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Remediation of Cr(VI) from chromium slag by biocementation

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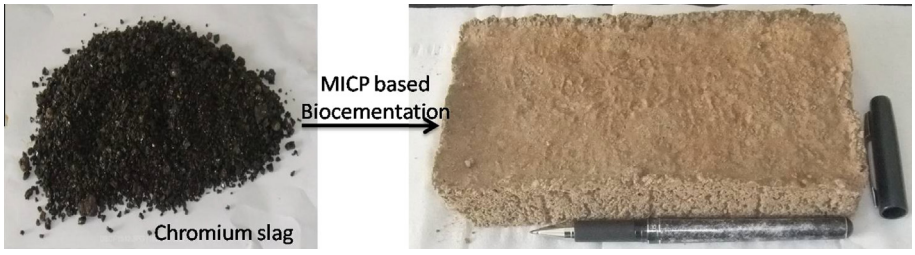
HIGHLIGHTS

- Microbially induced calcite precipitation to immobilize Cr(VI) from chromium slag.
- Biocementation/bio-consolidation of Cr(VI) from chromium slag.
- Incorporation of Cr(VI) into $CaCO_3$ to form a strong complex.
- Cr(VI) mobility was significantly decreased in the exchangeable fraction.
- Cr(VI) concentrations in the leachate decreased significantly after biocementation.

GRAPHICAL ABSTRACT



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ABSTRACT

Here we demonstrate a calcifying ureolytic bacterium *Bacillus* sp. CS8 for the bioremediation of chromate (Cr(VI)) from chromium slag based on microbially induced calcite precipitation (MICP). A consolidated structure like bricks was prepared from chromium slags using bacterial cells, and five stage Cr(VI) sequential extraction was carried out to know their distribution pattern. Cr(VI) mobility was found to significantly be decreased in the exchangeable fraction of Cr slag and subsequently, the Cr(VI) concentration was markedly increased in carbonated fraction after bioremediation. It was found that such Cr slag bricks developed high compressive strength with low permeability. Further, leaching behavior of Cr(VI) in the Cr slag was studied by column tests and remarkable decrease in Cr(VI) concentration was noticed after bioremediation. Cr slags from columns were characterized by SEM–EDS confirming MICP process in bioremediation. The incorporation of Cr(VI) into the calcite surface forms a strong complex that leads to obstruction in Cr(VI) release into the environment. As China is facing chromium slag accidents at the regular time intervals, the technology discussed in the present study promises to provide effective and economical treatment of such sites across the country, however, it can be used globally.

1. Introduction

The rapid pace of industrialization and its resulting by-products have affected the environment by producing hazardous wastes, which are being released in the environment (Khin et al., 2012). Contamination of the environment by Cr, especially chromate (Cr(VI)), has become a major area of concern (Cheung and Gu, 2003; Zayed and Terry, 2003). Among the different forms of chromium, Cr(VI) is highly mobile, most toxic and carcinogenic due to its high solubility in water, rapid permeability through biological membranes, and subsequent interactions with nucleic acids and proteins (Kamaludeen et al., 2003; Volpe et al., 2013), and posses a significant threat to the environment and public health. Cr(VI) contaminates through various industrial processes such as electroplating, metal finishing, leather, mining, and many others (Gao and Xia, 2011).

China is the largest amount of Cr slag producing country with discharges of slag 0.45 Mt annually by various industries (Gao and Xia, 2011). Most Cr industry operations lack appropriate disposal facilities, and hence; chromium slag has become a serious

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problem as a contaminant in soil or water (Huang et al., 2009). Serious Cr slag contamination accidents are occurring frequently in China. The impact of such an accident can be known in terms that made Xinrong in Yunnan province of China, the nearest village to the chemical industry, as "dead village" (Gao and Xia, 2011). In 2011, a total of 0.25 Mt of chromium residue, the byproduct of a local chemical factory, remains unprocessed on a hill in Lianggou village of Yima city in Henan province of China. The toxic chemical leaked into the soil and groundwater due to long rainy season, causing serious environmental problems. Further, high concentrations of chromate are found around many parts of China such as Hunan, Tianjin, Jinzhou and Yunnan ranging from 250–7060 mg kg^{-1} Cr(VI) in soil (Wang et al., 2011; Xu et al., 2011).

