00.
Azubuike, C.C., Chikere, C.B., Okpokwasili, G.C., 2016. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J. Microbiol. Biotechnol. 32, 180. https://doi.org/10.1007/s11274-016-2137-x.
Ballesteros, S., Rincón, J.M., Rincón-Mora, B., Jordán, M.M., 2017. Vitrification of urban soil contamination by hexavalent chromium. J. Geochem. Explor. 174, 132–139. https://doi.org/10.1016/j.gexplo.2016.07.011.
Bansal, N., Coetzee, J.J., Chirwa, E.M.N., 2019. In situ bioremediation of hexavalent chromium in presence of iron by dried sludge bacteria exposed to high chromium concentration. Ecotoxicol. Environ. Saf. 172, 281–289. https://doi.org/10.1016/j.ecoenv.2019.01.094.
Barnie, S., Zhang, J., Wang, H., Yin, H., Chen, H., 2018. The influence of pH, co-existing ions, ionic strength, and temperature on the adsorption and reduction of hexavalent chromium by undissolved humic acid. Chemosphere 212, 209–218. https://doi.org/10.1016/j.chemosphere.2018.08.067.
Batool, R., Hasnain, S., Batool, R., Yrjälä, K., 2014. Impact of environmental stress on biochemical parameters of bacteria reducing chromium. Braz. J. Microbiol. 45, 573–583. https://doi.org/10.1590/S1517-83822014000200028.
Batool, R., Yrjälä, K., Hasnain, S., 2015. Study on cellular changes and potential endotrophy of wheat roots due to colonization of Chromium reducing bacteria. Int. J. Environ. Sci. Technol. https://doi.org/10.1007/s13762-015-0757-6.
Batool, R., Yrjälä, K., Hasnain, S., 2012. Hexavalent chromium reduction by bacteria from tannery effluent. J. Microbiol. Biotechnol. https://doi.org/10.4014/jmb.1108.08029.
Bianco Prevot, A., Ginepro, M., Peracaciolo, E., Zelano, V., De Luca, D.A., 2018. Chemical vs bio-mediated reduction of hexavalent chromium. An in-vitro study for soil and deep waters remediation. Geoderma 312, 17–23. https://doi.org/10.1016/j.geoderma.2017.09.032.
Caravanos, J., Gualtero, S., Dowling, R., Ericson, B., Keith, J., Hanrahan, D., Fuller, R., 2014a. A simplified risk-ranking system for prioritizing toxic pollution sites in low- and middle-income countries. Ann. Glob. Health 80, 278. https://doi.org/10.1016/j.aogh.2014.09.001.
Caravanos, J., Gutierrez, L.H., Ericson, B., Fuller, R., 2014b. A comparison of burden of disease from toxic waste sites with other recognized public health threats in India, Indonesia and the Philippines. J. Health Pollut. 4, 2–13. https://doi.org/10.5696/2156-9614-4-7.2.
Chatham Stephens, K., Caravanos, J., Ericson, B., Sunga-Amparo, J., Susilorini, B., Sharma, P., Landrigan, P.J., Fuller, R., 2013. Burden of disease from toxic waste sites in India, Indonesia, and the Philippines in 2010. Environ. Health Perspect. 121, 791–796. https://doi.org/10.1289/ehp.1206127.
Chau, J.F., Bagtzoglou, A.C., Willig, M.R., 2011. The effect of soil texture on richness and diversity of bacterial communities. Environ. Forensics 12, 333–341. https://doi.org/10.1080/15275922.2011.622348.
Chen, F., Li, X., Zhu, Q., Ma, J., Hou, H., Zhang, S., 2019. Bioremediation of petroleum-contaminated soil enhanced by aged refuse. Chemosphere 222, 98–105. https://doi.org/10.1016/J.CHEMOSPHERE.2019.01.122.
Chen, Z., Huang, Z., Cheng, Y., Pan, D., Pan, X., Yu, M., Pan, Z., Lin, Z., Guan, X., Wu, Z., 2012. Cr(VI) uptake mechanism of Bacillus cereus. Chemosphere 87, 211–216. https://doi.org/10.1016/j.chemosphere.2011.12.050.
