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Nano-sized Fe_2O_3/Fe_3O_4 facilitate anaerobic transformation of hexavalent chromium in soil–water systems

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ABSTRACT

The purpose of this study is to investigate the effects of nano-sized or submicro Fe_2O_3/Fe_3O_4 on the bioreduction of hexavalent chromium (Cr(VI)) and to evaluate the effects of nano-sized Fe_2O_3/Fe_3O_4 on the microbial communities from the anaerobic flooding soil. The results indicated that the net decreases upon Cr(VI) concentration from biotic soil samples amended with nano-sized Fe_2O_3 (317.1 ± 2.1 mg/L) and Fe_3O_4 (324.0 ± 22.2 mg/L) within 21 days, which were approximately 2-fold of Cr(VI) concentration released from blank control assays (117.1 ± 5.6 mg/L). Furthermore, the results of denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing indicated a greater variety of microbes within the microbial community in amendments with nano-sized Fe_2O_3/Fe_3O_4 than the control assays. Especially, *Proteobacteria* occupied a predominant status on the phylum level within the indigenous microbial communities from chromium-contaminated soils. Besides, some partial decrease of soluble Cr(VI) in abiotic nano-sized Fe_2O_3/Fe_3O_4 amendments was responsible for the adsorption of nano-sized Fe_2O_3/Fe_3O_4 to soluble Cr(VI). Hence, the presence of nano-sized Fe_2O_3/Fe_3O_4 could largely facilitate the mobilization and biotransformation of Cr(VI) from flooding soils by adsorption and bio-mediated processes.

Introduction

Chromium (Cr) has been listed as one of the top 20 contaminants in the superfund priority list of hazardous substances (Dhal et al., 2013). For centuries, the anthropic activities, *e.g.*, mining, processing, and smelting activities have greatly contaminated the soil and water resources, resulting in an unavoidable introduction of Cr-contaminants (Wang et al., 2007). Geochemical processes and weathering, acting upon metallurgical wastes and by-products, initiate transport of Cr from contaminated areas and redistribution to surrounding soils, streams, and groundwater. Thus, Cr will be accumulated in soils. Its presence could be regarded as one of potential largest environmental risks due to the deposit/precipitation of Cr-contaminants, especially emerged in the soils close to Cr mines areas (Desjardina et al., 2002). Therefore, there was an urgent need to pay the efforts on investigating the transformation of Cr from soil–water systems as seeking the feasible remediation on Cr pollution in the environment (Teng et al., 2013).

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In general, the main species of Cr were presented with trivalent (Cr(III)) and hexavalent (Cr(VI)). Cr(VI) is highly soluble and toxic. However, the reduced form, Cr(III), is relatively in soluble and in a low toxicity (Arias and Tebo, 2003). In alkaline soils, such as the soil from Cr mine, the predominant state is hexavalent Cr(VI) and existed with the form of CrO_4^{2-} and $Cr_2O_7^{2-}$ (Døssing et al., 2011). In soil–water systems, microorganisms play important roles in the transformation of Cr(VI) and Cr(III). Recently, microbial mediation on the transformation of Cr(VI) in soil–water systems has been well studied (Samuel et al., 2012; Wang and Shen, 1995). Many microorganisms could directly reduce Cr(VI), such as *Proteobacteria*, *Shewanellaalga*, *Bacillus* spp., and several new isolated bacterial (Basu et al., 1997; Garavaglia et al., 2010; Shen and Wang, 1993). Guha et al. (2001) have found that oxygen could also serve as electron acceptors and subsequently competed with Cr(VI) in the systems containing high Cr(VI) concentrations. Moreover, Cr(VI) in estuarine soils was reported to be permanently reduced to Cr(III) under anaerobic conditions (Wadhawan et al., 2013). The regarding surveys illustrated that some limited factors, such as oxygen and electron donors, affected the bio-mediated process on the speciation and mobilization of Cr(VI) from soil.

