

Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions

J. Jeyasingh, Ligy Philip*

Department of Civil Engineering, Indian Institute of Technology, Chennai 600 036, India

Received 26 April 2004; received in revised form 7 October 2004; accepted 9 October 2004

Available online 8 December 2004

Abstract

Bacterial strains were isolated and enriched from the contaminated site of Tamil Nadu Chromates and Chemicals Limited (TCCL) premises, Ranipet, Tamil Nadu, India. The strain which was isolated from the highly contaminated location had shown high Cr(VI) reduction potential. Cr(VI) reduction was evaluated both in aerobic and anaerobic conditions. Though the aerobic system performed better than the anaerobic one, further study were carried out in the anaerobic condition due to its economic viability. At higher initial concentration, Cr(VI) reduction was not complete even after 108 h, however, specific Cr(VI) reduction, unit weight of Cr reduced/unit weight of biomass was greater at higher concentration. It was found that a bacterial concentration of 15 ± 1.0 mg/g of soil (wet weight) 50 mg of molasses/g of soil as carbon source were required for the maximum Cr(VI) reduction. The bioreactor operated at these conditions could reduce entire Cr(VI) (5.6 mg Cr(VI)/g of soil) in 20 days. The Cr(III) thus formed was found to be strongly attached to the soil matrix and the mobility of Cr(III) was negligible as evident from the low concentration of Cr(III) in the leachate. This study showed that bioremediation is a viable, environmental friendly technology for cleaning-up the chromium contaminated site at TCCL, Ranipet, Tamil Nadu, India, and optimal operating conditions under laboratory conditions were evaluated.

© 2004 Published by Elsevier B.V.

Keywords: Chromium(VI); Bioremediation; Contaminated soil; Optimum conditions

1. Introduction

Hexavalent chromium is widely used in many industrial processes such as electroplating, wood preservation, etc. Commercially available forms of hexavalent chromium (Cr(VI)) are potassium chromate and potassium dichromate. The chromium manufacturing industry produces a large quantity of solid and liquid waste containing hexavalent chromium. The treatment of these wastes is essential before discharging them to the environment. Cr(VI) compounds are highly water soluble, toxic and carcinogenic in mammals. In contrast, trivalent chromium is considered to be nontoxic as it precipitates at pH higher than 5.5 with the formation of

insoluble oxides and hydroxides in soil and water systems [1].

The remediation of chromium contaminated sites poses a number of unique challenges. Many technologies are currently used to clean up heavy metal contaminated soils. The most commonly used ones are soil removal and land filling, stabilization/solidification, physico-chemical extraction, soil washing, flushing and phytoremediation. None of these techniques are completely accepted as best treatment option [2] because either they offer a temporary solution, or simply immobilize the contaminant or costly when applied to large areas.

Bioremediation is one of the promising technologies that is expected to play an important role in waste site clean up. The bioremediation strategy is to detoxify Cr(VI) in the soil to reduce it to Cr(III), so that it gets immobilized in the soil matrix. Besides eliminating the toxicity of Cr(VI)

* Corresponding author. Tel.: +91 44 22578318; fax: +91 44 22578281.
E-mail address: ligy@civil.iitm.ernet.in (L. Philip).

by its reduction to Cr(III) the latter forms a particularly insoluble Cr(OH)₃ in the pH range of 6–9 (K_{sp} , 6.7×10^{-31}) [3] severely restricting its ability to migrate to ground water.

Many microbes were reported to reduce Cr(VI) under aerobic and anaerobic conditions [4–9]. Bader et al. [8] studied the potential of aerobic reduction of Cr(VI) by an indigenous soil microbial community and found that Cr(VI) in the soil was reduced by 33% within 21 days and suggested that Cr(VI) reducing microbial populations is widespread in soil. Studies have shown that certain bacterial species on surfaces of geologic materials can detoxify the compounds by reducing them to relatively insoluble and hence significantly less harmful trivalent chromium compounds [10]. Thus, treatment of Cr(VI) containing wastes consists primarily of reducing toxic and mobile Cr(VI) to nontoxic and immobile Cr(III). However, the potential of this bioremediation strategy for the clean up of Cr(VI) contaminated soil is to be still engineered.

The objective of this paper is to isolate and enrich Cr(VI) reducing microbes to bioaugment a soil bioreactor and to study its performance under optimal conditions.

