Characterization of Chromium Waste Form Based on Biocementation by *Microbacterium* sp. GM-1

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Abstract This paper demonstrated a biocementation technology for chromium slag by strain GM-1, a calcifying ureolytic bacterium identified as *Microbacterium*, based on microbially induced calcium carbonate. The characterization of *Microbacterium* sp. GM-1 was assessed to know the growth curve in different concentrations of Cr(VI). *Microbacterium* sp. GM-1 was tolerant to a concentration of 120 mg/L Cr(VI). Chromium waste forms were prepared using chromium, sand, soil and bacterial culture. There we had three quality ratios (8:2:1; 8:1:1; 8:2:0.5) of material (chromium, sand and soil, respectively). Bacterial and control chromium waste forms were analyzed by thermal gravimetric analyzer. All bacterial forms (8:2:1; 8:1:1; 8:2:0.5 J) showed sharp weight loss near the decomposition temperature of calcium carbonate between 600 and 700 °C. It indicated that the efficient bacterial strain GM-1 had induced calcium carbonate precipitate during bioremediation process. A five step Cr(VI) sequential extraction was performed to evaluate its distribution pattern in chromium waste forms. The percentage of Cr(VI) was found to significantly be decreased in the exchangeable fraction of chromium waste forms and subsequently, that was markedly increased in carbonated fraction after biocementation

by GM-1. Further, compressive strength test and leaching test were carried out. The results showed that chromium waste forms after biocementation had higher compressive strength and lower leaching toxicity. Additionally, the samples made of 8:1:1 (m/m/m) chromium + sand + soil were found to develop the highest compressive strength and stand the lowest concentration of Cr(VI) released into the environment.

Keywords Chromium slag · Cr(VI) · Biorecimentation · Calcium carbonate · Waste form

Introduction

Chromium slag is collectively known as the waste emissions in the production of metal chromium, chromium salt and ferrochrome [1]. Cr(VI) from chromium slag is most toxic due to its strong oxidation, carcinogenic properties, mutagenesis effects and corrosion, leading the damage of organism [2]. In the rain days, Cr(VI) from chromium slag which open to the sky released into the environment, polluting the soil and groundwater [3]. Chromium treatment is lacked of facilities, and chromium slag has become a serious problem as a contaminant in soil or water [4]. The method of chromium slag remediation should focus on reducing the content of Cr(VI) leaching into the environment. Physical methods consume high energy and chemical methods require amount of chemical reagents, which generates second pollution [5]. In order to overcome the limitation of these methods, there is a considerable interest in the use of biocementation. Cr(VI) is the main toxic form in chromium slag, so the method should pay more attention to containment or immobilization of this toxic species.

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The biocementation of *Microbacterium* sp. GM-1, based on microbially induced calcium carbonate precipitation, should prevent toxic effects of Cr(VI) from chromium slag to the environment. Calcium carbonate have higher strength and durability [6]. The method of biocementation has incomparable superiority and was widely used in civil engineering. It was found concrete material after biocementation greatly resisted the damage from acid, alkali, freeze–thaw cycle and dry shrinkage deformation [7]. Calcite particle induced by bacteria was tightly bound, which can effectively improve the permeability resistance of specimen [8]. What's more, it could be found that calcium carbonate crystal stuck on the surface of specimen by ultrasonic technology, improving carbonation resistance [9].

In the present study, the ability of Cr(VI) resistant for *Microbacterium* sp. GM-1 was evaluated. Chromium waste form was prepared using *Microbacterium* sp. GM-1 and a five-step Tessier sequential extraction procedure was adopted to analyze the geochemical speciation of Cr(VI). Further, test was done to understand the compressive strength of chromium waste forms against vertical loading, while leaching test were to assess the long term stability of Cr(VI).

Materials and Methods

Strain and Culture

Microbacterium sp. GM-1, a Gram-Positive strain, was isolated from active sludge and identified to hydrolysis urea in previous study. The purity of strain GM-1 was checked by 16S rRNA gene sequence analysis. The partial 16S rRNA gene sequence was analyzed with BLASTN program online [10] and deposited in Genbank (accession number KT893328). In this study, *Microbacterium* sp. GM-1 was cultured with NB (Nutrient Broth) containing (L^{-1}) 10 g peptone, 3 g beef extract and 5 g NaCl.