The choice of the appropriate method of remediation is governed largely by the mobility, distribution and speciation of Cr in environment. The physico-chemical methods for the remediation of Cr(VI) often require high energy and large quantities of chemical re-agents, which generates environmentally hazardous chemical byproducts (Jeyasingh and Philip, 2005). Thus, these technologies are not completely applied on a large scale. There has been a considerable interest in the use of bioremediation and phytoremediation to treat Cr-contaminated soils, sediments, and waters (Chandra et al., 1997; Lytle et al., 1998). However, the current bioremediation techniques fail mainly because of limitation of phytoremediation in arid area, re-release of immobilized or adsorbed heavy metals by some bacteria in environment, microbial sensitivity to redox potential change and changes into the valence state of particular toxic metal (Achal et al., 2012b). In order to overcome the drawbacks of most of the bioremediation techniques, it is necessary to look for some advanced remediation technologies. Cr(VI) is major toxic form present in Cr slag, so technology should focus on containment or immobilization of this toxic species. Technology with the combination of physical, chemical and biological methods might be most suitable one to prevent environment from Cr slag toxicity. It is important for Cr slag aggregate not to have high water absorption or permeability. A physical barrier over the Cr slag surface will protect it from rainfall, while urease based biochemical reaction might be helpful in the immobilization of Cr(VI). Such protection will prevent the re-release of Cr(VI) and even during heavy rainfall it will not be easy for Cr(VI) to leach out in water bodies or environment.

The method of biocementation, based on microbially induced calcite precipitation (MICP), through the participation of ureolytic bacteria should constitute an alternative method to prevent toxic effects of Cr(VI) from Cr slag to environment. Biocementation, contrary to other concepts, is a natural method and, in principle, makes less severe with the environment, because all the components used for cultivating the substrates as well as and the strain itself, naturally occur in the environment (Achal et al., 2009). Biocementation method has already found practical application in civil engineering (De Muynck et al., 2010; Van Tittelboom et al., 2010; Achal et al., 2011a) and to some extent, in bioremediation of some metals (Pan, 2009; Achal et al., 2011b, 2012a,b). However, to the best of our knowledge such biocementation technology has never been used before to prevent the toxicity effects of Cr slag to the environment.

In the present study, the ability of an ureolytic bacterium, *Bacillus* sp. CS8 isolated from Cr slag has been evaluated in the immobilization of Cr(VI) from Cr slag. A brick of Cr slag was prepared using bacterial culture and a five-step Tessier sequential extraction procedure was adopted to analyze the geochemical speciation of Cr(VI). Further, Cr slag column leaching experiments were performed to know leachability of Cr(VI) into the environment. Chromium slags collected from columns were characterized by SEM–EDS to confirm MICP process.

2. Materials and methods

2.1. Isolation of bacteria

The urease producing chromate resistant bacteria were isolated from chromium slag collected from a steel alloy factory in Hunan province in central southern part of China. The ureolytic bacterial cultures from Cr slag were enriched prior to bacterial isolation by adding 25 g slags into 50 mL of acetate minimal media supplemented with 4% urea in a flask. The acetate minimal media contained (g L^{-1}), NH_4Cl, 1.0; $CaCl_2{\cdot}H_2O$, 0.001; $MgSO_4{\cdot}7H_2O$, 0.2; $FeSO_4{\cdot}7H_2O$, 0.001; sodium acetate, 5; yeast extract, 0.5; K_2HPO_4, 0.5 (pH 7.0). The flask was incubated at 30 °C for 1 week under shaking condition at a speed of 130 rpm. For isolation and enumeration of chromate resistant bacterial strains, the supernatant from enrichment flask was diluted and spread onto acetate minimal agar media supplemented with $K_2Cr_2O_7$. $K_2Cr_2O_7$ was filter sterilized and added to the medium for the initial concentration of 50–250 mg L^{-1}. The plates were incubated at 30 °C overnight. Bacterial isolates that could tolerate the highest chromate concentration were selected. Subsequently, the colonies were transferred onto urea agar base, urease selective medium, to check the production of urease.

2.2. Identification of bacterial isolate

Genomic DNA was extracted from overnight grown bacterial cells using TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China). The 16S rRNA gene from the genomic DNA was amplified by PCR using the following primers: 5′-AGA GTT TGA TCC TGG CTC AG-3′ and 5′-AAG GAG GTG ATC CAG CCG CA-3′ corresponding to the forward and reverse primers of 16S rDNA, respectively. PCR amplification was performed using a LongGene MG96 + thermocycler (Hangzhou, China) as described by Achal and Pan (2011). 16S rRNA amplicon was gel eluted and ligated into the pTZ57R/T vector as per manufacturer's instruction (Fermentas, USA). The samples were sent to Beijing Genomics Institute (BGI, Beijing, China) for sequencing. A BLAST search was performed to find the possible sister groups of the newly sequenced taxa. The sequences were edited with BioEdit 5.0.6 and aligned using MAFFT v 6.240 with other sequences obtained from GenBank. Bayesian phylogenetic analysis was done in MrBayes v. 3.1.2. Two simultaneous independent replicates of five were run for 5 million generations with sampling at every 100th generation, and the convergence of the runs visualized using Tracer ver. 1.4.