2. Materials and methods

2.1. Soil sample

The soil samples were collected from seven different locations of the contaminated site located at Ranipet, Tamil Nadu, India in clean polyethylene bags and preserved in a deep freezer (APNA Scientific Supplies, Chennai). The nature of the soil/deposited sludge was not uniform through out the area. The samples were collected from representative regions, i.e.: (1) open well inside the factory premises, (2) grass root sample outside the premises (eastern side), (3) grass root sample outside the premises (southern side), (4) grass root sample in the SIPCOT drain, (5) sample from mat formation near ETP, (6) sample from waste dump site (eastern side), (7) sample from waste dump site (western side). All these sites were highly contaminated with Cr(VI). An amount of 150 g of soil from each site is taken, mixed well and dried in the oven at 60 °C for 24 h, crushed the sample using a crusher to remove the boulders. A representative sample of the soil was characterized in the laboratory as per standard procedure [11]. The soil characteristics thus determined are given in Table 1.

2.2. Nutrient media

The nutrient medium (N1) for bacterial growth consisted of peptone 10 g, beef extract 2 g, yeast extract 1 g, and sodium chloride 5 g in 1 l of distilled water and the mineral medium (M1) consisted of K₂HPO₄-2.12 g, KH₂PO₄-2.12 g, NaCl-2 g, MgSO₄-7H₂O-1 g, CaCl₂-0.1 g, and KNO₃-4 g in 1 l of distilled water. The pH was maintained at 7 ± 0.2 by using

Table 1
Soil characteristics

Parameter	Value
Soil organic matter	6.9 ± 0.5%
Cr(VI)	2.5–5.5 mg/g of soil
Total chromium	9 ± 12 mg/g of soil
Coefficient of curvature	1.067
Uniformity coefficient	6.67
pH	9.5–10.5
Sand	56 ± 1.5%
Silt	38 ± 1%
Clay	6 ± 0.3%

HCl or NaOH. Molasses was used as a carbon source. Sterilized media were used for all the studies.

2.3. Analytical procedures

2.3.1. Extraction and analysis of Cr(VI) and total chromium

For the extraction of Cr(VI) and total chromium from soil, an alkaline digestion method and nitric acid/sulfuric acid digestion method as per Standard Methods [12] were used, respectively. The hexavalent chromium was measured colorimetrically at 540 nm by reaction with diphenyl carbazide in acidic conditions. In the case of Cr(III), potassium permanganate was used to oxidize Cr(III) to Cr(VI).

2.3.2. Measurement of cell density

Overnight cultures were centrifuged, and the cell pellets was washed with physiological saline water thrice, resuspended in saline water, homogenized and was used as stock solution. Different dilutions were made from the stock solution. A known volume of these solutions were filtered through 0.45 µm filter paper (Millipore, USA) to find out the dry weight of cells. Corresponding absorbance was measured at 440 nm using a spectrophotometer. This information was used to prepare a calibration curve, dry weight versus absorbance. For unknown samples, the absorbance was measured at 440 nm and was converted to dry weight using absorbance versus dry weight calibration curve.

2.4. Experimental methods

2.4.1. Enrichment of the Cr(VI) reducing bacterial strains

Bacterial strains were isolated from the soil samples collected in and around the contaminated site. About 1 g of soil sample was added to 100 ml of growth medium (N1) and incubated for 24 h in facultative condition. The shake flask cultures were closed using Teflon stoppers. Then, 1 ml of this was transferred to 100 ml nutrient broth (N1) containing 100 ppm Cr(VI). This procedure was repeated by progressively increasing Cr(VI) in the nutrient medium up to 500 mg/l. A loopful from the above mixture was streaked on

Table 2
Bacterial strains employed for the present study

Sl. no.	Sample location	Strain identification name
1	Open well inside the factory premises	B2
2	Grass root sample outside the premises (eastern side)	C2
3	Grass root sample outside the premises (southern side)	D2
4	Grass root sample in the SIPCOT drain	F1
5	Sample from mat formation near ETP	H1
6	Sample from waste dump site (eastern side)	I2
7	Sample from waste dump site (western side)	J2

agar slants, incubated for 24 h and stored in the freezer at 4 °C for further use.

2.4.2. Screening of the enriched cultures

The enriched bacterial strains were named based on the locations at which they were collected. The details are given in Table 2. These cultures were used for the Cr(VI) reduction studies. This study was carried out in shake flasks with nutrient medium (N1) spiked with a Cr(VI) concentration of 50 mg/l.

The enriched cultures obtained from seven locations of the contaminated site were evaluated for their chromium(VI) reduction potential based on the kinetics of Cr(VI) reduction and their specific Cr(VI) reduction potential. Specific Cr(VI) reduction potential was calculated as Cr(VI) reduction by unit biomass in unit time. Further studies were conducted using the most promising bacterial strain emerging in the screening test. For the screening test, eight conical flasks with 100 ml of autoclaved nutrient medium (N1) spiked with 50 mg/l Cr(VI) were inoculated with same quantity (measured as optical density) of previously grown bacterial cultures isolated from seven locations. The residual Cr(VI) concentration in the liquid phase was measured with respect to time. Further studies to evaluate the effect of initial Cr(VI) concentration, Cr(VI) inhibitions, etc. were conducted using the selected bacterial isolate. For this, six conical flasks with 100 ml autoclaved nutrient medium (N1), were inoculated with the previously grown bacterial culture in log growth phase and incubated at 35 °C for 24 h. Then stock potassium dichromate solution (stock solution) was added to the mixture so that the Cr(VI) concentration in conical flasks was 50, 100, 150, 200, 300 and 400 mg/l, respectively. Initial bacterial concentration was also measured. The Cr(VI) concentration, COD and bacterial concentrations were determined at regular intervals. A control (all conditions were same except there was no bacterial addition) was always employed to quantify the abiotic reduction of Cr(VI).