Cr(VI)-Resistant Experiment

Cells of *Microbacterium* sp. GM-1 were grown at 30 °C for 24 h in 100 mL NB medium. To assess the Cr(VI)-resistance of *Microbacterium* sp. GM-1 in different concentrations of Cr(VI), Cr(VI) were filter sterilized and added to the medium. The initial concentrations of Cr(VI) were 0, 50, 100, 200 and 120, 140, 160, 180 mg/L. *Microbacterium* sp. GM-1 was incubated on a shaker at 120 rpm at 30 °C. A microplate reader was used to determine the optical density at 600 nm for the concentrations of microorganisms.

Chromium Waste Form Preparation

The select strain GM-1 was metured in NB (nutrient broth) media containing 2 % urea at 30 °C under shaking condition (120 rpm). The equimolar $CaCl_2$ with urea was added into fresh NB as nutrient solution. A chromium waste form of 2 cm × 2 cm × 2 cm (l × w × h) was constructed using chromium slag, sand, soil and nutrient solution. Chromium slag, sand and soil were mixed in three quality ratios (8:2:1; 8:1:1; 8:2:0.5). Then, overnight grown bacterial cells (equivalently $OD_{600} = 0.751$) and nutrient solution were added into the mixture. Control forms were also simulated in a similar way without adding bacterial cells. All the chromium waste forms were incubated in room temperature for 28 days. Nutrient solution was overlaid on the samples at an interval of 7 days.

Thermogravimetric Analysis

Both bacterial and control chromium waste forms were analyzed by thermal gravimetric analyzer (TGA/1600LF, CH). Weight loss of samples at different temperature (100–1000 °C) was recorded. The difference between bacterial and control chromium waste forms were analyzed.

Cr(VI) Sequential Extraction and Analysis

The five-stage Tessier sequential extraction method was used for Cr(VI) fraction in chromium waste forms. Cr(VI) fraction were exchangeable, carbonate bound, Fe–Mn oxides bound, organic matter bound and residual fractions [11]. The procedure of Tessier sequential extraction method was as followed:

- Exchangeable. The sample (1 g) was extracted at room temperature for 1 h with 8 ml of magnesium solution (1 M $MgCl_2$, pH = 7.0).
- Carbonate bound. The residue from (a) was leached at room temperature with 8 ml of 1 M NaOAc adjusted to pH = 5.0 with acetic acid (HOAc). Continuous agitation was maintained and the time necessary for complete extraction was evaluated.
- Fe–Mn oxides bound. The residue from (b) was extracted at room temperature for 16 h with 20 mL of 0.3 M $Na_2S_2O_4$ + 0.175 M Na-citrate + 0.025 M H-citrate.
- Organic matter bound. The residue from (c) was added 3 mL of 0.02 M HNO_3 and 5 mL of 30 % H_2O_2 adjusted to pH = 2 with HNO_3 , and the mixture was heated to 85 ± 2 °C for 2 h with occasional agitation. A second 3 mL of 30 % H_2O_2 (pH = 2 with HNO_3) was then added and the sample

was heated again to 85 ± 2 °C for 3 h with intermittent agitation. After cooling, 5 mL of 3.2 M NH_4OAc in 20 % (v/v) HNO_3 was added and the sample was diluted to 20 mL and agitated continuously for 30 min.

- (e) Residual fractions. The residue (0.9 g) from (d) was extracted with 20 ml (1 + 10) H_3PO_4 at 121 °C for 30 min to analyze total metal [12].

Otherwise, the Cr(VI) concentration was estimated by the diphenylcarbazide method.

Compressive Strength of Chromium Waste Form

Aiming at understanding the compressive strength of chromium waste forms against vertical loading, the test was done. Pressure value (F) was the experimental results. The computational formula of compressive strength (P) was as follows: $P = F/S$, where S was stressed area of samples. The value was expressed in MPa.

Leaching Toxicity Test

Chromium waste forms were crushed. Sulfuric acid–nitric acid method was used for the particles of chromium waste forms to determine the static leaching toxicity. 5 g samples were mixed with 50 ml extraction agent and placed in shaking condition for 16 h. The supernatant liquid was collected finally [13]. In order to evaluate the long-term stability of the specimens, dynamic leaching toxicity test was carried out. The entire chromium waste forms were covered with deionized water for 7 days. The leaching solution was collected at an interval of 1 days [14]. The Cr(VI) concentrations from supernatant liquid and leaching solution were estimated by the diphenylcarbazide method [15].

Results and Discussion

Cr(VI)-Resistance of *Microbacterium* sp. GM-1

Growth curve reflected the colony growth of microorganism under certain environmental conditions. According to the different growth rate, the growth curve generally divided into lag phase, logarithmic phase, stationary phase and decline phase. The length of these four times changed with the difference of bacterial hereditary, bacterial inoculum size and culture conditions. For Cr(VI) was heavy metal ions, determining the growth curve of GM-1 in different concentrations of Cr(VI), help in understanding its growth regularity and Cr(VI)-resistance characteristics. These benefited the choice to logarithmic phase and culture

condition of GM-1 in later experiments. When Optical density (OD) was 600 nm, concentration of bacteria suspensions was directly proportional to OD_{600} value. This paper measured the absorbance values in 600 nm of GM-1 in different concentrations of Cr(VI).