2.3. Cr slag brick preparation

A brick of size 18 cm × 9.5 cm × 3.5 cm (l × w × h) was constructed using Cr slag, sand, soil and bacterial culture. An amount of 800 g Cr slag, 200 g sand and 100 g soil was mixed with 100 mL of overnight grown bacterial cells of cfu equivalent to 5×10^7 CFU mL^{-1}. The selected bacterial strain CS8 was inoculated into NB (nutrient broth) media containing 2% urea and 25 mM $CaCl_2$ (hereafter name is used as NBU media), incubated at 30 °C under shaking condition (130 rpm). Sand and soil were sterilized properly prior to use to remove any microbial cell. The soil contained 19.2 mg kg^{-1} of total chromium out of which 8.1 mg kg^{-1} was Cr(VI). In control experiment, no bacterial cells were added. A specifically fabricated formwork was used to prepare such Cr slag bricks. The specimens were incubated at 30 °C for 1 month. NBU media were overlaid on the bricks at an interval of 7 d. After the termination of experiment, Cr slag bricks were subjected to chromium sequential extraction analysis, compressive strength and water absorption tests.

2.4. Cr(VI) sequential extraction and analysis

The five-stage Tessier sequential extraction method was used for Cr(VI) fractionation in Cr slag bricks specimens. The following Cr(VI) fractionation was obtained: exchangeable, carbonate bound, Fe–Mn oxides bound, organic matter bound and residual fractions (Tessier et al., 1979).

The Cr(VI) concentration was estimated by the diphenylcarbazide method. Briefly, 0.25 mL of solution (0.5 g of diphenylcarbazide in 10 mL of acetone) was added to 5 mL of sample and 0.05 M H_2SO_4 was used to acidify the samples, and the absorbance was measured spectrophotometrically at 540 nm (Urone, 1955).

2.5. Compressive strength of Cr slag brick

This test was done in order to determine the compressive strength of the Cr slag bricks against vertical loading. Compression testing was performed using a manual compression-testing machine (769YP-15A, Tianjin, China). The specimen was placed into machine and was gradually loaded until it failed (cracked). The ultimate load was recorded. The values were expressed in Mpa.

2.6. Water absorption test

To determine the increase in resistance toward water penetration, a sorptivity test was carried out as discussed in previous literature with slight modification (Achal et al., 2011a). The Cr slag bricks specimens were exposed to 10 ± 1 mm of water. At regular time intervals (15 min up to 12 h), the specimens were removed from the water and weighed after drying the surface with a wet towel. Immediately after the measurement, the test specimens were submerged again. The sorption coefficient, k (cm $s^{-½}$), was obtained by using the following expression:

$Q/A = k\sqrt{t}$, where Q = amount of water absorbed (cm^3); A = cross section of the specimen that was in contact with water (cm^2); t = time (s); Q/A was plotted against the square root of time, then k was calculated from the slope of the linear relation between the former.

2.7. Cr slag column leaching experiment

For studying leaching behavior of Cr(VI) in the Cr slag, column tests were conducted. Experimental set up consisted of glass column (3 cm diameter and 20 cm height) packed with 90 g of Cr slag. The slag was added to the columns in 10 g portions and was placed between additions by shaking the columns. The walls of columns were covered with single layer of sterilized sand from inside. A fine textured synthetic cloth acted as a 6 μm filter for the leachate. The glass columns were saturated well with overnight grown bacterial cells of cfu equivalent to 5×10^7 CFU mL^{-1}. Bacterial cells were not added into control columns. The columns were incubated at 30 °C. After 48 h, leaching of column was started. Milli Q water was used as leaching solutions. The glass columns were continuously fed for 168 h at an interval of 24 h with leaching solution. Cr(VI) in the leachate was determined at regular time interval by the diphenylcarbazide method.

