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Study on Bioremediation using Iron bacteria for Artificially Polluted Soil with Heavy Metals Cd^{2+} or Cr^{3+}

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Currently, the problem of heavy metals soil pollution is getting increasingly serious in China and bioremediation has received much attention on heavy metals removal from soil. Bio-grouting using iron-based complexes produced by the metabolism of iron bacteria A was conducted to remediate soil polluted artificially by heavy metals Cd^{2+} or Cr^{3+} and the effect of bioremediation was investigated. Results showed that the available contents of Cd^{2+} and Cr^{3+} in soil reduced by 38.6% and 50.5% respectively after bio-grouting once, and reduced by 49.9% and 58.7% after bio-grouting twice. Afterwards, Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) were used to identify the composition and structure of the iron-based complexes and to analyze the deposition mechanism of heavy metal. Microscopy analyses showed that the iron-based complexes, which contain Ferrum oxydatum phosphate, Schwertmannite, as well as other substances with excellent flocculation efficiency and large specific surface area, had poor crystalline form, but could fix heavy metals in a contaminated environment through adsorption and co-precipitation.

Key words: Iron bacteria; Heavy metals; Bioremediation; Iron-based complexes; Adsorption; co-precipitation.

Most heavy metals (e.g. Cadmium, Chrome) exist naturally at low concentrations in soils but at levels too low to cause toxicity. Since the industrial revolution, human activity have caused the concentration of heavy metals in soils to become significantly higher than the natural background values through agricultural production, industrial activities, transportation, and other aspects of atmospheric deposition. According to the survey, nearly 20 million hectares of arable land in China have been polluted by heavy metals, more than 20 percent of the total cultivated area[1-2]. Therefore, treatment of heavy metals pollution has been urgent.

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Using traditional physical-chemical methods to remediate heavy metal pollution usually has shortcomings such as high energy consumption, limited scale of operation, inclination to cause the secondary pollution, etc. While phytoremediation technology is also restricted by its environmental impact, long growth cycle, limited ability of activating heavy metals, and other aspects[3]. Since the 21st century, bioremediation techniques have shown superiorities in the control of heavy metal pollution with short processing cycle, less investment, high efficiency, no secondary pollution, etc.

The results of recent research have shown that the mechanism of heavy metals removal by microbes mainly consists the following two variations: (a) biological absorption; through polysaccharides, proteins, or microorganisms secreted by microbes as bio-adsorbents, heavy metals can be enriched inside the cell or at the

surface. The use of extracellular polymeric ESP to fix Pb^{2+}, Cu^{2+} and Mg^{2+} achieved good adsorption effects, in which Pb^{2+} has a higher affinity (Pulsawat *et al* 2003[4]).

(b) biological transformation, including redox, complexation, etc. By mineralization, covalent transformation, and other ways, chemical forms of heavy metals can be changed to reduce their ecological toxicity. Applications of microorganism-induced carbonate precipitation (MICP) has proven to be an effective means of removing heavy metal contaminants, such as Ni, Cu, Pb, ect., from soil or water. (Fujita *et al*.,2000, 2004; Qian *et al*.,2012;LI *et al*.,2013[5-9]). However, the majority of bioremediation technologies still remain in the laboratory stage, and removal mechanisms have not yet been fully proven, Thus there are still many problems needing to be solved before implementing large-scale application sites.

Previous studies have shown that iron bacteria A can decompose nutrients through enzyme catalytic reaction, eventually forming iron-based complexes which have great similarities with ferric hydroxide, as well as excellent reactivity and adsorbability[10]. In this paper, bio-grouting is introduced for remediating artificially contaminated soil of of Cd^{2+} or Cr^{3+}. Iron-based complexes produced by the metabolism of iron bacteria A are used to adsorb and fix heavy metals, changing the available form of heavy metals to steady state so as to achieve the purpose of remediation of the environment.

