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Immobilization of Cd in river sediments by sodium alginate modified nanoscale zero-valent iron: Impact on enzyme activities and microbial community diversity

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Abstract

This paper investigated how sodium alginate (SA)-modified nanoscale zero-valent iron (NZVI), play a constructive role in the remediation of cadmium (Cd) contaminated river sediments. The changes of the fraction of Cd, enzyme activities (urease, catalase, dehydrogenase) and bacterial community structures with the treatment by SNZVI were observed. The sequential extraction experiments demonstrated that most mobile fractions of Cd were transformed into residues (the

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maximum residual percentage of Cd increases from 15.49% to 57.28% after 30 days of incubation at 0.1 wt% SA), with the decrease of bioavailability of Cd. Exclusive of dehydrogenase, the activities of the other two enzymes tested were enhanced with the increase of incubation time, which indicated that dehydrogenase might be inhibited by ferric ions formed from SNZVI whereas no obvious inhibition was found for other enzymes. Polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) analyses were used for the detection of microbial community changes, and the results showed that SNZVI and NZVI could increase bacterial taxa and improve bacterial abundance. All the experimental findings of this study provide new insights into the potential consequences of SNZVI treatments on the metal Cd immobilization in contaminated river sediments.

Keywords
Nanoscale zero-valent iron; Sodium alginate; Cd; Enzyme activities; PCR-DGGE

1. Introduction
River sediments are basic components of our environment (Akcay et al., 2003).

Recently, the sediment with heavy metal pollution has attracted more and more widespread attention due to its high toxicity, and even at a low concentration it can cause a great harm to living organisms (De Jonge et al., 2012; Olivares-Rieumont et al., 2005; Zhang et al., 2016a). Cadmium (Cd) is one of the major environmental pollutants in China and other countries/regions of the earth (Fassett et al., 1975; Huang et al., 2015). Pollution of sediments with Cd causes its incorporation into the food chain, which could result in a wide variety of adverse effects in animals and
humans, especially because it is a cumulative contaminant (Vinodhini et al., 2008). Consequently there is an imperative need to remediate Cd-contaminated sediments. Recent researches show that nanoscale zerovalent iron (NZVI) is promising in removing contaminants including heavy metals, the treatments of which make the heavy metals immobile and prevent their entering into the deeper sediment layers, rivers, and groundwater (Zhang et al., 2016b; Dror et al., 2012; Xu et al., 2012; Feng et al., 2010). NZVI is composed of a Fe (0) core and an iron oxide shell. The core acts as an electron donor source, promoting reduction of compounds and the shell enables sorption, surface complexation and electron transport from and to the core (Calderon and Fullana, 2015). And it has been proposed as an efficient material for Cd immobilization (Calderon and Fullana, 2015; Su et al., 2014). But due to the small particle sizes and large specific surface areas of NZVI, it is probable that NZVI is easy to aggregate (Cumbal et al., 2003). Maintaining a stable small particle diameter is important to achieve sufficient mobility to reach the target contaminants (Su et al., 2015; Kharisov et al., 2012). To avoid an agglomeration of the particles, surface stabilizers (e.g., polyelectrolyte, surfactant, biopolymer) can be used that have some special performance with electrostatic repulsion or steric stabilization (Dong and Lo, 2013; Sirk et al., 2009). They can be coated onto the surface of the NZVI to decrease agglomeration and enhance the mobility of NZVI (Kim et al., 2009). As one of surface stabilizers, sodium alginate (SA) is a linear copolymer and natural anionic macromolecules found in the cell walls of brown algae, and each monomeric unit of sodium alginate contains one carboxylate and two hydroxyl groups. Its general
structure is comprised of 1,4-linked-α-L-guluronic acid (G) and β-d-mannuronic acid (M) in alternating blocks of GG, MM and MG arranged in an irregular pattern (Borba et al., 2016; Zia et al., 2015). This chemical conformation is good for chemical reactions and linkages in view of the presence of reactive sites, such as hydroxyl and carbonyl groups along the backbone (Zia et al., 2015). Researchers have reported that sodium alginate (SA) can effectively eliminate heavy metal ions, such as Pb\(^{2+}\), Cu\(^{2+}\), and Cd\(^{2+}\) (Gong et al., 2016), which hence could be a promising polymeric material to coat NZVI for immobilizing metal ions in contaminated sediments. The molecular structures of sodium alginate (SA) and the schematic diagram of SA modified NZVI is shown in (Fig. 1).