2.8. Characterization of Cr slag from leachate experiment

The Cr slag samples collected from control and bioremediated columns were analyzed in a SEM (Zeiss SUPRA 55VP, Germany). The samples were mounted on an aluminum holder with a carbon conductive tape, and later covered with a gold layer, approximately 200 Å in thickness, in an Emitech K575 sputtering system. Prior to scanning, the samples were dried at room temperature for a week. The elemental composition of the same samples was determined by EDS with an energy-dispersive X-ray analyzer.

2.9. Statistical analysis

All the experiments were performed in triplicate. The data were analyzed by ANOVA, and the means were compared by Tukey's test using GraphPad Prism (version 4.0) software.

3. Results and discussion

3.1. Isolation and identification of Cr(VI) reducing bacteria

To assess the possible effect of chromium stress on the isolation of the bacteria, Cr(VI) at different concentrations (50–250 mg L^{-1}) was introduced into the isolation medium to obtain Cr(VI) resistance. Among the different bacteria isolated, one bacterial strain designated as CS8 was selected based on higher urease production and ability to grow luxuriously in media amended with higher concentrations of Cr(VI). This isolate may have developed Cr(VI) resistance systems in an attempt to protect sensitive cellular components. Generally, the activity of microbial cells, those grow at high metal concentration, is found coupled with a variety of specific mechanisms of resistance and environmental factors (Gadd, 2000).

The bacterial strain CS8 was identified based on 16S rDNA sequencing analysis. The sequences generated from the 16S rDNA contained 1435 bp, the aligned dataset contain 1422 characters and there were 388, 318 and 716 constant, parsimony-uninformative and parsimony-informative characters, respectively. Gaps were treated as missing. The details of branch-and-bound search that yielded into parsimony trees are provided in Supplementary Materials 3.1. Maximum likelihood analysis recovered as single topology ($-\ln L = 11\,538$). The resulting maximum parsimony and maximum likelihood topologies did not differ significantly. The isolate CS8 formed a well-supported monophyletic lineage (Fig. 1). Most single species clades received moderate to strong support (61–91% Bootstrap Support, 67–100% Posterior Probability), although the nodes indicating relationship amongst them generally received less support.

The phylogenetic analysis showed that the isolate CS8 belongs to the phylum Firmicutes and the family Planococcaceae. Nine sequences were included in the dataset, *Sporolactobacillus inulinus* was used as outgroup taxon for rooting purposes. The phylogenetic analysis grouped CS8 into *Bacillus aryabhattai*, *Bacillus megaterium*, and *Bacillus flexus*, thus identified as *Bacillus* sp. (Fig. 1). The 16S rRNA gene sequence for the same was deposited in GenBank of NCBI under the accession number JX861098.

3.2. Cr(VI) analysis in Cr slag brick

Chromium slag brick contained Cr slag, sand, soil and bacterial culture; however there was no bacterial cells added in control specimens. A representation of specimen is shown in Fig. 2. There was no significant difference observed by naked eyes between control and bacterially prepared specimens, however clear white precipitate (due to MICP) and more compactness was noticed in those specimens prepared by *Bacillus* sp. CS8. Sand or soil was used as additive during preparation of Cr slag bricks as it induces calcifying ability of ureolytic bacteria (DeJong et al., 2010). Additionally such experiment would also give a comprehensive picture about bioremediation of soil contaminated with Cr slags.

After incubating all the Cr slag bricks for a month, Cr(VI) was estimated from bacterially prepared specimens and results were also compared with controls. To provide a comprehensive picture