MATERIALS AND METHODS

Physiological characteristics of iron bacteria A

Iron bacteria A was isolated from soil by using standard culture medium which consisted of the following: ferric ammonium citrate, magnesium sulfate, ferrous ammonium sulfate, dipotassium hydrogen phosphate, calcium chloride, and sodium nitrate, PH 6.8 to 7.0. The target strains were cultured at 30°C, 180 rpm, and ultimately formed the biological slime that was iron-based complex mentioned above. The metabolism of iron bacteria and biological slime were shown in Fig.1.

When the iron bacteria A was in the process of cultivating at 30°C and 180 rpm, the growth characteristics were monitored through dilution plate count method (Fig. 2). After the first 3 days, the amount of iron bacteria A began to multiply and grew exponentially. Two days later, strain concentrations were up to 1.2 × 109 cell / mL. With the consumption of nutrients, the bacteria entered a recession period, and the iron-based complexes were gradually deposited. Lastly, the bacterium solution revealed apparent color layering and the upper portion became clear. It was found at the same time that if the provision of nutrients were later continued, sediment quantity would continue to rise, which provide conditions for repetitive bio-grouting to remediate heavy metals in contaminated soil. The pH value changes in the process of bacteria growth were also measured. As shown in Fig.3, the pH value of the bacteria solution increased from the initial 7.0 to 8.6, which is of great significance for the formation of iron-based complexes

Bioremediation of Cd or Cr contaminated soil

Contaminated soil simulation

Soil samples were obtained from the middle and lower reaches of the Yangtze river. Available contents of common heavy metals in the original soil were measured in Table 1. The contaminated soil was prepared by mixing a $Cd(NO_3)_2$ or $Cr(NO_3)_3$ solution into the original soil samples, where the available contents of Cd^{2+} and Cr^{3+} were 2.89 mg/kg and 543.18mg/kg respectively. The values of the Cd and Cr available contents in soil was determined according to "soil environmental quality standards" (GB15618-1995) standard, and by taking into account the strain's tolerance to Cd and Cr.

Remediation methods

Soils contaminated by Cd and Cr were divided into three groups respectively, and three different methods were selected to remediate them. In method M-1, a 250ml bacteria solution cultured for 3 days was uniformly poured into 250g of contaminated soil, then left to stand for 10 days at 30°C. M-2 was a two-step process, where the first step was the same as M-1. The second step consisted of pouring another 250 ml of bacteria solution for secondary remediation until the water evaporated. In method M-3, 250 ml of standard culture solution, after sterilization, was directly added to 250g of contaminated soil. Available Cd and Cr contents was measured by atomic

absorption spectrophotometry before and after remediation.

Remediation Effect

The removal results of the three different methods for contaminated soils were shown in Tables 2 and 3. Due to good adsorption in the soil, Cd^{2+} and Cr^{3+} available contents in the soil before remediation decreased by 13.29% and 26.36% respectively compared with simulation theoretical values. Therefore removal calculations were based on the measured value before remediation. Available contents of Cd^{2+} and Cr^{3+}dramatically decreased by 38.6% and 50.5% respectively after a single bio-grouting, and by up to 49.9% and 58.7% after a second bio-grouting. Comparative results showed that removal rate by M-3 was much lower than that of M-1 and M-2, which illustrated that the reduction of heavy metals was associated with bacterial metabolic processes and products.

Heavy metals deposition mechanism analysis

In order to investigate the mechanism of deposition of heavy metals by iron bacteria, two conical flasks of 300 ml, numbered I and II, were used for liquid experiments. The first step was to add 50 ml of 20mg /L $Cd(NO_3)_2$ and 30mg/L $Cr(NO_3)_3$ to I and II respectively. Next, Add to each flask 250 ml of bacterial solution that had been incubated for 3 days, and then let them stand for 5-10 days. Ultimately, the mixed solution showed obvious stratification and a large number of redish-brown material was deposited at the bottom of the flask (as shown in Fig.4,5). The microstructure and phase composition of the precipitate were observed and analyzed by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR).