A successful immobilization remediation technique must maintain reasonable low solubility and bioavailability of heavy metals (Rutten et al., 2010). However, it is not completely achievable to judge and measure their toxicity, mobility and bioavailability on the basis of the total concentrations of the metals (Jain, 2004; Prica et al., 2010), and moreover detecting the metals speciations is indispensable, which is channelled back into remediation of river sediments. Enzymatic activities and microbial communities can directly address biological availability and toxicity of heavy metals, and help define the acceptable cleanup standards (Kumpliene et al., 2006). Enzyme activities are credible designators for the process of biological conversion in the river sediment (Zhou et al., 2005). Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) as a powerful molecular method for rapid detection of microbial community changes or comparative
analysis of environmental samples offers more accurate information about distribution
and composition of microbial species (Aydin et al., 2015).

Nanomaterials applied to contaminated river sediments can induce an important
change in the mobility and bioavailability of the heavy metal with potential
consequences on ecosystem health (Zou et al., 2016). In this study, sodium alginate
(SA)-modified nanoscale zero-valent iron (NZVI) was synthesized and the
performance of SNZVI in the remediation of Cd contaminated river sediments was
investigated. The mobility and bioavailability of sediment Cd was investigated using
the optimized European Community Bureau of Reference (BCR) three-step sequential
extraction procedure. Sediment enzymatic activities and microbial community
diversity were also studied to assess the effectiveness of Cd immobilization
remediation using SNZVI.

2. Materials and methods

2.1. Sediment characteristics

Sediments were sampled from the Xiangjiang River, one of the tributaries of the
Yangtze River in Hunan province in southern China. It drains an area of
approximately 94,600 km² and has a total length of approximately 856 km (Zhang et
al., 1989). In Hunan Province, there are abundant reserves of non-ferrous metals, and
most of the ores used for mining, mineral processing and smelting of non-ferrous and
rare metals are found in the middle and lower reaches of the Xiangjiang River; and
the effluents from these intensive mining and industrial activities are discharged into
the river (Zhang et al., 2009). With the development of industrial production and
enlargement of the cities, the lower reaches of the Xiangjiang River have been
polluted seriously day by day in recent years (Zeng et al., 2006). For the present study
contaminated sediments (0-20 cm depth) were collected from Changsha, which is
located in the lower Xiangjiang River. Samples were air dried, crushed and sieved (75
µm) and stored at 4 °C prior to the experiments.

The concentrations of metals in the sediment were determined after nitric acid
spectrophotometer (AAS, Agilent 3510, USA) was used to detect the concentrations
of metals in the samples. Potential ecological risk (PER) index developed based on
sedimentary theory was introduced to assess the ecological risk degree of heavy
metals in the present sediment. Risk index (RI) can be calculated by the formulas
proposed by Hakanson (Hakanson, 1980). Details about the procedures here used are
given in the Supplementary Materials. According to the PEI index, the potential risk
of the metals was 38 (Pb), 17 (Cu), 7 (Cr) and 1900 (Cd), showing the individual risk
of being low, low, low, and very high. The excess Cd results in an overall considerable
risk of the sediment (Supplementary Materials, Table S1). It shows that Cd
contributes the most to the potential environmental risk at the study region. In
addition, the fractionation pattern of the metals in the sediment samples, are given in
Fig. S1 (Supplementary Materials). Whereas the most Cd in the sediment was found
in the acid-soluble fraction (49.23%) (Fig. S1), indicating its higher mobility and
bioavailability compared to the other three metals (Peng et al., 2009). Thus, Cd was
selected as the main object of this study. The physical and chemical characteristics of
tested sediment were analyzed according to Bao (2000). The basic physiochemical properties of the tested sediments are listed in Table 1.

2.2. Preparation of NZVI and modified NZVI

Ferrous sulfate heptahydrate (99%), sodium borohydride (98.5%), and SA used for the preparation of NZVI and SNZVI were purchased from Jingkang New Material Technology Co., Ltd (Changsha, China). Ultra-pure water (18.2 MΩ cm, Barnstead D11911), ethanol and other solutions were deoxygenated before the reaction by introduction of nitrogen gas. All reagents for the experiments were of reagent grade and all solutions and dilutions were prepared in ultra-pure water.

NZVI nanoparticles were then prepared by reducing Fe$^{2+}$ ions to Fe$^0$ using borohydride solution at a BH$_4$-/Fe$^{2+}$ molar ratio of 2.0 (He and Zhao, 2007). An aqueous solution of 0.05 M FeSO$_4$·7H$_2$O was continuously mixed while 0.1 M NaBH$_4$ was added into a three-necked flask meanwhile continuously stirred with mechanical agitator under nitrogen protection, followed by an hour of mixed reaction. After synthesis, nanoparticles were separated magnetically and then washed three times with deoxy ultrapure water and ethanol in order to remove the remaining borohydride and dried under vacuum drying oven (DZF-6020, Shanghai) and stored in brown bottles filled with nitrogen gas.