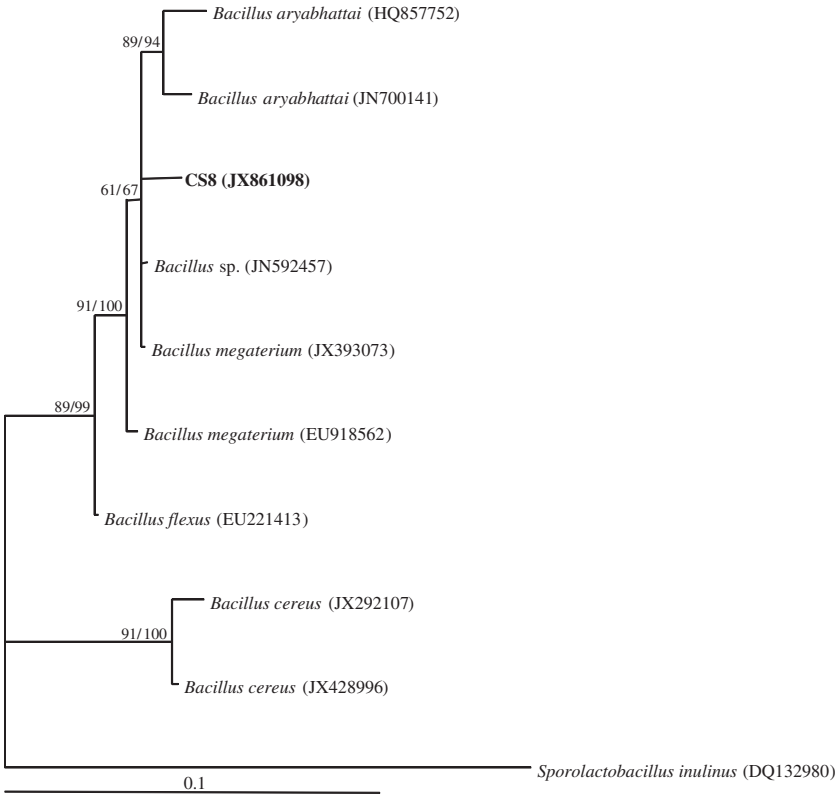

Fig. 1. Phylogeny of CS8 generated from Bayesian analysis of 16S rDNA sequences rooted with *Sporolactobacillus inulinus*. Bayesian posterior probability (PP) values, >50%, are given at the internodes (BS/PP).

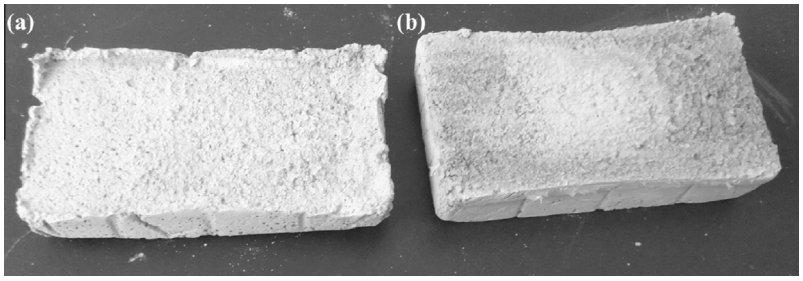

Fig. 2. (a) Bacterial Cr slag brick and (b) control specimen.

of Cr(VI) bioavailability and other potential risks, the Cr(VI) concentration in different fractions was determined by sequential extraction. The remediation of exchangeable Cr(VI), carbonate bound Cr(VI), Fe–Mn oxides bound Cr(VI), organic matter bound Cr(VI) and residual Cr(VI) by *Bacillus* sp. CS8 in the Cr slag bricks were investigated. The results are presented in Fig. 3. The control specimens contained 124.8 mg kg^{-1} Cr(VI) in exchangeable fraction that decreased to 2.6 mg kg^{-1} in bacterial specimens. Significantly higher ($p < 0.05$) exchangeable Cr(VI) fraction in control indicates that abiotic matter revealed no ability of Cr(VI) remediation (Chai et al., 2009). The residual fraction of Cr(VI) did not change significantly in control and bacterial Cr slag brick specimens as it was 61 and 66%, respectively. The heavy metals in the residual fraction are tightly bound and would not be expected to be released under natural conditions (Xian, 1989).

Furthermore, carbonate bound Cr(VI) concentration increased significantly ($p < 0.05$) in bacterial specimens compared to control. Such results imply the possibility of calcite precipitation based on ureolytic activity by *Bacillus* sp. CS8. Cr(VI) is found to be preferentially incorporated into the calcite surface during crystal growth

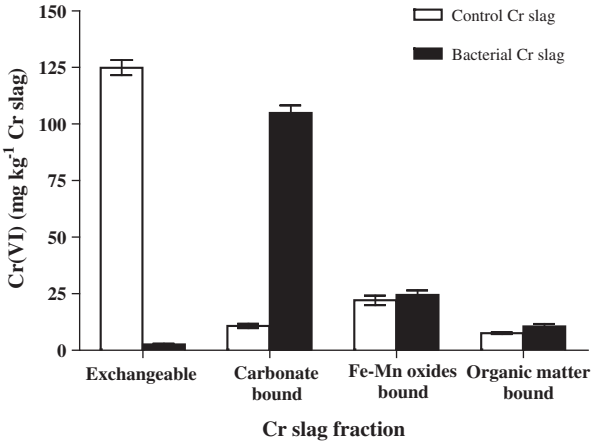

Fig. 3. Cr(VI) concentration in Cr slag bricks for the control and bacterial specimens.