X-ray diffraction (XRD)

XRD spectrum showed that bacterial metabolites or iron-based complexes were mainly amorphous materials. However, two weak peaks at 26°and 28°indicated that among them there were partially crystalline phases which were identified as Ferrum oxydatum phosphate ($Fe_5(PO_4)_4(OH)_3.2H_2O$) using the ICDD database (JCPDS) (as shown in Fig.6). Excellent flocculation efficiency of Ferrum oxydatum phosphate contributed to the adsorption of free cations in the water or soil, forming aggregates and then co-precipitation. Co-precipitated products from iron bacteria and heavy metals also had poor crystallinity and contained

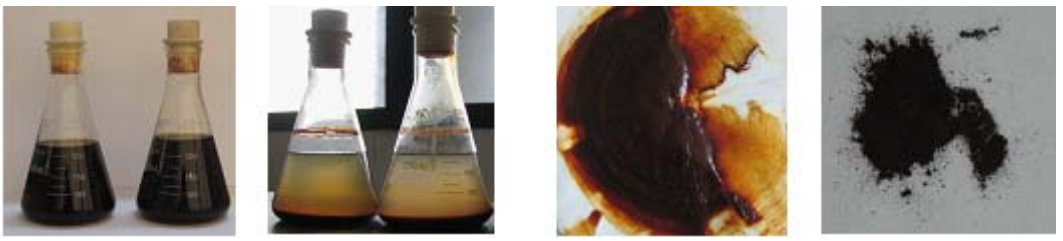


Fig. 1. Formation process of sediment through strain metabolism

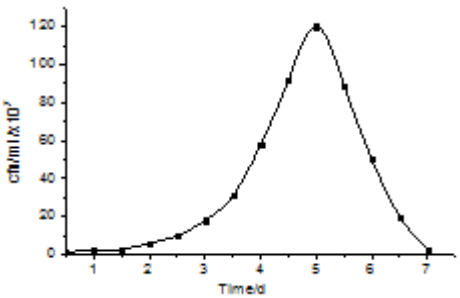


Fig. 2. Growth curve of iron bacteria A

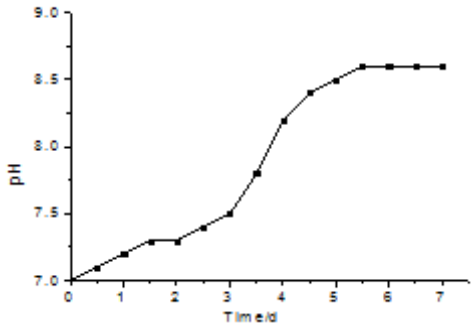


Fig. 3. Bacterium solution pH changes in the process of iron bacteria A metabolic

large amounts of amorphous materials(as shown in Fig 7, 8).

Fourier Transform Fnfrared Spectroscopy (FTIR)

The structure of iron-based complexes was characterized using FTIR(Fig.9,10). IR showed a strong and wide absorption peak of O-H at 3391.9 cm^{-1}, indicating the iron-based complexes contained large amounts of -OH functional groups. Through comparison with the standard in Table 4, most of absorption peaks' position and characteristics agreed well with schwertmannite[11-14]. So far natural or synthetic schwertmannite has all been found as amorphous or poor crystalline substances[15], which was also consistent with the XRD analysis results. In addition, the absorption peak at 1386.3cm^{-1} belonging to the stretching vibration of -COOH was related to the existence of ferric ammonium citrate in the culture medium.

Scanning Electron Microscopy (SEM)

Samples of precipitates that formed in the reaction vessels were examined by SEM. It can be seen from Fig.11(a) that iron-based complexes are characterized with flakiness, dispersion and no obvious crystallographic morphology. The SEM of Cd and Cr by iron bacteria in Fig.11(b,c) showed that a large number of tiny spherical particles, approximately 20-30 nm in size, were adsorbed and fixed tightly onto the flaky surface of iron-based complexes in the form of bio-aggregates, which proved that the reduction of Cd and Cr was associated with bacterial metabolic process.