SNZVI was prepared by dispersing NZVI particles in aqueous SA to result in suspensions comprising iron nanoparticles (0.5 g/L) and SA of various concentrations (0, 0.05, 0.1, 0.15, 0.2 wt%) individually, followed by sonication for 30 min.

2.3. Characterization
SEM images of NZVI and SNZVI were determined using a scanning electron microscope (SEM) (Quanta TM 250, USA). NZVI and SNZVI particle hydrodynamic diameters were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern). Images of the materials were obtained at an accelerating voltage of 20 kV. X-ray diffraction (XRD) patterns of NZVI and SNZVI samples were studied using AXS D8 Advance, LynxEye array detector equipped with Cu-Ka radioactive source (\(\lambda=0.154\) nm). The angle of diffraction was varied from 10° to 80° at the speed of 2°/min. Fourier transform infrared spectra of NZVI and SNZVI were obtained using 5700 FTIR Spectrometer (NICOLET, USA). 32 scans were taken.

2.4. Experimental design

Different fractions of Cd, enzyme activities and bacterial community diversity were analyzed with sediment samples respectively treated by adding 0.5 g / 2 g / 2 g (dry weight) of sediments and 5 mL / 20 mL / 20 mL of the SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%) suspension in 50 mL centrifuge tubes with screw caps, resulting in a suspension-to-sediment ratio of 10:1 (mL/g) in each sample. The mixtures were sealed and then placed at room temperature (23 ± 1 °C) for aging without any prior pH adjustment. Control experiments with sediment samples were also conducted using ultra-pure water instead of the SNZVI suspensions. For the purpose of investigating the effect of reaction time on Cd-immobilization efficiency, the sediment reaction time was undertaken on day 0, 1, 3, 5, 7, 10, 15, 30 for the analysis of metal fraction and enzyme assays and the diversity of bacterial communities. In order to ensure the quality of the data, all sediment treatments were performed in triplicate.
Illustration of the preparation procedure and the whole experiment procedure are presented in Fig. 2.

2.5. Sequential extraction of sediment-sorbed Cd$^{2+}$

Different fractions of Cd in the samples were determined by the procedure of selective sequential extraction (SSE). The procedure adopted in our experiment was the three-step extraction of the European Measurements (Salomons, 2006). Four different fractions are considered: i) Soluble species, carbonates, cation exchange sites (hereafter defined as acid-soluble), extracted utilizing 0.11 M acetic acid, pH 2; ii) iron and manganese oxides fraction (i.e. reducible fraction), extracted with 0.5 M hydroxylammonium chloride, pH 2; iii) organic and sulfide fraction (i.e. oxidizable fraction), extracted by hydrogen peroxide 30% and treated with 1 M ammonium acetate at pH 2, and iv) the residual fraction, that remains in the solid (i.e., the metals in the crystalline lattice of primary and secondary minerals), extracted by aqua regia. 0.5 g treated sediment was put in a 50 mL Teflon centrifuge tube and the first step of extract fluid mixed with samples of supernatant fluid. For each step, the extract fluid was decanted, filtered through a 0.45 µm filter membrane, and then the filtrate was analyzed for Cd by AAS. All of the extractions were performed in triplicate.

2.6. Enzyme activity assays

Enzyme activity can be used as a good indicator for studying the activity of microorganisms, and it also represents the scope of nutrient cycling and the process of decomposition. Urease activity was assayed with method described by Hu et al. (2014) expressed as NH$_4$-N mg/g. Catalase activity was analyzed by titration with 0.1 mol/L
193  KMnO$_4$ (Sun et al., 2012), expressed as mL/g. Dehydrogenase (DEH) activity was
194  determined as described by Casida Jr et al. (1964) and the reddish color intensity of
195  the filtrate was measured with a ultraviolet-visible spectrophotometer (UV-2700,
196  SHIMADZU) at a wavelength of 485 nm and methanol was used as a blank. All the
197  enzyme activities assays were applied to the moist sediment samples in triplicate.

2.7. DNA extraction and PCR-DGGE analysis

DNA was extracted from the sediment samples using the Soil DNA Extraction Kit
(MoBio Laboratories), according to the manufacture instructions. Previous studies
showed that the kit provides estimates of bacterial diversity equal to those obtained
using other in situ lysis procedures (Luna et al., 2006). The extracted DNA was stored
at -20 °C for future applications. Confirmation of the extraction and integrity of DNA
was performed in agarose gel with ethidium bromide staining.

Bacterial 16S rRNA genes were amplified by using the universal forward primer
PRBA338F (5’-ACTCCTACGGGAGGCAGCAG-3’) and PRUN518R (5’-ATTACC
GCGGCTGCTGG-3’) primers with a GC clamp attached to the forward primer
(Ovreås et al., 1997). PCR and DGGE were performed by the method of Liu et al.
(2014).