Figures 1 and 2 showed the growth curve of the *Microbacterium* sp. GM-1 in NB medium containing different concentrations of Cr(VI). In the low initial Cr(VI) concentrations (0, 50, 100, 120 mg/L), the growth rate of *Microbacterium* sp. GM-1 was decreased a little. In 0, 50 mg/L Cr(VI), growth curve of GM-1 was similar, which did not present a lag phase. GM-1 grew rapidly into the logarithmic phase. However, in 100, 120 mg/L Cr(VI), there was a lag phase obviously and bacterial growth was slow. Because the bacteria need to adapt to the high concentrations of Cr(VI). In contrast, higher concentrations of Cr(VI), such as 140, 160, 180, 200 mg/L, was resulted in a obvious decline over time. The growth of GM-1 was significantly inhibited and OD_{600} was almost close to 0. This might be explained as an increased time period for adaptation or DNA repair during the exposure to high levels of Cr(VI) in the NB medium. Understanding Cr(VI)-resistance ability of *Microbacterium* sp. GM-1 was benefited to the remediation for chromium slag in later, due to Cr(VI) was the main contaminant in it.

Fig. 1 The growth curve of *Microbacterium* sp. GM-1 at different Cr(VI) concentrations. Overnight culture of *Microbacterium* sp. GM-1 were cultivated in NB medium containing different concentrations of Cr(VI) (0, 50, 100 and 200 mg/L respectively) and the concentrations of microorganisms was detected once every 2 or 4 h. This paper measured OD_{600} . When Optical density (OD) was 600 nm, concentration of bacteria suspensions was directly proportional to OD_{600} value. Where error bars are shown, values are the average of triplicates and error bars represent the range

Fig. 2 The growth curve of *Microbacterium* sp. GM-1 at different Cr(VI) concentrations. Overnight culture of *Microbacterium* sp. GM-1 were cultivated in NB medium containing different concentrations of Cr(VI) (120, 140, 160 and 180 mg/L respectively) and the concentration of microorganisms was detected once every 2 or 4 h. This paper measured OD₆₀₀. When Optical density (OD) was 600 nm, concentration of bacteria suspensions was directly proportional to OD₆₀₀ value. Where error bars are shown, values are the average of triplicates and error bars represent the range

Thermogravimetric Analysis

Bacterial and control chromium waste forms were analyzed by thermal gravimetric analyzer (TGA/1600LF, CH). Weight loss of samples at different temperature (100–1000 °C) was recorded. Figures 3, 4, 5 showed the different weight loss between bacterial and control chromium waste forms. All bacterial forms (8:2:1; 8:1:1; 8:2:0.5 J) showed sharp weight loss near the decomposition temperature of calcium carbonate between 600–700 °C, but all control forms (8:2:1; 8:1:1; 8:2:0.5 W) didn't show weight loss during the process of heating obviously. The thermal decomposition temperature of calcium carbonate ranged 600–900 °C. The reason for quality reduction in bacterial samples is the thermal decomposition, which generated gaseous carbon dioxide. The result indicated that the efficient bacterial strain GM-1 had induced calcium carbonate precipitate during bioremediation process.

Cr(VI) Analysis in Chromium Waste Forms

After incubated for 28 days, Cr(VI) in treatments receiving bacteria and control samples was assessed. The contents of Cr(VI) in five fractions were tested. Finally, the percentage of Cr(VI) in total was calculated. Figure 6 showed fraction

Fig. 3 Thermogravimetric analysis for chromium waste forms in 8:2:1. Chromium waste forms in 8:2:1 was broke after incubating for 28 days. Thermal gravimetric analyzer (TGA/1600LF, CH) recorded the quality along with the changes of temperature (the change rate was 10 °C/min and the temperature ranged 30–1000 °C)

Fig. 4 Thermogravimetric analysis for chromium waste forms in 8:1:1. Chromium waste forms in 8:1:1 was broke after incubating for 28 days. Thermal gravimetric analyzer (TGA/1600LF, CH) recorded the quality along with the changes of temperature (the change rate was 10 °C/min and the temperature ranged 30–1000 °C)

distributions of Cr(VI) in different samples. The percentage of Cr(VI) from control samples was higher than that from treatments receiving bacteria in exchangeable fraction. The maximum value of control groups was up to 26.62 % (8:1:1 W). While the minimum value of treatments receiving bacteria was only 10.70 % (8:2:0.5 J). Significantly higher exchangeable Cr(VI) fraction indicated that abiotic matter revealed no ability of Cr(VI) remediation.