Some materials, such as carbon sources, electron shuttles, and iron minerals affect Cr(VI) transformation under anaerobic conditions (Field et al., 2013). Further, given the extensive use of nanoparticles (Xu et al., 2012) recently, large amounts of nanoparticles have now been released into aquatic environments and soil systems. It will lead to an unexpected ecological and environmental outcome because of the unique physical and chemical properties of nanoparticles (Jiang et al., 2013; Zhu et al., 2016). Therefore, it is critical to explore how nanoparticles affect soil microbial communities, and the potential effects on Cr(VI) transformation. Additionally, the recent studies have demonstrated that nanoparticles facilitated extracellular electron transfer in microbial fuel cells and soil systems. The addition of magnetite nanoparticles into soils seemed to increase the activity of methanogens through shifting the microbial abundances of acetate-oxidizing bacteria, propionate-oxidizing bacteria, and methanogenic archaea (Yamada et al., 2015; Cutting et al., 2010). Because nanoparticles might potentially influence the microbes directly (*e.g.*, *via* serving as electron shuttles to transport electrons), the possible mediating effects derived from of iron oxide nanoparticles on Cr(VI) reduction should deserve to be studied. For instance, Rao et al. (2013) has demonstrated that a more significant modification to the yeast cells in presence of phyto-inspired Fe^0/Fe_3O_4 nanoparticles during Cr(VI) reduction than in absence of Fe^0/Fe_3O_4 nanoparticles. In addition, nanoscale zero-valent iron and biogenetic nano-magnetite were capable of removing aqueous Cr(VI) from alkaline groundwater (Watts et al., 2015; Liu et al., 2010; Li et al., 2011). Although there have been studies showing that iron oxide nanoparticles mostly affected Cr(VI) reduction process, there was no clear evidence regarding roles of nanoparticles during Cr(VI) reduction in soil (Singh et al., 2012). Exactly, our previous study demonstrated that the addition of nano-sized Fe_2O_3, Fe_3O_4 and SiO_2 could potentially stimulate bacterial growth, and then changed the arsenic transformation in soil (Dong et al., 2014). Furthermore, Kato et al. (2010) reported that nano-sized Fe_3O_4 and nano zero valent iron oxide could also be used as electron conduits to dramatically improve microbial extracellular electron transfer in soil. Therefore, iron oxide nanoparticles, for example nano-sized Fe_3O_4, may influence microbial respiratory of Cr(VI) in soil–water systems. However, there was severely poor studies could powerfully illustrate how nano-sized Fe_2O_3/Fe_3O_4 shift the microbial community composition during the process of Cr(VI) transformation.

Hence, the study presented the aims to investigate Cr(VI) transformation from Cr-contaminated soil–water systems in presence of nano-sized or submicro Fe_2O_3/Fe_3O_4, particularly emphasizing on the role of nano-sized Fe_2O_3/Fe_3O_4. Additionally, microbiota in soil sample was analyzed by denaturing gradient gel electrophoresis (DGGE) and using pyrosequencing after treatment of nano-sized Fe_2O_3/Fe_3O_4. Finally, the involving mechanisms regarding nano-sized Fe_2O_3/Fe_3O_4 affecting Cr(VI) transformation and microbial communities in soil were committed to be illuminated.

1. Experimental

1.1. Test site

Cr-contaminated soil samples were collected from a tailings at a Cr-enriched mine area in Qujing, Yunnan Province, China. The total Cr content in the experimental samples used was 12,202.5 mg/kg (Granadlllo et al., 1994). The initial water-extraction Cr(VI) concentration in soil was 750 mg/kg and the initial water-extraction Cr(VI) concentration of each reactor was 325 mg/kg (Padarauskas et al., 1998). Content of other elements were negligible in soil. The pH of the soil was 8.9.

Soil samples from tailings were carefully collected and stored in sterile polyethylene bags and transported to the laboratory. Those not-dried samples to permitted for the microbiological analysis and used for the batches of soil microcosms culture incubation. The remained moist soil samples from tailings were stored in polyethylene vinyl containers at 4°C to maintain the original environment and survival of indigenous bacteria.

1.2. Sample preparation and treatment

Nano-sized Fe_2O_3 (30 nm)/Fe_3O_4 (20 nm) and submicro Fe_2O_3/Fe_3O_4 were purchased from Aladin company. The morphology of the above particles was characterized by scanning electron microscopy (SEM) (Appendix A Fig. S1). The specific surface area of nano-sized Fe_2O_3, nano-sized Fe_3O_4 submicro Fe_2O_3 and submicro Fe_3O_4 were 108.44m^2/g, 8.92m^2/g, 1.05m^2/g and 0.04m^2/g, respectively. All experimental operations were conducted under obligate anaerobic conditions. The treatment conditions were set up as follows: (1) anaerobic, 20 ± 1 g soil, treated with 0.2 g of submicro Fe_2O_3/Fe_3O_4 and 24 mL deionized water; (2) anaerobic, 20 ± 1 g soil, treated with 0.2 g of nano-sized Fe_2O_3/Fe_3O_4 and 24 mL deionized water; and (3) anaerobic, 20 ± 1 g soil, treated with 24 mL deionized water. In order to clarify if Cr(VI) could be abiotically reduced, another triplicate anaerobic samples with same amendments

were incubated under the abiotic condition. The sterile treatments were autoclaved at 120°C for 20 min. The mixture of soil/water slurries were placed in 105 mL serum bottles, bubbled with N_2 for 30 min, fitted with a butyl rubber stopper, sealed with an aluminum clamp under an N_2 atmosphere, and finally incubated at 30°C in the dark. Soil microcosms were subsampled at discrete time points.

In the other experiments exploring the adsorption of Cr(VI) to nano-sized or submicro Fe_2O_3/Fe_3O_4, 100 mg/L Cr(VI)(K_2CrO_4) was added to the anaerobic reactors containing 0.2 g Fe(III) and Fe(II,III) oxides. The adsorption was carried out at 30°C without shaking. Chemicals of analytical-grade or better were used in this study.