Choppala, G., Bolan, N., Lamb, D., Kunhikrishnan, A., 2013. Comparative sorption and mobility of Cr(III) and Cr(VI) species in a range of soils: implications to bioavailability. Water Air Soil Pollut. 224, 1–12. https://doi.org/10.1007/s11270-013-1699-6.
Choppala, G., Kunhikrishnan, A., Seshadri, B., Park, J.H., Bush, R., Bolan, N., 2018. Comparative sorption of chromium species as influenced by pH, surface charge and organic matter content in contaminated soils. J. Geochem. Explor. 184, 255–260. https://doi.org/10.1016/J.GEXPLO.2016.07.012.
Chromium in Drinking-Water Background Document for Development of WHO Guidelines for Drinking-Water Quality.
CGWB, 2016. Ministry of Water Resources, River Development and Ganga Rejuvenation, Government of India, States/Districts from where Chemical Constituents in Ground Water beyond Bureau of Indian Standards (BIS). New Delhi.
Dhal, B., Thatoi, H.N., Das, N.N., Pandey, B.D., 2013. Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: a review. J. Hazard Mater. 250–251, 272–291. https://doi.org/10.1016/j.jhazmat.2013.01.048.
Dowling, R., Caravanos, J., Grigsby, P., Rivera, A., Ericson, B., Amoyaw-Osei, Y., Akuffo, B., Fuller, R., 2017. Estimating the prevalence of toxic waste sites in low- and middle-income countries. Ann. Glob. Health 82, 700. https://doi.org/10.1016/j.aogh.2016.07.008.
Dzionek, A., Wojcieszyńska, D., Guzik, U., 2016. Natural carriers in bioremediation: a review. Electron. J. Biotechnol. 23, 28–36. https://doi.org/10.1016/j.ejbt.2016.07.003.
Eary, L.E., Rai, D., 1991. Chromate reduction by subsurface soils under acidic conditions. Soil Sci. Soc. Am. J. 55, 676. https://doi.org/10.2136/sssaj1991.03615995005500030007x.
Emenike, C.U., Agamuthu, P., Fauziah, S., 2017. Sustainable remediation of heavy metal polluted soil: a biotechnical interaction with selected bacteria species. J. Geochem. Explor. 182, 275–278. https://doi.org/10.1016/J.GEXPLO.2016.10.002.
Emenike, C.U., Agamuthu, P., Fauziah, S.H., 2016. Blending Bacillus sp., Lysinibacillus sp. and Rhodococcus sp. for optimal reduction of heavy metals in leachate contaminated soil. Environ. Earth Sci. 75, 26. https://doi.org/10.1007/s12665-015-4805-9.
EPA, 2016. For underground storage tank sites A guide for corrective action plan reviewers. How to Eval. Altern. Cleanup Technol. Undergr. Storage Tank Sites.
Ericson, B., Caravanos, J., Chatham Stephens, K., Landrigan, P., Fuller, R., 2013. Approaches to systematic assessment of environmental exposures posed at hazardous waste sites in the developing world: the Toxic Sites Identification Program. Environ. Monit. Assess. 185, 1755–1766. https://doi.org/10.1007/s10661-012-2665-2.
Fernández, P.M., Viñarta, S.C., Bernal, A.R., Cruz, E.L., Figueroa, L.I.C., 2018. Bioremediation strategies for chromium removal: current research, scale-up approach and future perspectives. Chemosphere 208, 139–148. https://doi.org/10.1016/J.CHEMOSPHERE.2018.05.166.
Freundlich, H., 1906. Over the adsorption in solution. J. Phys. Chem. 57, 1100e1107.
Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122, 109–119. https://doi.org/10.1016/j.geoderma.2004.01.002.
Gomes, H.I., Dias-Ferreira, C., Ribeiro, A.B., 2013. Overview of in situ and ex situ remediation technologies for PCB-contaminated soils and sediments and obstacles for full-scale application. Sci. Total Environ. 445–446, 237–260. https://doi.org/10.1016/j.scitotenv.2012.11.098.