2.4.3. Effect of pH

The effect of pH on Cr(VI) reduction was studied by varying the reaction mixture pH from 4 to 10. The experi-

ments were carried out with an initial Cr(VI) concentration of 50 mg/l.

2.4.4. Cr(VI) reduction in aerobic and anaerobic conditions

Cr(VI) reduction potential was studied under aerobic and anaerobic conditions with an initial Cr(VI) concentration of 50 mg/l in nutrient medium (N1). Two conical flasks were filled with 100 ml of nutrient medium (N1), autoclaved, and 1 ml of previously grown H1 bacterial strains were added in each conical flasks, and incubated at 35 °C for 24 h. Then potassium dichromate solution (stock solution) was added so that the Cr(VI) concentration in each conical flask was 50 mg/l. To achieve anaerobic condition one of the conical flasks with the reaction mixture was flushed with N₂ through a filter for 5 min. Air tight caps were employed. The samples were withdrawn from this flask using syringes.

2.4.5. Evaluation of optimum conditions using miniature reactors

To evaluate the optimum conditions for bioremediation, miniature reactors were employed. Soil employed for all the biotransformation studies were sterilized in a hot air oven by keeping it at 150 °C for 2 h. The miniature reactors were of 500 ml capacity glass beakers containing 25 g of contaminated soil. These received different concentrations of molasses and were inoculated with bacteria. The performance of the reactors was monitored regularly under anaerobic conditions. The schematic diagram of the miniature reactor is shown in Fig. 1.

2.4.6. Soil reactors for Cr(VI) reduction

In order to study the effectiveness of bioremediation of Cr(VI) contaminated soils, experiments were carried out in two laboratory scale soil reactors. A schematic diagram of the reactor is shown in Fig. 2. The reactors were made up of 3 mm thick acrylic transparent sheet. The top compartment was of 10 cm diameter and 25 cm height with four sample collection ports located at 5, 10, 15, 20 cm from the top. The bottom compartment was 12 cm in diameter and 10 cm in height which was used as leachate collector. Filter media consisting of gravel, glass beads, and sand were packed to a height of 4.5 cm from the bottom of the top compartment over which 3 kg of the contaminated soil was loosely packed

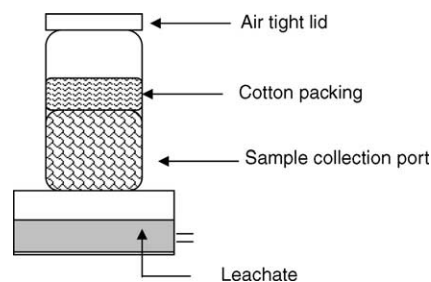


Fig. 1. Schematic diagram of miniature soil bioreactor.

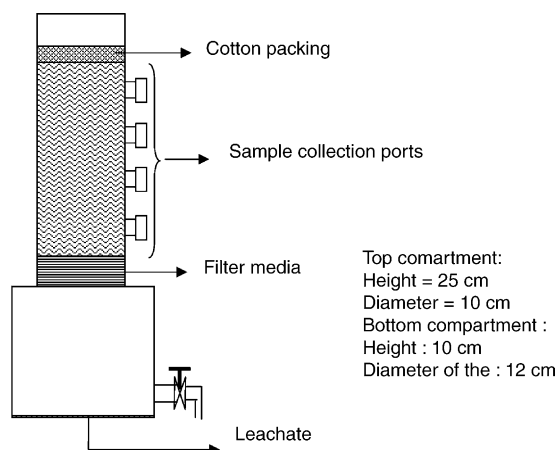


Fig. 2. Schematics diagram of the soil bioreactor.

to a height of 18 cm. Soil employed for all the biotransformation studies were sterilized in a hot air oven by keeping it at 150 °C for 2 h. One of such reactors was supplemented with molasses, mineral medium (M1), enriched microorganisms along with contaminated soil whereas the second reactor contained all except enriched microorganisms. Operating conditions of both the reactors were identical. Cr(VI) concentrations in the solid phase and liquid phase (leachate) were analyzed regularly in both the reactors. A mass balance of Cr(VI) and total Cr for the system was also made. The top of the reactor was covered with wet cotton to maintain the moisture content in the range of 50–52%. The reactors were fitted with air tight caps.