(Tang et al., 2007). The Cr(VI) in calcium carbonate structure was resistant to gaseous reductants or solution-phase extractants (Thornton and Amonette, 1999; Hua et al., 2007), implying the long-term stability of Cr(VI) incorporated in the calcium carbonate. The strong Cr(VI)-calcite complex precipitation leads to obstruction in Cr(VI) release into the environment.

However, there were no much differences in control and bacterial specimens in terms of Fe–Mn oxides bound Cr(VI). Cr(VI) from both specimens can react with iron sulfide, and many other hydrous inorganic oxides such as Fe, Mn, Al oxides (Peterson et al., 1997; Kendelewicz et al., 1999; Wei et al., 2002). The species of Cr(VI) associated with organic matter was found to be only 7.6 mg kg^{-1} in control while bacterial specimens contained slightly higher content i.e., 10.3 mg kg^{-1}. Metal species associated with organic matter are either complexed or adsorbed, thus they are tightly held and their release into the solution is slow (Achal et al., 2012b).

3.3. Compressive strength of Cr slag brick

Compressive strength test was done in order to know effectiveness of MICP. It may be noteworthy to say that control specimens were not able to take load from compression testing machine and it failed to give any value. The compressive strength had been significantly increased ($p < 0.05$) for the Cr slag bricks that contained microbial cells. The compressive strength recorded in specimens prepared with bacterial cells was 0.36 (±0.02) MPa. The compressive strength of Cr slag bricks was pronounced and similar or little higher with that of strength with mud bricks and unburned bricks.

The improvement in compressive strength by *Bacillus* sp. CS8 is due to the deposition of $CaCO_3$ on the cell surfaces and within the pores of Cr slag–sand–soil matrix, which plugs the pores within the whole specimens (Ramakrishnan et al., 1998; Ramachandran et al., 2001; Ghosh et al., 2005; Achal et al., 2009). Increases in compressive strength, based on MICP, of cement mortar by different bacteria *Sporosarcina pasteurii* and *Shewanella* sp. were also reported by some researchers (Ramachandran et al., 2001; Ghosh et al., 2005). Biocementation forms calcium carbonate inside and between aggregate of Cr slag that leads to increase in compressive strength of Cr slag bricks. The process may be summarized according to following equations:

$$\textit{Bacillus}\ \text{sp. CS8} + Ca^{2+} \rightarrow \textit{Bacillus}\ \text{sp. CS8} - Ca^{2+}$$

$$H_2N - CO - NH_2 \leftrightarrow 2NH_4^+ + CO_3^{2-}$$

$$\textit{Bacillus}\ \text{sp. CS8} - Ca^{2+} + CO_3^{2-} \rightarrow \textit{Bacillus}\ \text{sp. CS8} - CaCO_3 \downarrow$$

Bacillus sp. CS8 can attract Ca^{2+} ions present in media or subsequent environment, which react with CO_3^{2-} originating from urea hydrolysis. Simultaneously, NH^{4+} increase pH value in surrounding medium, which improves calcite precipitation efficiency (Stocks-Fischer et al., 1999; Achal et al., 2013).

3.4. Water absorption test

The water absorption test was carried out on both control and bacterially prepared Cr slag bricks over a period of 12 h, however the control specimens collapsed during this test after 6 h. The presence of bacterial cells resulted in a significant decrease of the water uptake compared to control specimens. Over a period of 6 h, the Cr slag brick specimens treated with *Bacillus* sp. CS8 absorbed nearly four times less water than the control specimens (Fig. 4).

The deposition of a layer of calcium carbonate crystals on the surface resulted in a decrease of the sorptivity. Previously a decrease in the permeability of sandstone was noticed by injecting $CaCO_3$-forming reactants (Nemati and Voordouw, 2003) while Van Tittelboom et al. (2010) reported that the crack sealing by *Bacillus sphaericus* based on MICP resulted in a decrease in water permeability.

From this experiment, it is clear that the presence of a layer of carbonate crystals on the surface of Cr slag by bacterial isolate has the potential to improve its performance to combat with degradation processes. As a consequence, the leachability behavior of Cr(VI) would be limited, and will prevent water body or ground water to get contaminated with this toxic form of chromium, especially during rainy season. Cr slag bricks based on MICP shall be of sufficient durability to withstand the effect of driving rain appropriate to their exposure situation and will develop resistance to erosion by rainfall.