Gong, Y., Werth, C.J., He, Y., Su, Y., Zhang, Y., Zhou, X., 2018. Intracellular versus extracellular accumulation of Hexavalent chromium reduction products by Geobacter sulfurreducens PCA. Environ. Pollut. 240, 485–492. https://doi.org/10.1016/j.envpol.2018.04.046.
Groudev, S., Georgiev, P., Spasova, I., Nicolova, M., 2014. Decreasing the contamination and toxicity of a heavily contaminated soil by in situ bioremediation. J. Geochem. Explor. 144, 374–379. https://doi.org/10.1016/J.GEXPLO.2014.01.017.
Hamdan, S.S., El-Naas, M.H., 2014. Characterization of the removal of Chromium(VI) from groundwater by electrocoagulation. J. Ind. Eng. Chem. 20, 2775–2781. https://doi.org/10.1016/j.jiec.2013.11.006.
Hashim, M.A., Mukhopadhyay, S., Sahu, J.N., Sengupta, B., 2011. Remediation technologies for heavy metal contaminated groundwater. J. Environ. Manag. 92, 2355–2388. https://doi.org/10.1016/j.jenvman.2011.06.009.
He, M., Li, X., Liu, H., Miller, S.J., Wang, G., Rensing, C., 2011a. Characterization and genomic analysis of a highly chromate resistant and reducing bacterial strain Lysinibacillus fusiformis ZC1. J. Hazard Mater. 185, 682–688. https://doi.org/10.1016/j.jhazmat.2010.09.072.
He, Y., Gong, Y., Su, Y., Zhang, Y., Zhou, X., 2019. Bioremediation of Cr (VI) contaminated groundwater by Geobacter sulfurreducens: environmental factors and electron transfer flow studies. Chemosphere 221, 793–801. https://doi.org/10.1016/J.CHEMOSPHERE.2019.01.039.
Hemkemeyer, M., Dohrmann, A.B., Christensen, B.T., Tebbe, C.C., 2018. Bacterial preferences for specific soil particle size fractions revealed by community analyses. Front. Microbiol. 9, 149. https://doi.org/10.3389/fmicb.2018.00149.
Ingle, K.N., Harada, K., Wei, C.N., Minamoto, K., Ueda, A., 2011. Policy framework for formulating environmental management strategy for sustainable development of tanneries in India. Environ. Health Prev. Med. 16, 123–128. https://doi.org/10.1007/s12199-010-0168-8.
Jin, X., Jiang, M., Du, J., Chen, Z., 2014. Removal of Cr(VI) from aqueous solution by surfactant-modified kaolinite. J. Ind. Eng. Chem. 20, 3025–3032. https://doi.org/10.1016/j.jiec.2013.11.038.
Jobby, R., Jha, P., Yadav, A.K., Desai, N., 2018. Biosorption and biotransformation of hexavalent chromium [Cr(VI)]: a comprehensive review. Chemosphere 207, 255–266. https://doi.org/10.1016/J.CHEMOSPHERE.2018.05.050.
Juarez, S., Nunan, N., Duday, A.C., Pouteau, V., Schmidt, S., Hapca, S., Falconer, R., Otten, W., Chenu, C., 2013. Effects of different soil structures on the decomposition of native andadded organic carbon. Eur. J. Soil Biol. 58, 81–90. https://doi.org/10.1016/j.ejsobi.2013.06.005.
Kamaludeen, S.P.B., Megharaj, M., Juhasz, A.L., Sethunathan, N., Naidu, R., 2003. Chromium-Microorganism Interactions in Soils: Remediation Implications 93–164. https://doi.org/10.1007/0-387-21728-2_4.
Kantar, C., Demiray, H., Dogan, N.M., 2011. Role of microbial exopolymeric substances (EPS) on chromium sorption and transport in heterogeneous subsurface soils: II. Binding of Cr(III) in EPS/soil system. Chemosphere 82, 1496–1505. https://doi.org/10.1016/j.chemosphere.2010.11.001.
Karnakar Reddy, T., 2014. A News Article: Toxic Waste Poses Major Threat. The Hindu, Nalgonda, India. https://www.thehindu.com/todays-paper/tp-national/tp-andhrapradesh/toxic-waste-poses-major-threat/article6648075.ece.