1.3. Chemical analyses

The concentrations of Cr_{total} and soluble Cr(VI) in samples were analyzed. For soil microcosm experiments, samples were subsampled by asepsis injector. The mixture slurry of soil/water were concentrated (3500 × *g*, 15 min) followed after shaking by constant temperature oscillator at 30°C for 2 min. After treatment, the concentrations of Cr_{total} and soluble Cr(VI) of the samples were determined using Atomic absorption spectroscopy(AA-6860,Shimadzu,Japan) and diphenylcarbazide spectrophotometric methods, respectively.

1.4. PCR-DGGE

DNA in soil was extracted using the Fast DNA Spin Kit for Soil (MPBiomedical) according to the manufacturer's instructions. The forward primer was F357GC, *i.e.*, 5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC C CCT ACG GGA GGC AGC AG-3′. The reverse primer was R518, *i.e.*, 5′-ATT ACC GCG GCT GCT GG-3′. The PCR experiments was done as previously described (Dong et al., 2014).

1.5. DNA recovery, cloning, sequencing and phylogenetic analysis

Predominant DGGE bands were excised under a UV lamp, eluted overnight in sterilized Milli-Q water at 4°C, and reamplified using F357GC and R518 primers. Before cloning, the PCR products were purified using a PCR purification kit (Sangon Biotech, China) according to the manufacturer's instructions.

The purified products and contrast DNA fragments were cloned into a pMD18-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* JM109 competent cells. Subsequently, the cloned 16S rDNA gene fragments were sequenced by Sangon (Shanghai, China). Sequences were analyzed against the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) using the BLAST program. Besides, sequence alignment and phylogenetic analyses were performed using Mega 6 software. After the phylogenetic tree was constructed in Mega using the neighbor-joining method (Dong et al., 2013), the sequence was uploaded to GenBank (http://www.ncbi.nlm.nih.gov/genbank).

1.6. Pyrosequencing and data processing

Methods for DNA extraction, pyrosequencing and data processing were described by Shen et al. (2014). The original pyrosequencing data were available at the European Nucleotide Archive by accession PRJEB8317. (http://www.ebi.ac.uk/ena/data/view/PRJEB8317).

1.7. Statistical analysis

Data were analyzed using Origin 8.0 and Canoco. Each data point shown in the figures represents an average value. The standard deviations (SD) for replicate samples are shown in the figures as error bars. The treatment results were examined using analysis of variance (ANOVA). To analyze the statistical significance ($p < 0.05$) between pairs, the least significance difference (LSD) multiple range test was performed. Canoco was utilized to analyze the relationship between nano-sized Fe_2O_3/Fe_3O_4 and microorganism composition (redundancy analysis (RDA analysis)).

2. Results and discussion

2.1. Effects of nano-sized Fe_2O_3/Fe_3O_4 on Cr(VI) transformation in soil–water systems

The experiments were carried out to evaluate the influence of nano-sized and submicro Fe_2O_3/Fe_3O_4 on Cr(VI) transformation in Cr-contaminated soils. As shown in Fig. 1A, in biotic samples, the final Cr(VI) concentration in samples amended with nano-sized Fe_2O_3 (14.3 ± 20.2 mg/L) or nano-sized Fe_3O_4 (7.4 ± 0.1 mg/L) were much lower than that in the blank sample (214.4 ± 16.7 mg/L) within 21 days. However, the decrease in Cr(VI) concentration was negligible after 16.5 days, though the net loss in Cr(VI) concentration was 117.1 ± 5.6 mg/L in biotic blank sample. Besides, the Cr(VI) concentration in biotic samples amended with submicro Fe_2O_3 and submicro Fe_3O_4 were almost close to the concentration released from the blank sample in the same condition (Fig. 1C). Additionally, in those abiotic samples amended with nano-sized Fe_2O_3 and Fe_3O_4, there were only 62.3 mg/L and 49.6 mg/L of Cr(VI) net decreased, respectively, but no significant decrease could be observed in abiotic blank samples (Fig. 1B). On the contrary, in submicro Fe_2O_3/Fe_3O_4, sterile treated samples, there was a negligible decrease in Cr(VI) concentration (Fig. 1D).

2.2. Analysis of microbial community composition by PCR-DGGE

To identify possible relationships between microbial communities and the performance of nano-sized Fe_2O_3/Fe_3O_4, subsets of PCR-DGGE fingerprints were analyzed from the varied soil microcosms, as illustrated in Fig. 2a. For each treatment condition, the corresponding bands indicting the most abundant populations in each profile could be visually inspected. Correspondingly, a deficiency of intense bands indicated the growth lackness or a similar selection of varied types of microbes. There were a greater number of bands in samples amended with nano-sized or submicro Fe_2O_3/Fe_3O_4 than that the blank samples. More importantly, two bands namely CN3 and CN4, were more visually abundant in samples amended with nano-sized Fe_2O_3/Fe_3O_4 than that in other samples. In