(a) After mixing 1d (b) After mixing 7d

Fig. 4. Changing process after bacteria mixing with Cd $(NO_3)_2$ solution

(a) After mixing 1d (b) After mixing 7d

Fig. 5. Changing process after bacteria mixing with Cr $(NO_3)_3$ solution

Table 1. Available contents of common heavy metals in original soil

Heavy metal	Cd	Cr	Pb	Cu	Zn
Available contents (mg/kg)	0.04	16.51	2.38	5.90	18.35

Table 2. Test results of available Cd^{2+} in soil

Contaminated soil	Methods	Before (mg/kg)	After (mg/kg)	Removal rate (%)
Cd^{2+}	M-1	1.58	1.37	13.29
	M-2		0.97	38.6
	M-3		0.79	49.9

Table 3. Test results of available Cr^{3+} in soil

Contaminated soil	Methods	Before (mg/kg)	After (mg/kg)	Removal rate (%)
Cr^{3+}	M-1	400.00	294.58	26.36
	M-2		198.00	50.5
	M-3		165.2	58.7

Mechanism analysis

With the increase of OH^-'s concentration during iron bacteria metabolic process, Fe^{3+} in the environmental media could mightily attract both OH^- to generate dual-core, triple-core, and bridge chain coordination polymers[16], eventually linking together to become iron-based complexes. Among various kinds of anions, phosphate and sulfate radical have great effects on iron ion hydrolysis[17], they could replace some of the hydroxyls combining with iron ions to form Ferrum oxydatum phosphate, Schwertmannite and other substances.

