Bioremediation of Chromium Smelting Slag by Sulfate-Reducing Bacteria (SRB)

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Abstract: The remediation of chromium smelting slag was investigated by sulfate-reducing bacteria (SRB). This research showed that removal rate of Cr(VI) was 49.78% and 4.21% in remediation group and control group for 60 days, respectively. Analysis of the physiochemical index, chemical speciation and microbial community, the result indicated that redox potential decreased to -246 mV and pH value changed to neutral in remediation process. After the remediation of 60 days, chemical fractions of chromium had a noticeable change from dissolved state (water-soluble, exchangeable fraction and carbonate fractions) to stable state, chromium was most in the fraction of organics and residuals, which meant chromium was stable and would not be released into environment; simultaneously, microbial community structure had also significant different between remediation group and control group, sulfate-reducing bacteria (SRB) increased and became dominant microbial on the ratio of remediation group, therefore, the treatment of chromium contaminated soil by microbial remediation both removed Cr(VI) and decreased its environmental risks.

Keywords: Chromium smelting slag, Remediation, Sulfate-reducing bacteria, Solidification

1. Introduction

Chromium(Cr), resulting from chromite mines and its related industrial pollution, such as leather tanning, metallurgical, electroplating etc. is a public concern for potential damage to the natural environment [1]. Chromium occurs as both trivalent and hexavalent forms in the environmental system. Cr(III) plays an extremely important part in human metabolism, has a hand in the maintenance of glucose, cholesterol and triglyceride levels, and plays more essential role as one of the nutritional energy substances for live microorganism [2]. The latter is more toxic, carcinogenic, mutagenic and teratogenic to the living beings [3]. Therefore, it is crucial to find an effective method to treat chromium smelting slag from Cr(VI) to Cr(III) before they are accumulated and discharged into around environment. Physical and chemical approaches as traditional technologies are used for the remediation of chromium contaminated sites for a long time, but they are still some shortcomings in the whole remediation process, such as asking great
deal of energy, more expensive and easy creating secondary contamination, Microbial remediation is regard as one of the most interesting methods for cleaning polluted environments in recent years [4]. Several microorganisms have ability to tolerance and diminish the toxicity and bioavailability of heavy metals even in the presence of chromium pollutants [4]. In particular, some bacteria have been isolated and applied to remove and immobilize the more toxic form Cr(VI) as a cost-effective green technology for remediation of wastes contaminated with chromium. Bacillus amyloliquefaciens was isolated from soil samples of chromite mine of Sukinda by Das (2014) for removal Cr(VI) and investigation on mechanism of Cr(VI) reduction and removal [1]. Wu et al (2019) isolated Cr(VI) resistant bacteria, Bacillus sp CRB-7, from tannery waste disposal site containing Cr(VI), The strain CRB-7 could remove Cr(VI) at optimal condition and complete reduction of 120 mg L\(^{-1}\) Cr(VI) within 48 h at pH 7 and 37 °C [5].

2. Materials and methods

2.1. Samples collection
The smelting slag of our research was collected from a chromium metallurgy plant in Qinghai province of China. It is situated at 36°29 North latitudes and 101°49 East longitudes. The samples were collected from 30 cm beneath surface with pH 10.03, and divided into two parts and transferred into sterile polythene bags. A part was sent to the Sangon Biotech for high throughput sequencing, another was sent to the laboratory at 4 °C until being used for analysis. The chemical characteristics of sample were shown in Table 1:

|       | MgO | CaO | SO₃ | Fe₂O₃ | Cr₂O₃ | SiO₂ | Al₂O₃ | Na₂O | NiO | TiO₂ |
|-------|-----|-----|-----|-------|-------|------|-------|------|-----|------|
|       | 30.08 | 26.06 | 10.95 | 9.35 | 8.96 | 7.33 | 5.23 | 0.71 | 0.15 | 0.11 |

2.2. Microorganisms and remediation
The functional microorganisms used in the experiments were the strains of sulfate-reducing bacteria (SRB), which were previously isolated from contaminated sludge sample in our laboratory. It was enriched and cultivated in the improved Luria Bertani (LB) medium at 28 °C for 36 h, and the nutrients were included following composition: glucose 2.0 g, yeast extract 3.0 g, tryptone 6.0 g, and NaCl 8.0 g, distilled water 1.0 L and pH 8.0. 5 days after inoculation, the sulfate-reducing bacteria suspension was detected at 600nm (OD\(_{600}\)) using a UV-Vis spectrophotometer (TU-1810/DPC, Beijing Persee, China) for further investigation.