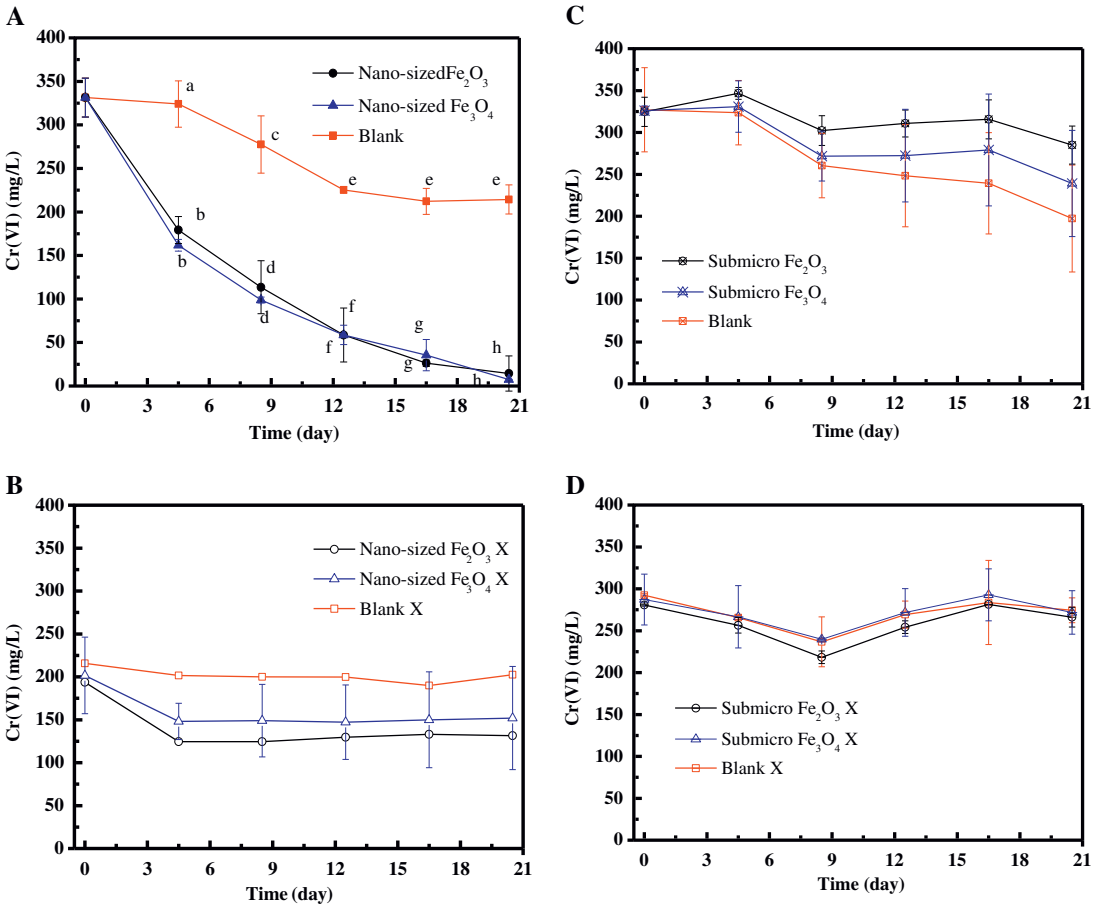

Fig. 1 – Soluble Cr(VI) concentration under biotic (A, C) and abiotic (B, D) condition from the chromium contaminated soil in presence of nano-sized and submicro Fe_2O_3 or Fe_3O_4 particles (X: sterile treatment). The lowercase letters shown in panel indicate a significant difference compared with the control at $p < 0.05$ level.

addition, the phylogenetic tree constructed from these data (Fig. 2b) illustrated that these representative bands were mostly affiliated with known members of *Proteobacteria* and *Bacteroidetes*. The results also showed that the *Proteobacteria* and *Bacteroidetes* spp. had significant changes in community compositions, especially *Proteobacteria* (Table 1).

2.3. High-throughput sequencing

Predominant phylotypes detected in these libraries are summarized in Table 2. The addition of nano-sized Fe_2O_3/Fe_3O_4 obviously caused shifts in bacterial communities after long-term exposure. *Proteobacteria* was the most abundant phylum in all samples with a relative abundance (>50%) in both treated samples; comparing with the blank sample (42.5% ± 5.0%). This was consistent with the result of DGGE analysis. Simultaneously, contrasting to the blank samples, the proportions of the phyla *Nitrospirae*, *Crenarchaeota* and *Acidobacteria* were decreased, whereas the proportions of other phyla, such as *Firmicutes*, *Chloroflexi*, *Eruyarchaeota* and *Actinobacteria*, were slightly increased. Lower-order taxonomic analysis demonstrated that specific subsets of *Proteobacteria*, including *Desulfuromonadales*, *Pelobacteraceae*, *Geobacter* and *Anaeromyxobacter*, were greatly enriched and frequently present in samples amended with nano-sized Fe_2O_3/Fe_3O_4 (with proportions of approximately 0% in blank samples).