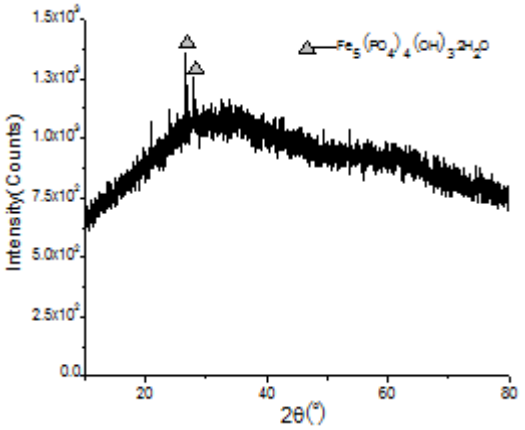


Fig. 6. XRD pattern of bio-metabolites

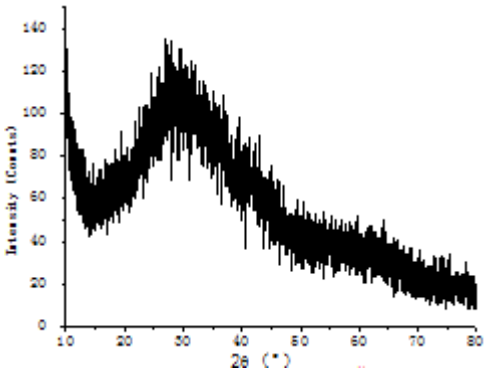


Fig. 7. XRD pattern of bio-metabolites and Cd^{2+} coprecipitates

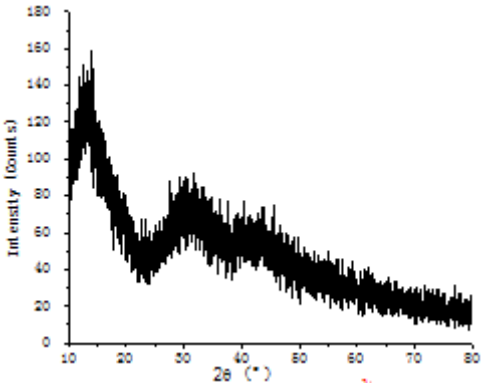


Fig. 8. XRD pattern of bio-metabolites and Cr^{3+} coprecipitates

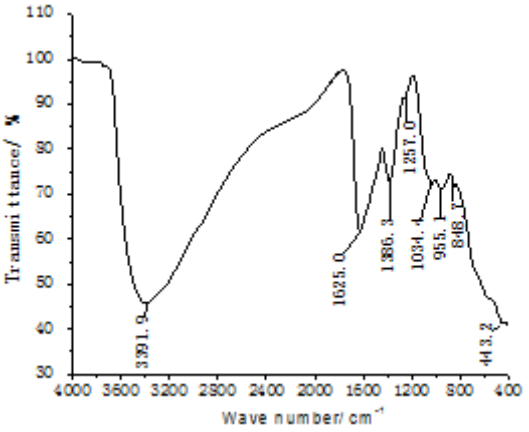


Fig. 9. FTIR spectrum of iron-based complexes

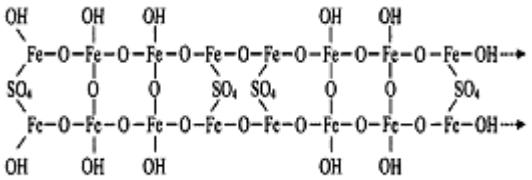
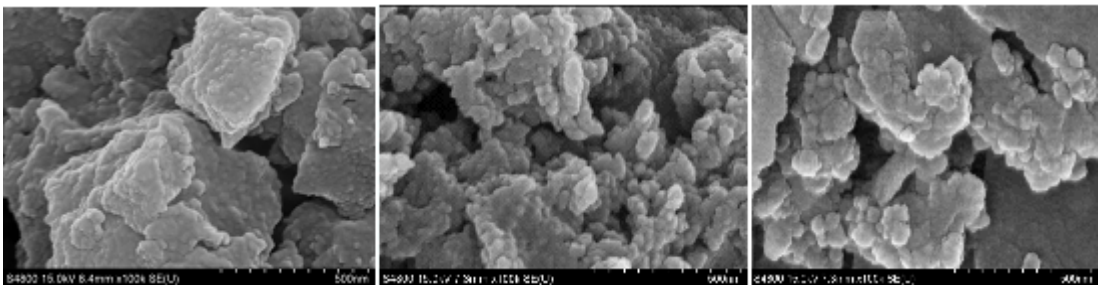


Fig. 10. Structure of schwertmannite

Table 4. IR absorbance characteristics of standard and synthetic schwertmannite

Substance	OH stretch	H_2O deformation	SO_4	FeO_6
Standard schwertmannite	3318	1629	702-1118	432
Iron-based complexes	3391.9	1625.0	848-1034	443.2



(a) Iron-based complexes (b) Co-precipitation with Cd^{2+} (c) Co-precipitation with Cr^{3+}

Fig. 11. SEM of precipitation

Ferrum oxydatum phosphate, Schwertmannite containing hydroxyl, sulfate and other groups have excellent flocculation performance and larger specific surface area of 100~200$m^2.g^{-1}$ [15-16]. Hence iron-based complexes with good adsorption properties and surface chemical activity have strong absorption capacities for heavy metals in contaminated water or soil. With the progress of bacterial metabolism, the iron-based complexes constantly adsorbed free state Cd^{2+} ,Cr^{3+} and fixed them by co-precipitation in the form of stable aggregates.

CONCLUSION

In this study, laboratory tests have shown that Cd or Cr contaminants could be adsorbed and precipitated using iron-based complexes produced by the metabolism of iron bacteria A. Further, the removal effects of Cd^{2+} and Cr^{3+} in contaminated soil increased with the amount of bio-grouting. XRD, FTIR and SEM analysis indicated that the iron-based complexes were amorphous, but contained Ferrum oxydatum phosphate and Schwertmannite, which had excellent flocculation efficiency and large specific surface area. Heavy metals in soil can be fixed by iron-based complexes and co-deposited to form stable agglomerates, which implies a transition of heavy metals from an effective state into stable state.

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