3.5. Cr slag column leaching experiment

Chromium slag column leaching experiment was performed to know whether MICP serves as barrier to resist water flowing through it or not. After 7 d of leaching experiment, clear white precipitate was observed in bacterially treated glass columns all around Cr slag that was absent in control (Fig. SM-1). It was noticed that flow rate through Cr slags from columns treated with *Bacillus* sp. CS8 was slower compared to control. The reduced permeability or deflected flow in bacterial columns can result from calcite layer. Calcium carbonate precipitation induced by bacterial cells will plug the pores around Cr slags that act as barrier to any ingress substances into it. Because of such barrier with

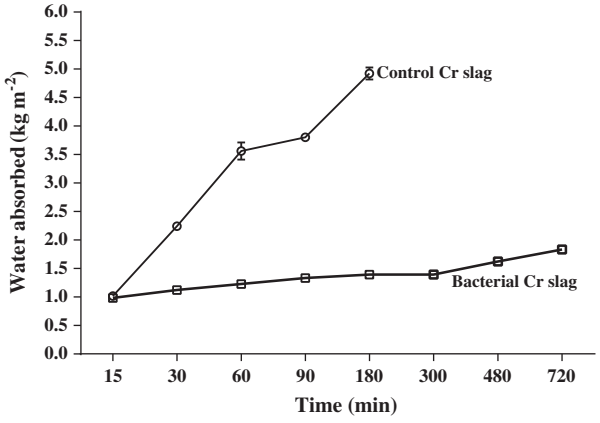

Fig. 4. The influence of the bacterial treatment on the rate of water absorption by Cr slag bricks at different time intervals.

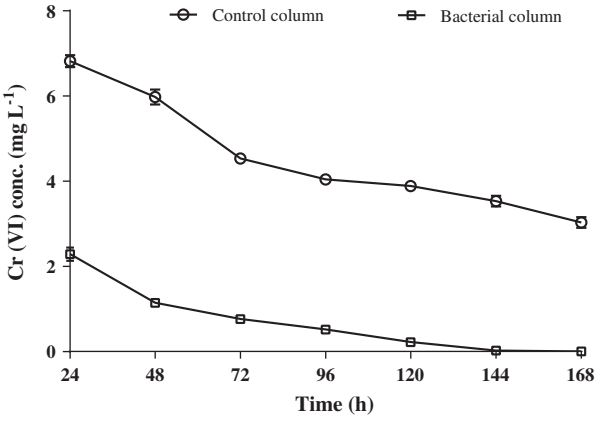

Fig. 5. Cr(VI) concentration in leachate collected from control and bacterial Cr slag columns.

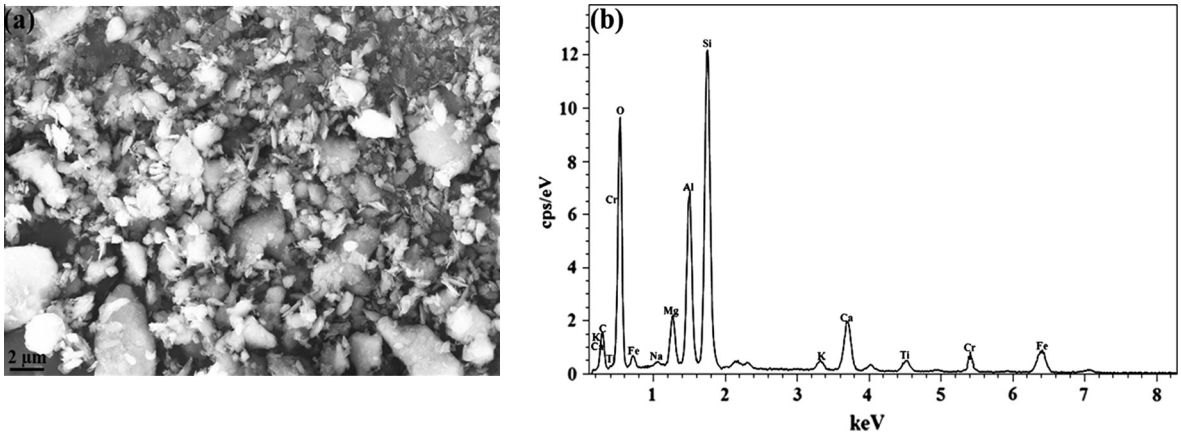

Fig. 6. (a) SEM micrograph of Cr slag collected from control column and (b) respective EDS spectrum showing a high abundance of the principal components of aluminosilicates along with sharp Cr peak.