Fig. 5 Thermogravimetric analysis for chromium waste forms in 8:2:0.5. Chromium waste forms in 8:2:0.5 was broke after incubating for 28 days. Thermal gravimetric analyzer (TGA/1600LF, CH) recorded the quality along with the changes of temperature (the change rate was 10 °C/min and the temperature ranged 30–1000 °C)

Furthermore, the percentage of Cr(VI) in carbonate bound increased significantly in treatments receiving bacteria compared to control groups. That of treatments receiving bacteria (8:2:1; 8:1:1; 8:2:0.5 J) were up to 21.36, 22.70 and 18.73 %, respectively. The differences among three treatments receiving bacteria were not obvious, but the samples in 8:1:1 J also reached the maximum value. Such result implied the possibility of calcium carbonate precipitation based on ureolytic activity by *Microbacterium* sp. GM-1. Cr(VI) is found to be preferentially incorporated into the calcium carbonate surface during crystal growth. The Cr(VI) in calcium carbonate structure was resistant to gaseous reductants or solution-phase extractants, indicating the long-term stability of

Cr(VI) incorporated in the calcium carbonate [16]. The strong Cr(VI)-calcite complex precipitation leads to obstruction in Cr(VI) released into the environment.

However, there were no much differences in treatments receiving bacteria and control samples in terms of Fe–Mn oxides bound. The percentage of Cr(VI) in Fe–Mn oxides bound was 45.56, 48.24, 54.27 % (8:2:1; 8:1:1; 8:2:0.5 J) and 55.43, 45.72, 51.14 % (8:2:1; 8:1:1; 8:2:0.5 W). Cr(VI) from both samples can react with iron sulfide, and many other hydrous inorganic oxides such as Fe, Mn, Al oxides [17–19]. It was seen that the percentage of Cr(VI) in organic matter bound was all low. The value was nearly 0.5 %. Samples associated with organic matter are either complexed or adsorbed, thus they are tightly held and their release into the solution is slow [20].

The residual fraction of Cr(VI) did not change significantly in treatments receiving bacteria and control samples as it was 45.56, 48.24, 54.27, 55.43, 45.72 and 51.14 %, respectively. The ones in treatments receiving bacteria were a little higher than that in control groups. The heavy metals in the residual fraction are tightly bound and would not be expected to be released under natural conditions [21].

Compressive Strength of Chromium Waste Forms

Compressive strength test was done in order to know effectiveness of calcium carbonate. Table 1 and Fig. 7 showed the result. The compressive strength of the chromium waste forms that contained *Microbacterium* sp. GM-1 were increased compared with that of control samples generally. The compressive strength recorded in samples prepared with bacterial cells was 0.35, 0.63, 0.38 MPa (8:2:1; 8:1:1; 8:2:0.5 J). The sample prepared with 8:1:1 (m/m/m) (chromium + sand + soil) reached the highest value. While the control ones were 0.31, 0.35, 0.18 MPa

Fig. 6 Cr(VI) fraction of chromium waste forms. All chromium samples were prepared in three proportion (8:2:1; 8:1:1; 8:2:0.5) of chromium, sand and soil. There were treatments receiving bacteria mixed with *Microbacterium* sp. GM-1 (J) and control groups mixed with water (W). The five-stage Tessier sequential extraction method was used for Cr(VI) fraction. The contents of Cr(VI) in five fractions were tested. Finally, the percentage of Cr(VI) in total was calculated

Table 1 Compressive strength of chromium waste forms

Number	Compressive strength (MPa)			Average
	1	2	3	
8:2:1 J	0.37	0.28	0.39	0.35
8:2:1 W	0.34	0.28	0.30	0.31
8:1:1 J	0.64	0.57	0.69	0.63
8:1:1 W	0.30	0.41	0.33	0.35
8:2:0.5 J	0.39	0.34	0.41	0.38
8:2:0.5 W	0.18	0.18	0.17	0.18

Fig. 7 The influence of the bacterial treatment on compressive strength by different chromium waste forms. Pressure value (F) was the experimental results. The compressive strength (P) was calculated by formula of $P = F/S$, where S was stressed area of samples. Where error bars are shown, values are the average of triplicates and error bars represent the range

(8:2:1; 8:1:1; 8:2:0.5 W). Such results indicated there was likely to be calcium carbonate precipitation induced by *Microbacterium* sp. GM-1 in treatments receiving bacteria and it contributed to improving the compressive strength of chromium waste forms. It could be seen that the difference between 8:2:1 J and 8:2:1 W samples was not significant. 8:2:1 may be the appropriate quality ratio, which provided appropriate cementation and skeleton. Furthermore, the best proportion of chromium slag, sand and soil is 8:1:1. Both treatments receiving bacteria and control samples, the one constructed in 8:1:1 were against most vertical loading.