2.4.7. Performance evaluation of soil reactor based on optimum conditions

Two reactors of 21 capacity were used for the present study. In one reactor a mixture of 1600 g of contaminated soil, 24 g (dry weight) of bacteria which was grown separately in mineral medium (M1), 80 g of molasses and 390 ml of mineral medium were added. In the other reactor, a mixture of 1600 g of contaminated soil, 80 g of molasses and 390 ml of mineral medium were added which was kept as control reactor. Fifty milliliter of mineral medium (M1) was added in both the reactors daily and the leachate from both the reactors were collected and analyzed for Cr(VI). Soil samples from the reactors were collected from various sampling ports using an auger and analyzed for Cr(VI). An equal amount of fresh soil was added to the system at each time.

3. Results and discussion

3.1. Screening of microbes for chromium(VI) reduction

Bacterial cultures isolated from seven locations of the contaminated site were screened based on their Cr(VI) reduction

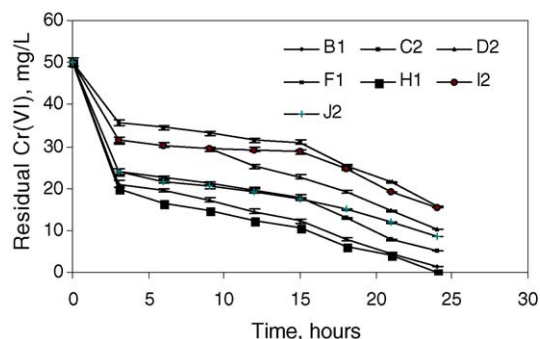


Fig. 3. Kinetics of Cr(VI) reduction by different bacterial strains.

capacity. Kinetics of Cr(VI) reduction was carried out under aerobic conditions with an initial Cr(VI) concentration of 50 mg/l for all the seven strains and the results are presented in Figs. 3 and 4. Among the seven strains screened, H1, the strain isolated from a clay mat near the old effluent treatment plant (ETP) showed highest Cr(VI) reduction potential. This strain was used for further studies.

The strain which had a history of exposure to high Cr(VI) concentration has exhibited an excellent Cr(VI) reduction potential. The environmental stress might have forced the strain to develop an effective detoxification mechanism to survive in the adverse condition. It was also reported that many microorganisms isolated from the contaminated sites showed high resilience to a toxicant and hence, better pollution remediation potential [13–16]. The specific Cr(VI) reduction potential was also higher for bacterial strain H1 (Fig. 4). Bacterial strains B1 and C2 also showed high specific chromium reduction.

3.2. Effect of initial Cr(VI) concentration on Cr(VI) reduction

In order to determine the effect of initial Cr(VI) concentration on microbial Cr(VI) reduction, studies were carried using H1 bacterial strain with different initial Cr(VI) concentrations (50–400 mg/l) under aerobic condition and the results are presented in Fig. 5(a). The specific growth rate with respect to different Cr(VI) concentrations are presented in Fig. 5(b).

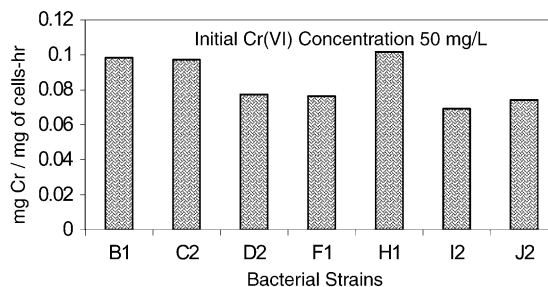


Fig. 4. Specific Cr(VI) reduction rate for different bacterial strains.

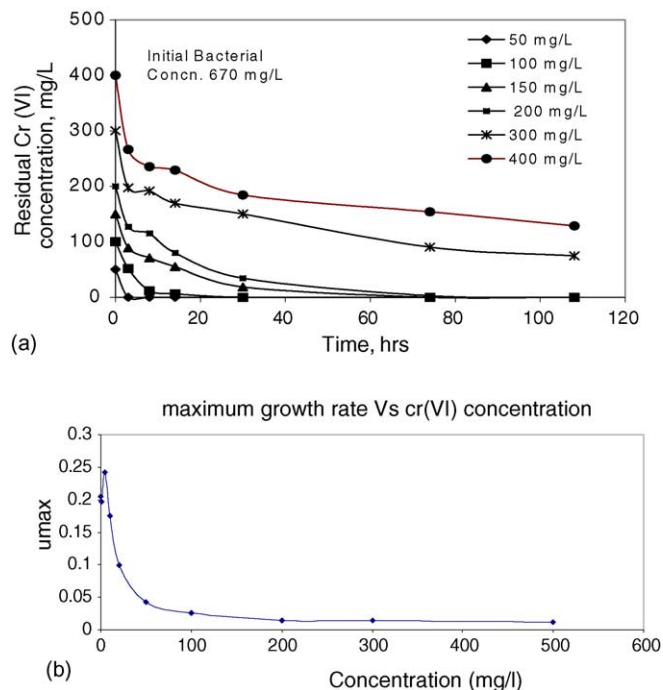


Fig. 5. (a) Effect of initial Cr(VI) concentration on Cr(VI) reduction. (b) Effect of initial Cr(VI) concentration on maximum specific growth rate.