Karthick, S., Sangeetha, C.J., Shashidhar, T., 2019. Lab-scale biodegradation study of BTEX under the unsaturated condition and its effect on soil matric potential. Soil Sediment Contam. An Int. J. 28, 171–183. https://doi.org/10.1080/15320383.2018.1556597.
Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I., Dumat, C., 2017. A comparison of technologies for remediation of heavy metal contaminated soils. J. Geochem. Explor. 182, 247–268. https://doi.org/10.1016/j.gexplo.2016.11.021.
Kirpnick-Sobol, Z., Reliene, R., Schiestl, R.H., 2006. Carcinogenic Cr(VI) and the nutritional supplement Cr(III) induce DNA deletions in yeast and mice. Canc. Res. 66, 3480–3484. https://doi.org/10.1158/0008-5472.CAN-05-3944.
Lee, S.E., Lee, J.-U., Chon, H.T., Lee, J.S., 2008. Reduction of Cr(VI) by indigenous bacteria in Cr-contaminated sediment under aerobic condition. J. Geochem. Explor. 96, 144–147. https://doi.org/10.1016/J.GEXPLO.2007.04.005.
Leita, L., Margon, A., Sinicco, T., Mondini, C., 2011. Glucose promotes the reduction of

hexavalent chromium in soil. Geoderma 164, 122–127. https://doi.org/10.1016/j.geoderma.2011.05.013.
Li, M., He, Z., Hu, Y., Hu, L., Zhong, H., 2019. Both cell envelope and cytoplasm were the locations for chromium(VI) reduction by Bacillus sp. M6. Bioresour. Technol. 273, 130–135. https://doi.org/10.1016/j.biortech.2018.11.006.
Liu, J., 2018. Soil remediation using soil washing followed by ozone oxidation. J. Ind. Eng. Chem. 65, 31–34. https://doi.org/10.1016/J.JIEC.2018.05.001.
Long, D., Tang, X., Cai, K., Chen, G., Shen, C., Shi, J., Chen, L., Chen, Y., 2013. Cr(VI) resistance and removal by indigenous bacteria isolated from chromium-contaminated soil. J. Microbiol. Biotechnol. https://doi.org/10.4014/jmb.1301.01004.
López, S., Prieto, M., Dijkstra, J., Dhanoa, M.S., France, J., 2004. Statistical evaluation of mathematical models for microbial growth. Int. J. Food Microbiol. 96, 289–300. https://doi.org/10.1016/j.ijfoodmicro.2004.03.026.
Luo, L., He, H., Yang, C., Wen, S., Zeng, G., Wu, M., Zhou, Z., Lou, W., 2016. Nutrient removal and lipid production by Coelastrella sp. in anaerobically and aerobically treated swine wastewater. Bioresour. Technol. 216, 135–141. https://doi.org/10.1016/j.biortech.2016.05.059.
Maggi, F., Porporato, A., 2007. Coupled moisture and microbial dynamics in unsaturated soils. Water Resour. Res. 43, 1–11. https://doi.org/10.1029/2006WR005367.
Malaviya, P., Singh, A., 2011. Physicochemical technologies for remediation of chromium-containing waters and wastewaters. Crit. Rev. Environ. Sci. Technol. 41, 1111–1172. https://doi.org/10.1080/10643380903392817.
Maleki, A., Hayati, B., Naghizadeh, M., Joo, S.W., 2015. Adsorption of hexavalent chromium by metal organic frameworks from aqueous solution. J. Ind. Eng. Chem. 28, 211–216. https://doi.org/10.1016/j.jiec.2015.02.016.
MOEF, 2015. Inventory and Mapping of Probably Contaminated Sites in India – Existing Data and General Information on Contaminated Sites. (New Delhi).
Mulligan, C.N., Yong, R.N., Gibbs, B.F., 2001. Remediation technologies for metal-contaminated soils and groundwater: an evaluation. Eng. Geol. 60, 193–207. https://doi.org/10.1016/S0013-7952(00)00101-0.