2.4. Redundancy analysis

RDA is usually employed to depict the community patterns following various treatments. RDA in this study was presented in Fig. 3, and showed that nano-sized Fe_2O_3/Fe_3O_4 had different effects on species found in the microbial communities. After treatment with nano-sized Fe_2O_3/Fe_3O_4, the major bacterial families associated with Cr(VI) transformation were *Pelobacteraceae*, *Azoarcus*, *Anaeromyxobacter*, *Geobacter*, *Anaerolinea* and *Symbiobacterium*. Furthermore, *Peloabacteraceae*, *Azoarcus*, *Anaeromyxobacter* and *Geobacter* increased following treatment with nano-sized Fe_2O_3. On the other hand, treatment with nano-sized Fe_3O_4 mainly stimulated growth of *Anaerolinea* and *Symbiobacterium*.

2.5. Discussion

The aim of this study was to evaluate effects of nano-sized Fe_2O_3/Fe_3O_4 on Cr(VI) transformation and microbial community composition. During incubation, nano-sized Fe_2O_3/Fe_3O_4 facilitated anaerobic transformation of Cr(VI) in biotic soil

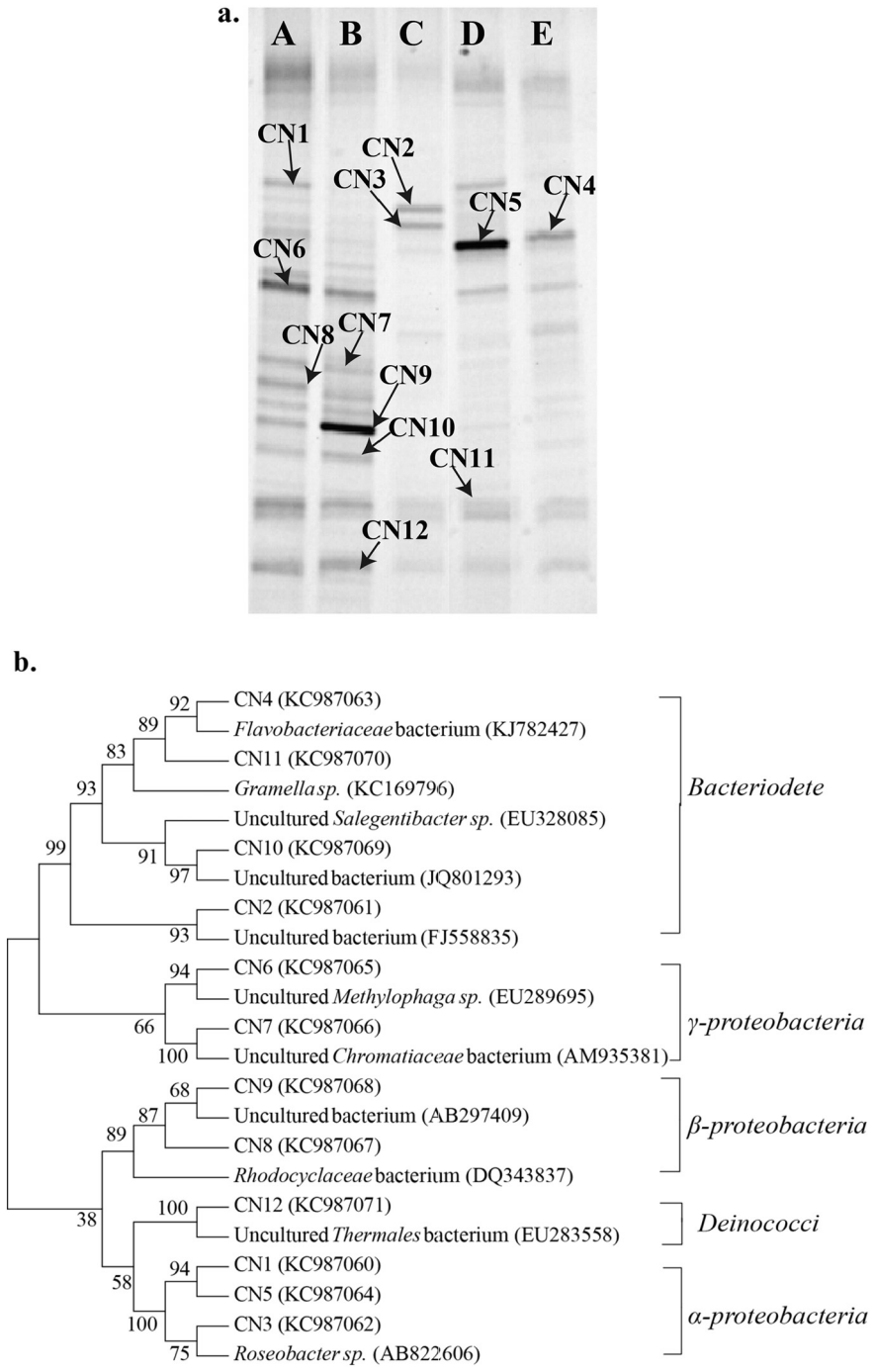

Fig. 2 – (a) The DGGE profiles of 16S rDNA fragments amplified from DNA extracted from sediment under different amendments (A: submicro Fe_2O_3; B: submicro Fe_3O_4; C: Control; D: nano-sized Fe_2O_3; E: nano-sized Fe_3O_4), and (b) Phylogenetic tree of 16S rDNA gene clones from the soil amended with nano-sized and submicro Fe_2O_3/Fe_3O_4.