In chromium waste forms, soil was binder, and sand provided a skeleton. The internal gap in chromium wastes from control groups was relatively wide. However, in treatments receiving bacteria, GM-1 successfully induced calcium carbonate precipitation inside and on the surface of chromium waste forms. Calcium carbonate is not only filled the gap on the surface and inside of chromium waste

forms, also had cementation, making loose particles more compacting. To some extent, Calcium carbonate induced by *Microbacterium* sp. GM-1 improved the compressive strength of chromium waste forms.

Leaching Toxicity Test

Leaching toxicity test was done for samples to assess the long-term stability. Figure 8 showed the concentrations of Cr(VI) in static leaching toxicity test. Those of Cr(VI) increased significantly in control samples compared to treatments receiving bacteria. The concentrations of Cr(VI) in control samples were up to 1.72, 1.29 and 1.82 mg/g. While it was found only 0.13, 0.05 and 0.17 mg/g in treatments receiving bacteria. The samples constructed in 8:1:1 had the best effect. It was found only 0.05 mg/g Cr(VI). Higher static leaching toxicity implied that there were more contaminant released into the environment and pollutant the soil and water in nature, which was banned by Environment Policy Act.

Figure 9 showed the concentrations of Cr(VI) in dynamic leaching toxicity test. It could be seen obviously that the level of Cr(VI) stood lower in treatments receiving bacteria. The contents did not change significantly for 7 days. The differences among three treatments receiving bacteria were little. It was said different proportions of materials did not change the value of the leaching toxicity. But those in all control samples were greatly high and up to more than 10 mg, though there was a trend of decrease after 2 days, because of the adsorbability of soil. Calcium carbonate induced by *Microbacterium* sp. GM-1 seem to be a physical barrier. It can protect Cr(VI) from rainfall. In

Fig. 8 Cr(VI) concentration in static leaching toxicity test collected from chromium waste forms. The contents of Cr(VI) was the result of static leaching toxicity. The Cr(VI) concentrations was calculated by formula of $p = m(\text{Cr})/m(\text{ch})$, where $m(\text{Cr})$ was the detecting value in static leaching toxicity test; $m(\text{ch})$ was the quality of chromium waste form

Fig. 9 Cr(VI) concentration in dynamic leaching toxicity test collected from chromium waste forms. The content of Cr(VI) was estimated by the diphenylcarbazide method

short, the ureolytic strain *Microbacterium* sp. GM-1 based biochemical reaction might be helpful in the immobilization of Cr(VI).

Microbacterium sp. GM-1 induced calcium carbonate precipitation inside chromium waste forms. On one hand, it not only plug the internal space, but also formed a thin film in the surface, which hindered the channel of migration of Cr(VI). On the other hand, Cr(VI) embedded in the calcium carbonate crystal lattice. Cr(VI) in crystal lattice resisted to extraction of gaseous reductant or solution. The structure of Cr(VI)–CaCO₃ were tight, and effectively prevent the Cr(VI) to release in the environment, resulting in improving its long-term stability.

Conclusions

This paper studied the characteristic of *Microbacterium* sp. GM-1, understanding its growth curve in different concentrations of Cr(VI). The result showed that *Microbacterium* sp. GM-1 grew well in the high concentration of Cr(VI) (120 mg/L). Additionally, *Microbacterium* sp. GM-1 was used for making chromium waste forms as it successfully induced calcium carbonate precipitation inside and on the surface of chromium waste forms. Calcium carbonate filled the gap inside and plugged the migration channels of Cr(VI). On the surface of the forms, it served as thin film, preventing toxic and harmful substances in chromium waste forms releasing to the environment. *Microbacterium* sp. GM-1 was benefit to solidifying

chromium, resulting in lower leaching toxicity. The chromium waste forms in 8:1:1 had the lowest balance leaching toxicity (0.05 mg/g), and that in 8:2:1 had lowest dynamic leaching toxicity (less than 2 mg). Furthermore, loose particles was cemented by calcium carbonate, improving the compressive strength of chromium waste forms. That in 8:1:1 reached the highest value (0.63 MPa).

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