The initial bacterial cell concentration was 670 mg/l. The time required for complete reduction of Cr(VI) increased with the initial Cr(VI) concentrations. Complete Cr(VI) reduction was achieved in 3 h for initial Cr(VI) concentration of 50 mg/l. For a high Cr(VI) concentration of 200 mg/l, it took 108 h. Beyond this Cr(VI) concentration, the complete Cr(VI) reduction was not observed. The specific Cr(VI) reduction rate for various concentrations of Cr(VI) is given in Fig. 6.

The specific Cr(VI) reduction rate increased with the initial Cr(VI) concentration. Even at high initial Cr(VI) concentration of 400 mg/l, Cr(VI) was still reduced at a high specific rate (0.062 mg/mg cell h). These results also showed that isolated microbial consortium was able to sustain a Cr(VI) concentration in the range of 400 mg/l without much adverse effect. This is an important observation especially when in situ

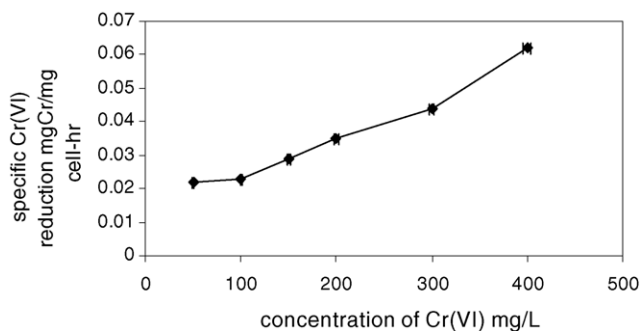


Fig. 6. Specific Cr(VI) reduction rate for different initial Cr(VI) concentrations.

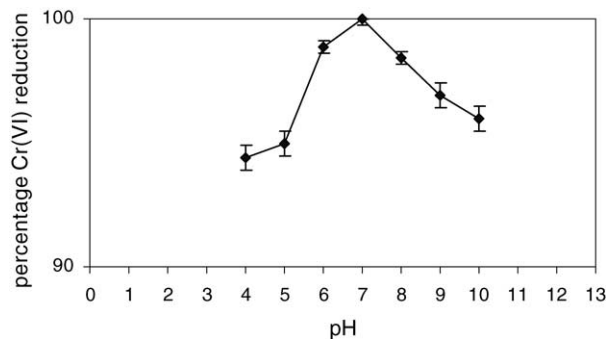


Fig. 7. Effect of pH on Cr(VI) reduction.

bioremediation is contemplated. The microbes are able to reduce/remediate Cr(VI) even at higher concentrations though it takes a long time. The cell yield was very low at high Cr(VI) concentrations due to the inhibition effect (Fig. 5b). This may be the reason for high specific Cr(VI) reduction rate at higher initial Cr(VI) concentrations.

3.3. Effect of pH

pH plays an important role in most of the biological systems. The preferable pH range in such systems is 6–8. The effect of pH on Cr(VI) reduction was studied for a wide range of pH 4–10 under aerobic conditions. The results are shown in Fig. 7. At neutral pH, the Cr(VI) reduction was found to be 100% within 3 h when an initial Cr(VI) concentration of 50 mg/l was exposed to a bacterial concentration of 670 mg/l. The reduction decreased with either increase or decrease of pH from neutral. Here, entire Cr(VI) was in liquid phase and when bioremediation of Cr(VI) for clean up of contaminated soils is opted, then, adsorption and desorption characteristics of Cr(VI) as influenced by pH may determine the performance of the system. It was reported that Cr(VI) could be desorbed from soil at a faster rate at elevated pH values [17].

3.4. Cr(VI) reduction in aerobic and anaerobic conditions

Bioremediation of Cr(VI) was studied under aerobic and anaerobic conditions with an initial Cr(VI) concentration of 50 mg/l and the results are shown in Fig. 8. It was observed

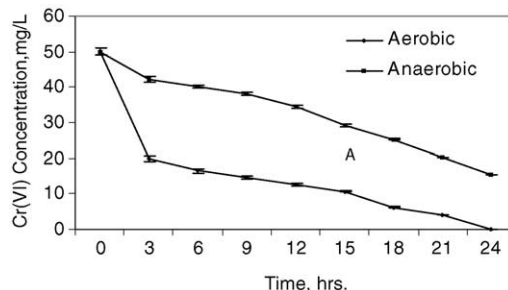


Fig. 8. Cr(VI) reduction in aerobic and anaerobic conditions.