Nam, A., Choi, U.S., Yun, S.T., Choi, J.W., Park, J.A., Lee, S.H., 2018. Evaluation of amine-functionalized acrylic ion exchange fiber for chromium(VI) removal using flow-through experiments modeling and real wastewater. J. Ind. Eng. Chem. https://doi.org/10.1016/j.jiec.2018.05.029.
Nicolova, M., Spasova, I., Georgiev, P., Groudev, S., 2017. Microbial removal of toxic metals from a heavily polluted soil. J. Geochem. Explor. 182, 242–246. https://doi.org/10.1016/J.GEXPLO.2016.11.003.
Olsen, R.A., Bakken, L.R., 1987. Viability of soil bacteria: optimization of plate-counting technique and comparison between total counts and plate counts within different size groups. Microb. Ecol. 13, 59–74. https://doi.org/10.1007/BF02014963.
Pattanapipitpaisal, P., Brown, N., Macaskie, L., 2001. Chromate reduction and 16S rRNA identification of bacteria isolated from a Cr(VI)-contaminated site. Appl. Microbiol. Biotechnol. 57, 257–261. https://doi.org/10.1007/s002530100758.
Pal, A., Paul, A.K., 2004. Aerobic chromate reduction by chromium-resistant bacteria isolated from serpentine soil. Microbiol. Res. 159, 347–354. https://doi.org/10.1016/j.micres.2004.08.001.
Pantsar-Kallio, M., Reinikainen, S.P., Oksanen, M., 2001. Interactions of soil components and their effects on speciation of chromium in soils. Anal. Chim. Acta 439, 9–17. https://doi.org/10.1016/S0003-2670(01)00840-6.
Pradhan, D., Sukla, L.B., Sawyer, M., Rahman, P.K.S.M., 2017. Recent bioreduction of hexavalent chromium in wastewater treatment: a review. J. Ind. Eng. Chem. 55, 1–20. https://doi.org/10.1016/J.JIEC.2017.06.040.
Pure Earth, 2007. A Site Clean-Up Project Report. Blacksmith Institute, New York. https://www.pureearth.org/project/ranipet/.
Pure Earth, 2010. An Annual Report. Blacksmith Institute, New York. http://www.pureearth.org/wp-content/uploads/2014/12/2010Annual-Report-Final-small-file.pdf.
Pure Earth, 2012. An Annual Report. Blacksmith Institute, New York. http://www.pureearth.org/wp-content/uploads/2014/12/Blacksmith_2012AnnualReport.pdf.
Reasoner, D.J., Geldreich, E.E., 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49, 1–7.
Reible, D.D., Lanczos, T., 2006. Assessment and Remediation of Contaminated Sediments. Springer.
Reyimu, Z., Özçimen, D., 2017. Batch cultivation of marine microalgae Nannochloropsis oculata and Tetraselmis suecica in treated municipal wastewater toward bioethanol production. J. Clean. Prod. 150, 40–46. https://doi.org/10.1016/J.JCLEPRO.2017.02.189.
Richard, F.C., Bourg, A.C.M., 1991. Aqueous Geochemistry of Chromium: A Review. Water Reshttps://doi.org/10.1016/0043-1354(91)90160-R.
Roy, W.R., 1991. Batch-type Procedures for Estimating Soil Adsorption of Chemicals: Technical Resource Document. Risk Reduction Engineering Laboratory, Office of Research and Development, US Environmental Protection Agency.
Saha, R., Nandi, R., Saha, B., 2011. Sources and toxicity of hexavalent chromium. J. Coord. Chem. https://doi.org/10.1080/00958972.2011.583646.
Saha, R., Saha, I., Nandi, R., Ghosh, A., Basu, A., Ghosh, S.K., Saha, B., 2013. Application of Chattim tree (devil tree, Alstonia scholaris) saw dust as a biosorbent for removal of hexavalent chromium from contaminated water. Can. J. Chem. Eng. https://doi.org/10.1002/cjce.21703.
Schindler, F., Merbold, L., Karlsson, S., Sprocati, A.R., Kothe, E., 2017. Seasonal change of microbial activity in microbially aided bioremediation. J. Geochem. Explor. 174, 4–9. https://doi.org/10.1016/J.GEXPLO.2016.04.001.
Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H., Kandeler, E., 2001. Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. Appl. Environ. Microbiol. 67, 4215–4224. https://doi.org/10.1128/AEM.67.9.4215-4224.2001.
Shashidhar, T., Murty Bhallamudi, S., Philip, L., 2007a. Development and validation of a model of bio-barriers for remediation of Cr(VI) contaminated aquifers using laboratory column experiments. J. Hazard Mater. 145, 437–452. https://doi.org/10.1016/J.JHAZMAT.2006.11.034.
Shashidhar, T., Nandanan, N., Philip, L., Murty Bhallamudi, S., 2007b. Design of a passive biobarrier system for chromium containment in confined aquifers. Pract. Period. Hazard. Toxic, Radioact. Waste Manag. 11, 216–224 10.1061, ASCE)1090-025X (2007)11:4(216.
Shashidhar, T., Philip, L., Murty Bhallamudi, S., 2006. Bench-scale column experiments to study the containment of Cr(VI) in confined aquifers by bio-transformation. J. Hazard Mater. 131, 200–209. https://doi.org/10.1016/j.jhazmat.2005.09.034.
Shen, H., Wang, Y.T., 1993. Characterization of enzymatic reduction of hexavalent chromium by Escherichia coli ATCC 33456. Appl. Environ. Microbiol. 59, 3771–3777.
Slater, L., Atekwana, E., 2013. Geophysical signatures of subsurface microbial processes. Eos (Washington. DC) 94, 77–78. https://doi.org/10.1002/2013EO080001.
Song, H., Liu, Y., Xu, W., Zeng, G., Aibibu, N., Xu, L., Chen, B., 2009. Simultaneous Cr(VI) reduction and phenol degradation in pure cultures of Pseudomonas aeruginosa CCTCC AB91095. Bioresour. Technol. 100, 5079–5084. https://doi.org/10.1016/j.biortech.2009.05.060.
Stanin, F.T., 2005. The transport and fate of chromium(VI) in the environment. In: Chromium (VI) Handbook, https://doi.org/10.1007/978-94-007-4375-5.
Stollenwerk, K.G., Grove, D.B., 1985. Adsorption and desorption of hexavalent chromium in an alluvial aquifer near telluride, Colorado1. J. Environ. Qual. 14, 150. https://doi.org/10.2134/jeq1985.00472425001400010030x.
Taylor, P., Kimbrough, D.E., Cohen, Y., Winer, A.M., Creelman, L., Kimbrough, D.E., 1999. Critical reviews in environmental science and Technology A critical assessment of chromium in the environment A critical assessment of chromium in the environment. Crit. Rev. Environ. Sci. Technol. 29, 1–46. https://doi.org/10.1080/10643389991259164.
Thiruvenkatachari, R., Vigneswaran, S., Naidu, R., 2008. Permeable reactive barrier for groundwater remediation. J. Ind. Eng. Chem. 14, 145–156. https://doi.org/10.1016/j.jiec.2007.10.001.
PA. Method 7196A: chromium, hexavalent (colorimetric), test methods for evaluating solid waste, physical/chemical methods. Washington, DC, https://www.epa.gov/hw-sw846/sw-846-test-method-7196a-chromium-hexavalent-colorimetric.
US EPA, 2004. SW-846 Test Method 3052: Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. Washington, DC. https://www.epa.gov/sites/production/files/2015-12/documents/3052.pdf.
US EPA, 2007. SW-846 Test Method 3015A: Microwave Assisted Acid Digestion of Aqueous Samples and Extracts, Revision 1. Washington, DC. https://www.epa.gov/hw-sw846/sw-846-test-method-3015a-microwave-assisted-acid-digestion-aqueous-samples-and-extracts.
US EPA, 2014. SW-846 Test Method 6020B. Inductively Coupled Plasma - Mass Spectrometry, Washington, DC. https://www.epa.gov/hw-sw846/sw-846-test-method-6020b-inductively-coupled-plasma-mass-spectrometry.
Wang, L.K., 2017. Handbook of Advanced Industrial and Hazardous Wastes Management. CRC Press.