2.3. Bioremediation assays
Incubation concentration of sulfate-reducing bacteria (SRB) was 10% (w/v) in the remediation group with 100 g chromium smelting slag at 30 °C for 60 days. In order to detect changes in pH and redox potential, samples were taken out at 10 mL and filtered by 0.45μm filter membrane every 5 days, The concentration of Cr(VI) was measured by 1.5-diphenylcarbazide method (GB7467-1987) according to national standard in China in different time (0 days to 60 days), after 60 days incubation, The chemical fraction of the samples were gradual separated by Tessier, Analysis the effect of SRB on microbial
community, microbial 16SrDNA gene were sequenced by miseq method, samples were collected, extracted DNA and constructed clone libraries, No inoculated SRB but with the same characteristics of the conditions tests were performed as the control group.

2.4. Detection of physicochemical properties
The pH and redox potential of the sample were quantified by a pH meter (S470 Seven Excellence, Switzerland) with a glass electrode and a oxidation-reduction potential meter (PC-320, Shanghai Honff, China) with a glass electrode. Cr(VI) was detected by the chromogenic reaction of diphenylcarbazide colorimetric using a UV-vis spectrophotometer at 540 nm (TU-1810/DPC, Beijing Persee, China). The chemical state of chromium was analyzed by Tessier method [6].

3. Results and discussion
3.1. Analysis of pH and redox potential
The pH and redox potential have significant changes on the solubility of chromium. Figure 1 was shown pH decreased to neutral environment from 10.03 to 7.96 in the remediation after 60 days of bioremediation by sulfate-reducing bacteria (SRB), but pH values of control group showed still alkalinity, and changed relative flat between 10 and 11 in the whole experimental process without sulfate-reducing bacteria (SRB). The redox potential had a tendency to increase at first 5 days, then decrease and trend to stable at last in 60 days remediation process by microorganism. The initial redox potential of Cr-contaminated site was 412mV, and redox potential decreased to under zero after 30 days of remediation and down to -246mV in 60days. However, redox potential was keeping between 300 to500 mV and always oxidation state in control group. The result of pH and redox potential shown that the bioremediation was an acid-consuming and forming reduction state process by utilizing sulfate ions from \( \text{SO}_4^{2-} \) to \( \text{S}^{2-} \) after a short period of adaptation by sulfate-reducing bacteria (SRB) , it can be increasing pH values to neutral environment and decreasing environmental pollution risks.

![Figure 1. The change of pH and redox potential in different time](image)

3.2. Chemical fractions of chromium analysis
To further investigate the chemical state of chromium influenced by sulfate-reducing bacteria (SRB), original, control and remediation were sequential extractions by Tessier improvement method, Figure 2 showed the change of chemical fractions. The percentage of water-soluble (T1), exchangeable fraction
(T2), carbonate fractions (T3), Fe-Mn oxides fraction (T4), organic fraction (T5), and residual fraction (T6) in the original chromium slag was 5.93, 8.72, 4.32, 37.91, 2.97, and 40.14 %, respectively. After 60 days in control group, the T1, T2 and T3 fractions increased and the stable state (T4, T5 and T6) decrease from 81.01 to 75.43%. However, After 60 days of treatment in remediation, the T1, T2 and T3 fractions decreased to 1.08, 1.65 and 5.12%, respectively. Chemical fractions of chromium was stable state (T4, T5 and T6) over 92%, The significant change of T1, T2 and T3 indicated that these states of chromium were highly unstable and kept mobilized. This result had agreement with Meng et al. (2017), microorganism were used for remediation chromium contaminated by changing active state (Cr (VI)) to stable state (Cr(III)) [7]. Our finding proved that sulfate-reducing bacteria (SRB) can reduce Cr (VI) to stable state by Tessier experiment. These results suggested that sulfate-reducing bacteria (SRB) might be used as a stabilizer for effective remediation of chromium contaminated sites.