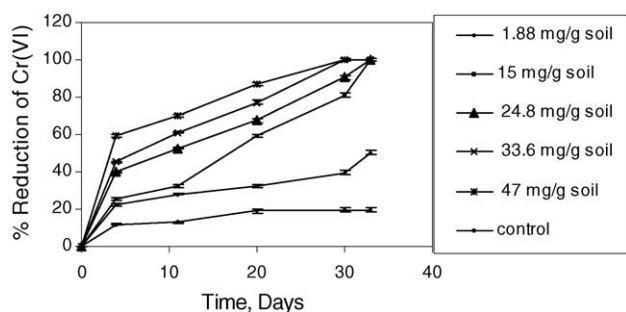


Fig. 9. Percentage reduction of Cr(VI) with different initial bacterial concentration.

that under aerobic conditions, Cr(VI) reduction was high. However, as large areas have to be bioremediated under field condition, provision of aeration may not be economically feasible. Therefore, the anaerobic reduction/bioremediation option is evaluated further though it is slightly less efficient (90%) than the aerobic system.

3.5. Evaluation of optimum conditions

Before evaluating anaerobic bioremediation technology both at bench scale and field scale, it is essential to determine the optimum conditions in terms of microbial concentration, pH, carbon source requirement, etc.

3.6. Optimum concentration of bacteria

A set of six small reactors (500 ml capacity) were charged with 25 g of contaminated soil and 10 ml of mineral medium (M1) which contained 20 g/l of molasses. The bacterial cells which were preacclimatized to 100 mg/l of Cr(VI) were added to first five reactors and thoroughly mixed to result in the initial bacterial concentration of 4.8 ± 0.5 , 15 ± 1.0 , 24 ± 1.3 , 33 ± 1.5 , and 47 ± 1.2 mg bacteria/g of soil, respectively and mixed well. The sixth reactor served as control without addition of microbial culture. All the reactors were operated under anaerobic conditions. The evaporation losses were made up by adding appropriate volume (2 ml) of M1. There was no provision for leachate collection. The Cr(VI) reduction profile as a function of initial anaerobic bacterial density is shown in Fig. 9. Even though the Cr(VI) reduction rate increased with an increase in bacterial concentration, the optimum bacterial concentration was observed as 15 mg/g of soil as there was 100% reduction of Cr(VI) in 33 days. The control reactor exhibited only 10% abiotic reduction in 33 days.

3.7. Optimum concentration of molasses

A set of 10 small reactors were operated with 25 g of contaminated soil and 15 mg bacteria/g of soil to determine the effect of carbon source on Cr(VI) reduction. Ten milliliter

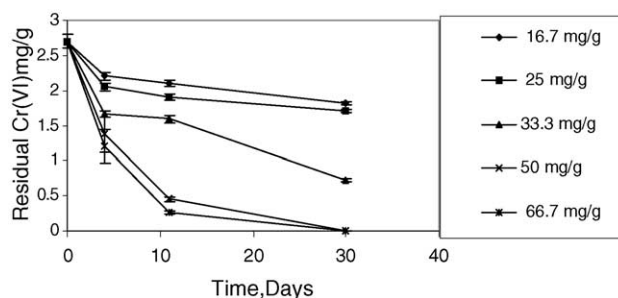


Fig. 10. Cr(VI) reductions with different molasses concentration.

of mineral medium (M1) containing 10, 15, 20, 30, 40 mg/l of molasses was added to each reactors, respectively. To the other set of five reactors, only the mineral medium containing the same concentrations of molasses was added as abiotic controls. All the reactors were operated under anaerobic conditions. The evaporation losses were made up by adding appropriate volume (2 ml on daily basis) of M1. The Cr(VI) reduction profile is shown in Fig. 10. Cr(VI) reduction rate increased with increase in molasses concentration. But the optimum molasses concentration was 34 mg/g of soil as the slope of the line is maximum. However, there was no appreciable abiotic reduction of Cr(VI) in control reactors.