samples, while submicro iron oxide had no effects on Cr(VI) transformation, suggesting that the nano-sized Fe_2O_3/Fe_3O_4 effects on Cr(VI) transformation were more important than submicro Fe_2O_3/Fe_3O_4. Previous studies had confirmed that microbial respiration was affected by certain physico chemical properties of nanoparticles, such as particle size, aggregation state, and minimal structure (Lovley, 1997; Lovley and Phillps, 1986). When iron oxide is supported with other substances, it can significantly improve the efficiency of reduction Cr(VI) (Li et al., 2010; Prus and Zhdanyuk, 2016). The reasons for nano-sized Fe_2O_3/Fe_3O_4 particles facilitating the transformation of Cr(VI) might be attributed to the special characteristics of nanoparticles. Therefore, it is necessary to investigate the mechanism regarding on nano-sized Fe_2O_3/Fe_3O_4 affecting Cr(VI) transformation.

A series of experiments were carried out to explore the mechanism responsible for the facilitation. The concentrations of Cr(VI) in abiotic samples in presence of nano-sized Fe_2O_3/Fe_3O_4 were lower than in blank sample, illustrating that nano-sized Fe_2O_3/Fe_3O_4 might absorb the soluble Cr(VI) in soil

Table 1 – Results of some partial 16S rRNA sequences using BLAST in GenBank.

Band	Phylogenetic clade	Best match	Similarity (%)
CN1 (KC987060)	*α-Proteobacteria*	Uncultured marine bacterium (HE9871589)	98
CN2 (KC987061)	*Bacteroidetes*	Uncultured bacterium (FJ558835)	94
CN3 (KC987062)	*α-Proteobacteria*	*Roseobacter* sp. (AB822606)	100
CN4 (KC987063)	*Bacteroidetes*	Uncultured *Salegentibacter* sp. (EU328085)	96
CN5 (KC987064)	*α-Proteobacteria*	Uncultured bacterium (JF341584)	96
CN6 (KC987065)	*γ-Proteobacteria*	Uncultured *Methylophaga* sp. (EU289695)	96
CN7 (KC987066)	*γ-Proteobacteria*	Uncultured *Chromatiaceae* bacterium (AM935381)	94
CN8 (KC987067)	*β-Proteobacteria*	*Rhodocyclaceae* bacterium (DQ343837)	96
CN9 (KC987068)	*β-Proteobacteria*	Uncultured bacterium (AB297396)	98
CN10 (KC987069)	*Bacteroidetes*	Uncultured bacterium (JQ801293)	100
CN11 (KC987070)	*Bacteroidetes*	*Gramella* sp. (KC169796)	95
CN12 (KC987071)	*Deinococci*	Uncultured *Thermales* bacterium (EU283558)	99

Table 2 – The relative abundance of major phyla of bacteria.

	Nano-sized Fe_3O_4	Nano-sized Fe_2O_3	Blank
Phylum *Firmicutes*	7.65 ± 2.90	6.20 ± 0.25	2.50 ± 1.12
Clostridium	0.95 ± 0.81	0.68 ± 0.29	0.83 ± 0.38
Symbiobacterium	2.33 ± 0.47	1.78 ± 0.26	0.039 ± 0.041
Sedimentibacter	0.11 ± 0.07	0.08 ± 0.0029	0
Phylum *Proteobacteria*	43.43 ± 5.30	53.99 ± 4.05	42.54 ± 4.97
Azoarcus	4.48 ± 2.06	8.81 ± 0.092	0.35 ± 0.13
Aquabacterium	3.96 ± 1.56	2.13 ± 1.36	3.36 ± 1.97
Desulfuromonadales	7.35 ± 3.17	3.03 ± 0.23	0
Pelobacteraceae	3.75 ± 0.56	6.33 ± 0.058	0.068 ± 0.023
Geobacter	1.81 ± 0.78	3.48 ± 0.34	0.24 ± 0.078
Anaeromyxobacter	1.27 ± 0.36	1.38 ± 0.089	0.23 ± 0.081
Stenotrophomonas	0.57 ± 0.15	0.51 ± 0.19	0.76 ± 0.022
Rhodoplanes	0.85 ± 0.24	0.49 ± 0.18	0.50 ± 0.20
Phylum *Chloroflexi*	15.07 ± 3.10	9.68 ± 0.75	6.17 ± 1.02
Anaerolinea	3.58 ± 0.51	1.73 ± 0.14	0.0085 ± 0.012
Anaerolinaceae;g__SHD-14	4.97 ± 1.57	3.29 ± 0.20	0.005 ± 0.0071
Phylum *Acidobacteria*	6.79 ± 1.06	4.39 ± 0.94	11.18 ± 0.54
Candidatus Solibacter	1.27 ± 0.043	1.032 ± 0.059	1.08 ± 0.043
Phylum *Actinobacteria*	3.46 ± 0.48	2.37 ± 0.21	2.9 ± 0.68
Gaiellaceae	0.84 ± 0.34	0.55 ± 0.079	0.68 ± 0.063
Rhodococcus	0.54 ± 0.26	0.046 ± 0.0043	0.071 ± 0.0042
Phylum *Nitrospirae*	1.21 ± 0.44	0.90 ± 0.14	2.07 ± 0.12
Nitrospira	0.66 ± 0.40	0.39 ± 0.02	1.17 ± 0.06
Phylum *Euryarchaeota*	3.35 ± 1.97	1.07 ± 0.60	0.050 ± 0.026
Methanoculleus	3.32 ± 1.94	0.99 ± 0.50	0
Phylum *Crenarchaeota*	0.66 ± 0.28	0.59 ± 0.015	1.01 ± 0.40
Candidatus Nitrososphaera	0.62 ± 0.22	0.52 ± 0.031	0.98 ± 0.42