![Figure 2. Percentages of chromium in different fractions](image)

### 3.3. The removal rate of Cr (VI) analysis

The effect of sulfate-reducing bacteria (SRB) on removal rate of Cr (VI) with remediation time was investigated in Figure 3. The result of removal rate of Cr (VI) was significant increasing, which was 3.77, 18.90, 29.48, 36.15, 44.32 and 49.78% from10 to 60 days (interval period was 10 days) in the bioremediation process by sulfate-reducing bacteria (SRB). The removal rate of Cr (VI) was only about 3% for 60 days in control group, and changing relatively gentle. This result was in good agreement with sequential extractions of chromium in the remediation. With gradually decreasing of dissolved chromium concentration in this system, dissolved fraction of Cr (VI) translated into stable fraction of Cr (III) by sulfate-reducing bacteria (SRB) in the bioremediation process. This trend was also reported in *Ochrobactrum sp.* with high Cr(VI)-reducing ability under alkaline conditions, it was tolerant to high concentration of Cr(VI) (800 mg.L\(^{-1}\)) and capable of reducing Cr(VI) to Cr (III) [8]. Previous study had shown that removal rate of Cr(VI) by using *Desulfovibrio desulfuricans* was 74.2% under the optimal conditions with the 100 mg.L\(^{-1}\) of Cr(VI), the mechanism revealed that *Desulfovibrio desulfuricans* remove Cr(VI) indirectly by H\(_2\)S products [9].
Figure 3. The change of removal rate Cr(VI) in different time

3.4. Analysis of microbial community structure

In this research, in order to analyze the effect of Cr(VI) on microbial community, 16S rDNA sequence of microorganisms was carried out by high-throughput technology [10], equivalent slags from chromium contaminated site were weighted and mixed to extract DNA and construct two clone libraries (control and remediation). Microbial community structure was also different on gene level (Figure. 4), Pseudomonas and Anoxybacillus as indigenous microorganism were the dominant microbe (59.00 and 5.33%) in the samples of control group, Pseudomonas was often the most common and numerically significant microorganism in heavy metal contaminated sites. We can see in the figure 4, microbial community structure had changed remarkably between control and remediation group. In bioremediation group, Pseudomonas, Enterobacter, Streptococcus and sulfate-reducing bacteria (SRB) accounted for 23.11, 11.45, 2.75 and 17.4% of all reads, respectively, and were the dominant microorganisms of the removal Cr(VI) from chromium contaminated sites. These results indicated that adding sulfate-reducing bacteria (SRB) to contaminated site could effectively remove Cr(VI) and constructed a healthy microbial community structure for perdurable and effective bioremediation system. It was agreement with the research of Liu et al. (2016) that the structure of microbial community changed significantly during bioleaching system, and the leaching rate was affected obviously [11].

Figure 4. Relative bacterial class abundance in each sample as determined from 16S gene pyrosequencing of DNA from control and remediation.
4. Conclusion
Chromium smelting slag was inorganized discharged and stored, which causes great harm to biology and surrounding soil. Bioremediation had attracted much attention in recent years for removal heavy metal. In this research, sulfate-reducing bacteria (SRB) was applied to bio-remediate Cr(VI) in the chromium smelting slag. The pH and redox potential of the bioremediation system changed significantly by sulfate-reducing bacteria (SRB), and decreased to neutral environment (-246mV), respectively. The result of chemical fractions by Tessier was shown that chromium was immobilized from dissolved state to stable state for 60 days. By construction of microbial community structure appropriately, functional bacteria turned into dominant microorganisms for a long time in the process of remediation. As the results suggested, we proposed that sulfate-reducing bacteria (SRB), as a new bioremediation technique for removal Cr(VI), could be applied in chromium contaminated sites with high efficiency. Moreover, bioremediation is well matched to the new concept of eco-friendly, low-cost and stably to remove pollution, and indicates promise as an emerging remediation technique that was applied for pollutant clean-up in industry.

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