3.8. Performance evaluation of soil reactor under optimum conditions

In one the two reactors a mixture of 1600 g of contaminated soil, 24 g (15 mg (wet weight)/g of soil dry weight) of bacteria was grown in mineral medium under anaerobic conditions with 100 ppm Cr(VI) concentration, 53 g (33.3 mg/g of soil) of molasses and 390 ml of mineral medium (M1). The second reactor was constituted as control reactor with all components except microbes. Both the reactors received 50 ml of mineral medium (M1) on a daily basis and the leachate was collected and analyzed for Cr(VI). Soil samples from the reactors were analyzed for Cr(VI). The performance of these reactors is presented in Fig. 11. In the bioreactor, a leachate Cr(VI) concentration of 1318 mg/l was observed in the second day and on the ninth day the Cr(VI) concentration was found to be nil, whereas in the control reactor initially the

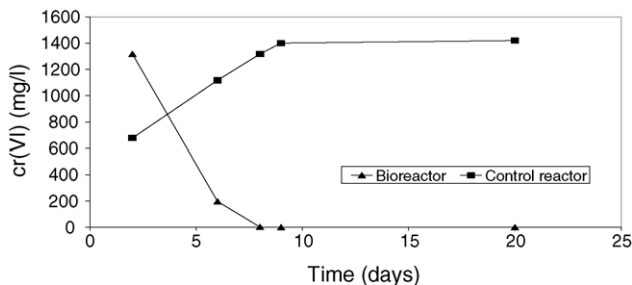


Fig. 11. Cr(VI) in leachate of the reactor under optimum molasses concentrations.

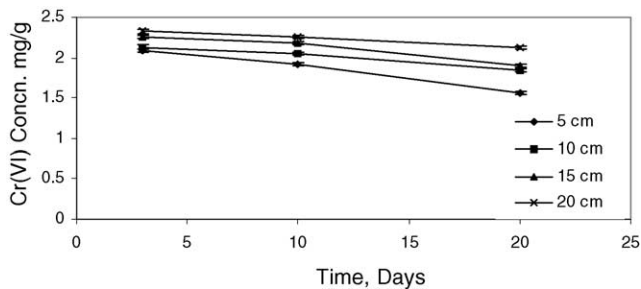


Fig. 12. Cr(VI) reductions in the soil with respect to depth in the control reactor.

Cr(VI) concentration was low and it increased with respect to time.

Similarly the soil samples at different depths were taken from the bioreactor and the control reactor and analyzed for Cr(VI). The results are presented in Figs. 12 and 13. The Cr(VI) reduction rate decreased with increase of depth. The soil samples collected from the bioreactor revealed that there was no Cr(VI) presence at different depths after 20 days. There was slight reduction of Cr(VI) observed in the control reactor. This might be due to abiotic reduction. The mass balance of Cr(VI) and total Cr is presented in Table 3. It was observed that $97 \pm 1.2\%$ reduction of Cr(VI) occurred in the bioreactor.

The initial mass of Cr(VI) present in the bioreactor was 4320 mg (2.7 mg/g) of soil in 1600 g of contaminated soil and the total Cr was 18 400 g (11.5 mg/g of soil). The amount of Cr(VI) and total Cr leached out along with the mineral medium was 123 ± 1.5 and 127 ± 1.7 mg, respectively. After 20 days the Cr(VI) present in the bioreactor was nil and total Cr was found to be 18 272 mg (average). It revealed that the Cr(VI) had converted to Cr(III). Hence percentage reduction of Cr(VI) to Cr(III) was observed as $97 \pm 1.2\%$. Similarly, the amount of Cr(VI) and total Cr present in the control reactor were observed and the reduction of Cr(VI) to Cr(III) was $12 \pm 0.8\%$.

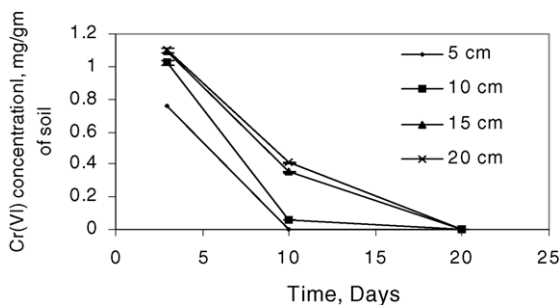


Fig. 13. Cr(VI) reductions in the soil with respect to depth in bioreactor under optimum conditions.

Table 3
Mass balance of reactor under optimum condition, period of study, 20 days

Reactor	Initial mass of Cr(VI) (mg)	Initial mass of total Cr (mg)	Mass of Cr(VI) leached out (average value) (mg)	Mass of total Cr leached out (mg)	Cr(VI) remained in the soil at the end of 20 days (mg)	Total Cr remained in the soil at the end of 20 days (mg)	Mass of Cr(VI) converted to Cr(III) (mg)	Reduction of Cr(VI) to Cr(III) (%)
Test	4320 ± 35	18400 ± 65	123 ± 9	127 ± 13	ND	18272 ± 52	4196 ± 25	97 ± 1.2
Control	4320 ± 35	18400 ± 65	803 ± 15	840 ± 18	2968	17560 ± 50	548 ± 20	12.7 ± 0.4