All data are presented as mean ± SD.

samples (Fig. 1B). Another experiments was processed to evaluate the absorption ability of nano-sized Fe_2O_3/Fe_3O_4 to Cr(VI), clearly illustrating that nano-sized iron oxide effectively absorbed Cr(VI). Previous studies have also demonstrated that Cr(VI) can be absorbed by iron nanoparticles (Zhang et al., 2010). However, submicro Fe_2O_3/Fe_3O_4 used in the present study haven't been suggested to own the capacity of absorbing Cr(VI) (Appendix A Fig. S2). This phenomenon was attributed to the specific surface area of the iron oxides. The specific surface area of nano-sized Fe_2O_3/Fe_3O_4 was more than 100-fold of submicro Fe_2O_3/Fe_3O_4. Large surface areas of nano-sized Fe_2O_3/Fe_3O_4 would facilitate the adsorption of heavy metal ions, which was in agreement with the several reports involving the absorption of Cr(VI) resulted from the addition of nano-sized Fe_2O_3/Fe_3O_4 (Akhbarizadeh et al., 2013; Chowdhury and Yanful, 2010). Besides, the net reduction of Cr(VI) concentration decreased after treating soil samples with nano-sized Fe_2O_3/Fe_3O_4 (Fig. 1A–B), demonstrating that nano-sized iron oxide improved Cr(VI) transformation mainly by biotic processes. Hence, the nano-sized Fe_2O_3/Fe_3O_4 facilitating Cr(VI) transformation was most probably functioned in both abiotic processes (Dhal et al., 2013) and biotic (*e.g.*, when nano-sized Fe_2O_3/Fe_3O_4 stimulated changes in the composition of microbial communities that contain Cr(VI)- and iron oxide-reducing bacteria).

Microorganisms played a predominant role in the process of Cr(VI) transformation. Many Cr(VI)-reducing bacteria, containing both Cr(VI)-respiring bacteria and iron reducing bacteria, have already been isolated from various environments, such as soils, alkaline lakes, and saline lakes (Okeke, 2008; Pinon-Castillo et al., 2010). These anaerobic bacteria have been regarded as the main force for Cr(VI) reduction. In the present work, the obtained results using DGGE further strengthened the evidence bridging the Cr(VI) reduction and the effects of indigenous bacteria. *Proteobacteria* and *Bacteroidetes* were proved to be the dominant microbial species in blank samples, illustrating that *Proteobacteria* and

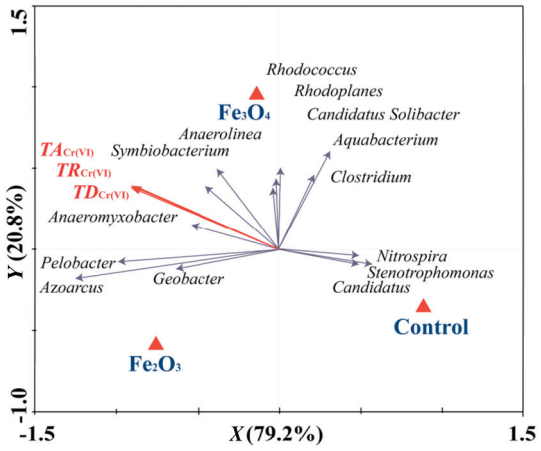

Fig. 3 – RDA plot indicates the correlation between the genera of microbial communities and relevant environmental factors under the varied amendments. The top 14 genera are listed in the map. The values on the axes indicate the percentages of the total variation explained by each axis.