Wang X., Z., Chen Q., J., Chai Y., L., Yang H., Z., Huang H., S., Zheng, Y., et al., 2011. Environmental impact and site-specific human health risks of chromium in the vicinity of a ferro-alloy manufactory, China. J. Hazard Mater. 190, 980–985. https://doi.org/10.1016/j.jhazmat.2011.04.039.
Wang, P.C., 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. Appl. Environ. Microbiol. 55, 1665–1669.
Wang, Y.-T., Xiao, C., 1995. Factors affecting hexavalent chromium reduction in pure cultures of bacteria. Water Res. 29, 2467–2474. https://doi.org/10.1016/0043-1354(95)00093-Z.
Xiao, W., Zhang, Y., Li, T., Chen, B., Wang, H., He, Z., Yang, X., 2012. Reduction kinetics of hexavalent chromium in soils and its correlation with soil properties. J. Environ. Qual. 41, 1452. https://doi.org/10.2134/jeq2012.0061.
Yan, Z., Liu, C., Liu, Y., Bailey, V.L., 2017. Multiscale investigation on biofilm distribution and its impact on macroscopic biogeochemical reaction rates. Water Resour. Res. 53, 8698–8714. https://doi.org/10.1002/2017WR020570.
Yang, S., Ngwenya, B.T., Butler, I.B., Kurlanda, H., Elphick, S.C., 2013. Coupled interactions between metals and bacterial biofilms in porous media: implications for biofilm stability, fluid flow and metal transport. Chem. Geol. 337–338, 20–29. https://doi.org/10.1016/J.CHEMGEO.2012.11.005.
Yuan, W., Xu, W., Wu, Z., Zhang, Z., Wang, L., Bai, J., Wang, X., Zhang, Q., Zhu, X., Zhang, C., Wang, J., 2018. Mechanochemical treatment of Cr(VI) contaminated soil using a sodium sulfide coupled solidification/stabilization process. Chemosphere 212, 540–547. https://doi.org/10.1016/j.chemosphere.2018.08.121.
Zeng, Q., Hu, Y., Yang, Y., Hu, L., Zhong, H., He, Z., 2019. Cell envelop is the key site for Cr(VI) reduction by Oceanobacillus oncorhynchi W4, a newly isolated Cr(VI) reducing bacterium. J. Hazard Mater. 368, 149–155. https://doi.org/10.1016/j.jhazmat.2019.01.031.
Zhang, P., Zheng, J., Pan, G., Zhang, X., Li, L., Rolf, T., 2007. Changes in microbial

community structure and function within particle size fractions of a paddy soil under different long-term fertilization treatments from the Tai Lake region, China. Colloids Surf. B Biointerfaces 58, 264–270. https://doi.org/10.1016/J.COLSURFB.2007.03.018.

Zhang, Q., Amor, K., Galer, S.J.G., Thompson, I., Porcelli, D., 2019. Using stable isotope fractionation factors to identify Cr(VI) reduction pathways: metal-mineral-microbe interactions. Water Res. 151, 98–109. https://doi.org/10.1016/J.WATRES.2018.11.088.

Zhitkovich, A., 2011. Chromium in drinking water: sources, metabolism, and cancer risks. Chem. Res. Toxicol. 24, 1617–1629. https://doi.org/10.1021/tx200251t.

Zhou, J., Xia, B., Treves, D.S., Wu, L.-Y., Marsh, T.L., O'Neill, R.V., Palumbo, A.V., Tiedje, J.M., 2002. Spatial and resource factors influencing high microbial diversity in soil. Appl. Environ. Microbiol. 68, 326–334. https://doi.org/10.1128/AEM.68.1.326-334.2002.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Res. 47, 4294–4302. https://doi.org/10.1016/J.WATRES.2013.05.004.

Zhu, W., Chai, L., Ma, Z., Wang, Y., Xiao, H., Zhao, K., 2008. Anaerobic reduction of hexavalent chromium by bacterial cells of Achromobacter sp. Strain Ch1. Microbiol. Res. 163, 616–623. https://doi.org/10.1016/J.MICRES.2006.09.008.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K., 1990. Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56, 1875–1881.