4. Conclusions

The present study focuses on the ability of a mixed population of microbial culture isolated from hexavalent chromium contaminated site to biotransform Cr(VI). Batch and continuous experiments were carried out to study the biotransformation pattern of Cr(VI) with molasses as the electron donor. Among the bacterial strains isolated from seven different locations of the contaminated site, the bacterial strain (H1) which was isolated from a clay mat near the effluent treatment plant (ETP) showed high Cr(VI) reduction potential. Optimum concentration of bacteria and molasses for the reduction of Cr(VI) to Cr(III) was observed as 15 and 34 mg/g of soil, respectively. The $97 \pm 1.2\%$ reduction of Cr(VI) was achieved within 20 days under optimum concentration of molasses and bacteria in anaerobic conditions. The results of the present study suggest that indigenous microorganisms are capable of anaerobic reduction of high concentration of Cr(VI) in contaminated soil. Reduction of Cr(VI) to Cr(III) is a microbially mediated process, and provision of a suitable electron donor to contaminated soils shall greatly speed up this reaction, thereby decreasing Cr(VI) toxicity and mobility. The biotransformed Cr(VI) remained in the soil as Cr(III).

References

- [1] J.M. Chen, O.J. Hao, Microbial chromium(VI) reduction, Crit. Rev. Environ. Sci. Technol. 28 (3) (1998) 219–251.
- [2] K. Yamamoto, J. Kato, T. Yano, H. Ohtake, Kinetics and modelling of hexavalent chromium reduction in *Enterobacter cloacae*, Biotechnol. Bioeng. 41 (1993) 129–133.
- [3] L.D. Benefield, J.K. Judkins, B.L. Weand, Process Chemistry for Water and Wastewater Treatment, Prentice-Hall, NJ, USA, 1982.
- [4] R. Gopalan, H. Veeramani, Studies on microbial chromate reduction by *Pseudomonas* sp. in aerobic continuous suspended growth cultures, Biotechnol. Bioeng. 42 (1994) 471–476.
- [5] Shen, Y.T. Wang, Hexavalent chromium removal in two stage bioreactor system, J. Environ. Eng. 11 (1995) 798–804.
- [6] P.C. DeLeo, H.L. Ehrlich, Reduction of hexavalent chromium by *Pseudomonas fluorescens* LB300 in batch and continuous cultures, Appl. Microbiol. Biotechnol. 40 (1994) 756–759.
- [7] E.I. Kvasnikov, V.V. Stepanyuk, T.M. Kiyushnikova, N.S. Serpokrylov, G.A. Simonova, T.P. Ksathina, L.P. Panchenko, A new chromium reducing gram-variable bacterium with mixed type of flagellation, Mikrobiologiya 54 (1985) 83–88.
- [8] J.L. Bader, G. Gonzalez, P.C. Goodell, A.S. Ali, S.D. Pillai, Aerobic reduction of hexavalent chromium in soil by indigenous microorganisms, Bioremed. J. 3 (3) (1999) 201–211.
- [9] P. Wang, T. Mori, K. Kamori, M. Sesatu, K. Toda, H. Ohtake, Isolation and characterization of an enterobacter cloacae strain that reduces hexavalent chromium under anaerobic conditions, Appl. Environ. Microbiol. 55 (1989) 1665–1669.
- [10] Y.T. Wang, C. Xiao, Factors affecting hexavalent chromium reduction in pure cultures of bacteria, Water Res. 29 (1995) 2467–2474.
- [11] Compendium of Indian Standards on Soil Engineering. Part 1. Bureau of Indian Standards, India, 1989.
- [12] Standard Methods for the Examination of Water and Wastewater, American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1995.
- [13] P. Wang, T.K. Mori, M. Kamori, K. Sesatu, H. Ohtake, Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic condition, Appl. Environ. Microbiol. 55 (1989) 1665–1669.
- [14] L.H. Bopp, Ehrlich, Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300, Arch. Microbiol. 150 (1998) 426–431.
- [15] L. Philip, L. Iyenkar, C. Venkobacher, Cr(VI) reduction by *Bacillus coagulans* isolated from contaminated soil, J. Environ. Eng., ASCE 124 (12) (1998) 1165–1170.
- [16] J.L. Bader, G. Gonzalez, P.C. Goodell, A.S. Ali, S.D. Pillai, Chromium resistant bacterial populations from a site heavily contaminated with hexavalent chromium, Water Air Soil Pollut. 109 (1999) 263–274.
- [17] C.H. Wang, C.P. Huang, P.F. Sanders, Transport of Cr(VI) in soils contaminated with chromite ore processing residue(COPR), Pract. Period. Hazard. Toxic Radioactive Waste Manage. 6 (2002) 6–13.