Bacteroidetes contributing to Cr(VI) bioreduction. Numerous reports have confirmed that *Bacteroidetes* and *Proteobacteria* are the main species in microbial communities subjected to long-term Cr contamination (Branco et al., 2005; Joynt et al., 2006). On the other hand, DGGE results showed that *Proteobacteria* and *Bacteroidetes* were enriched in Cr-contaminated soils after the treatment with nano-sized iron oxides, further proving that the alteration in microbial communities resulted from the important roles of nano-sized Fe_2O_3/Fe_3O_4 played in soil contamination. Besides, the type of nano-sized Fe_2O_3/Fe_3O_4 also affected Cr(VI) transformation at the first 4.5 days of the experiment period (Fig. 1A). The results of RDA confirmed that nano-sized Fe_2O_3/Fe_3O_4 had a varied effect on electron transfer, resulting in differential growth of various microbial species within the communities (Fig. 3).

Although recent studies have shown that nano-sized Fe_2O_3/Fe_3O_4 alter the compositions of soil microbial communities (Garcia et al., 2012; Ge et al., 2013), relatively few studies examined microbial community composition in relationship to Cr(VI) under the treatment with nanoparticles. Nemecek et al. (2014) reported that nanoscale zero-valent iron facilitated Cr(VI) removal and shifted the microorganism composition in this process. There was no influence on the abundance of G^- bacteria, while the abundance of G^+ bacteria was significantly increased. However, there was no detail information about the indigenous microbial community composition. To further investigate the effects of nano-sized Fe_2O_3/Fe_3O_4 on the abundance of important soil bacteria, high-throughput sequencing was used to determine the proportions of bacteria in Cr-contaminated soils in addition of nano-sized Fe_2O_3/Fe_3O_4. *Proteobacteria* was the main microorganism associated with Cr(VI) reduction. Subgroups of *Proteobacteria*, especially *Desulfuromonadales*, *Pelobacteraceae*, *Geobacter* and *Anaeromyxobacter* all exhibit strong abilities to reduce Cr(VI) (Garavaglia et al., 2010; Holmes et al., 2004; Lonegran et al., 1996; Papassiopi et al., 2009; Treude et al., 2003; Wu et al., 2006). Therefore, it was inferred that the addition of nano-sized Fe_2O_3/Fe_3O_4 could strongly enhance Cr(VI) reduction by enhancing the growth of *Proteobacteria*.

Summarizing the discussion above, a mechanism for Cr reduction in soils by biotic and abiotic interactions was proposed (Fig. 4). Cr(VI) could be directly bioreduced by indigenous bacteria and adsorbed by nano-sized iron oxides, which become the two crucial factors that accelerate Cr(VI) transformation. The toxicity of Cr(VI) would slow the rate of direct Cr(VI) reduction by microorganisms. However, nano-sized Fe_2O_3/Fe_3O_4 could act as absorbents to decrease the soluble of Cr(VI) at the beginning of the experiments. Thus, it suggested that nano-sized Fe_2O_3/Fe_3O_4 provided an indirect mechanism by which microbes resist the toxicity of Cr(VI). Simultaneously, nano-sized Fe_2O_3/Fe_3O_4 strongly stimulated microbial respiration that resulting in Cr(VI) reducing. In summary, the mechanism of Cr(VI) transformation in soil contains two aspects, *i.e.*, nano-sized Fe_2O_3/Fe_3O_4 absorbed Cr(VI) and stimulated respiration of Cr(VI) reducing bacteria.

3. Conclusion

This study documented the ability of nano-sized Fe_2O_3/Fe_3O_4 to facilitated Cr(VI) reduction in contaminated soils. Nano-sized Fe_2O_3/Fe_3O_4 played an important role on the process of Cr(VI) transformation. Cr(VI) transformation in soils in the presence of nano-sized Fe_2O_3/Fe_3O_4 showed that (1) Cr(VI) was initially adsorbed by nano-sized Fe_2O_3/Fe_3O_4; (2) nano-sized Fe_2O_3/Fe_3O_4 mainly stimulated the growth of phylum *Proteobacteria*, resulting in a rapid bio-mediated Cr(VI) reduction. Therefore, the addition of nano-sized Fe_2O_3/Fe_3O_4 to Cr-contaminated soils could provide an effective method to accelerate Cr(VI) transformation. Overall, this study showed the effects of nano-sized Fe_2O_3/Fe_3O_4 in regarding to the transformation of Cr(VI) from soils and the relevant information about the shift to the compositions of soil indigenous microbial community.

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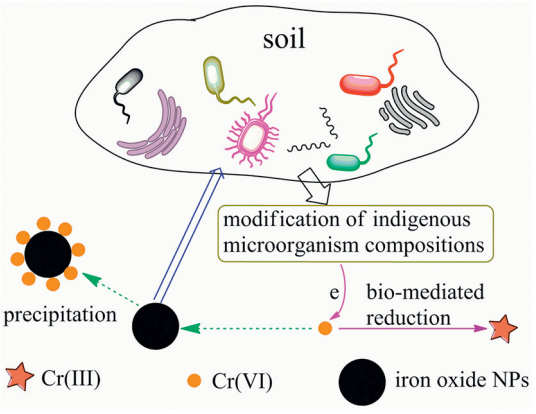

Fig. 4 – Schematic illustration of Cr(VI) removal mechanisms.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2017.01.007.

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