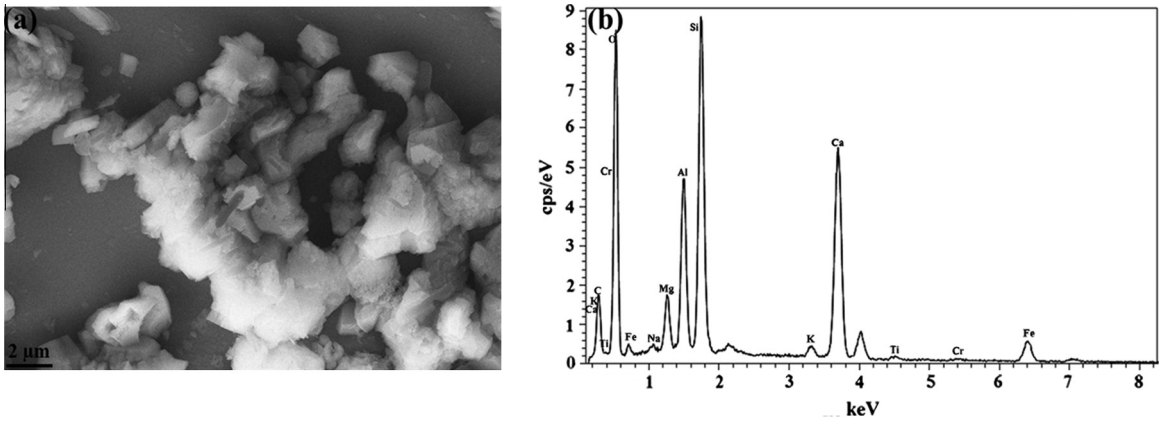

Fig. 7. (a) SEM micrograph of Cr slag collected from bacterially treated column showing rod-shaped structure housed by*Bacillus* sp. CS8 and (b) respective EDS spectrum showing negligible amount of Cr.

impermeable behavior, water system will not get easily contaminate with Cr(VI) even in rainy season.

The Cr(VI) concentrations in the leachate decreased strongly with respect to time in bioremediated columns and almost disappeared at the end of 168 h (Fig. 5). On the other hand, leachate from control columns contained significant amount of Cr(VI) over time. A much larger amount of Cr(VI) was leached out with water in control samples. This reduction in Cr(VI) release from the columns could have been due to incorporation of Cr(VI) into the calcite surface (Tang et al., 2007), induced by *Bacillus* sp. CS8.

3.6. Characterization of Cr slag from leachate experiment

Further to explicit the role of MICP, Cr slag samples collected from control and bacterial columns were examined under SEM. Fig. 6a shows a SEM of bacteria-free Cr slag, while Fig. 7a shows micrographs of the Cr slag treated with *Bacillus* sp. CS8. Control samples presented a typical morphology of Cr slag and appear as discrete particles. The preferential precipitation of calcite at the Cr slag contacts can be seen in bacterially treated samples. The bacterial treatment resulted in the prominent presence of crystalline deposits on the surface of Cr slag. The bacteria indentations can be seen, in Fig. 7a, as being dispersed into calcite structure with rod-shaped structures. It can be roughly assumed with these SEM observations that calcite was evenly distributed throughout the Cr slag samples treated with *Bacillus* sp.

The Cr slag samples were also analyzed by EDS. The Cr slags contained mainly elements such as Si, Mg, Al, Fe and O, which are the principal components of a typical Cr slag collected from mining area (Lan, 1998). As EDS is semi-quantitative technique, elemental quantity has not been shown here. Clear sharp chromium peaks were observed in the EDS spectrum of leachate collected from control soil (Fig. 6b). The EDS analysis demonstrated that MICP process was effective in immobilizing Cr(VI) from Cr slag, as negligible chromium was detected in the EDS spectra of Cr slag collected from bacterially treated columns (Fig. 6b).

4. Conclusions

This is first of its kind of study where MICP was proved as an effective process for the remediation of Cr(VI) from chromium slags using ureolytic chromate reducing *Bacillus* sp. CS8. The calcite deposition on the Cr slag surface reduces the permeability as it serves as a barrier to harmful substances to enter, will make it resistant to erosion by rainfall, thus preventing water-bodies to get contaminate with Cr(VI) pollution. Cr(VI) incorporated in the calcium carbonate lattice is also chemically stable. The technology has huge potential for its potential use in remediation of Cr slag contamination sites.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2013.08.008.

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