2.8. Statistical analysis

All univariate data were analyzed using the software package SPSS 16.0 (SPSS Inc,
Chicago, Illinois, USA). One-way analysis of variance (ANOVA) and Two-way
ANOVA were used to determine differences of urease activity, catalase activity,
dehydrogenase activity among the treatment groups and individual effect of time and
concentrations. Shannon-Wiener diversity index (H) for bacterial DGGE community fingerprinting calculated as follows:

\[ H = - \sum \left( \frac{N_i}{N} \right) \ln \left( \frac{N_i}{N} \right) \]  

(1)

Where \( N_i \) was the height of a peak of each band \( i \), \( i \) was the number of bands in each DGGE profile, and \( N \) was the sum of all peak heights in a given DGGE profile.

The correlation between the distributions of bacterial communities and the different SNZVI concentrations were assayed by principal component analysis (PCA) using CANOCO software V4.5 (Biometris, Wageningen, Netherlands) (Zhang et al., 2011a).

3. Results and discussion

3.1. Characterization

The SEM images of NZVI and SNZVI showed that the morphology and nanoparticle distribution of NZVI in the absence or presence of SA (Fig. 3). The synthesized NZVI in the absence of SA showed that NZVI particles were aggregated into a chain-like structure (Fig. 3a), that can lead to a decrease of its surface reactivity (He and Zhao, 2005). Therefore Fe\(^0\) nanoparticles are usually fixed on support materials such as resins or starch (He and Zhao, 2005; Li et al., 2007), considering it decreased the aggregation of Fe\(^0\) nanoparticles and improved its mechanical strength. Compared with Fig. 3a, the 0.1 wt% SNZVI presented in Fig. 3b was clearly well-dispersed, and on the surface of the zero-valent iron were spherical particles. The hydrodynamic diameter of the NZVI particles produced was less than 100 nm (Supplementary Materials, Fig. S2a), the distribution consisted primarily (35.1%) of particles 43.82
nm in diameter. Fig. S2b (Supplementary Materials) shows the hydrodynamic
diameter and size distribution of the 0.1 wt% SNZVI. The particle size of the SNZVI
was mainly distributed in the ranging from 107.67 nm to 110.23 nm. It means the
particle size distribution is narrower.

Fig. 4 presents the XRD pattern of NZVI (a) and synthesized SNZVI (b). The
diffraction peak at 44.9° (2θ) as shown in Fig. 4b, corresponded to the formation of
iron in its zero-valent form (Weng et al., 2013). This indicated that SA was coated
onto the NZVI surface. In addition, iron oxides were detected on the surface of the
NZVI: Fe$_3$O$_4$/γ-Fe$_2$O$_3$ morphology corresponding to 2θ at 35° and Fe\(^0\) at 30° (Zhang
et al., 2011b; Kanel et al., 2005) were observed in Fig. 4a. However, Fig. 4b shows
that these peaks of iron oxides were reduced or disappeared in SNZVI, where ferrous
oxide (FeO) magnetite/maghemite (Fe$_3$O$_4$/γ-Fe$_2$O$_3$), and lepidocrocite (γ-FeOOH)
were produced in NZVI. These corresponded to the peaks marked as “F”, “M”, “L” in
Fig. 4a (Kim et al., 2013; Zhang et al., 2011b). It is suggested that the SA used in the
synthesizing procedure might prevent NZVI particles from air oxidating. The
characteristic peak of SA (Gong et al., 2016) has not been detected because of its low
concentration. Based on these results, it was concluded that the surface of SNZVI
offers more stability, which was consistent with results obtained for NZVI supported
on materials such as resin, starch, or surfactant modified zeolite (He and Zhao, 2005;
Li et al., 2007; Ponder et al., 2000).

FTIR spectra for NZVI and SNZVI were scanned in the range of 4000-400 cm$^{-1}$
(Fig. 5), where Fig. 5a and Fig. 5b indicate NZVI and 0.1 wt% SNZVI, respectively.
Broad bands at 3500-3300 cm$^{-1}$ in NZVI and the composite (Fig. 5a and b) resulted from O-H stretching may be attributed to H$_2$O and M-OH, while the band at 1650 cm$^{-1}$ can be due to O-H bending (Mohapatra et al., 2010). Strong bands at < 900 cm$^{-1}$ in the NZVI alone (Fig. 5a), attributable in part to the presence of iron oxidation oxides (Zhang et al., 2011b), were weaker in the composite, indicating less oxidation of SNZVI. The SA support may reduce the generation of iron oxide. In addition, Fe-O stretches of Fe$_2$O$_3$ and Fe$_3$O$_4$ were observed at 469.00 cm$^{-1}$ and 540.60 cm$^{-1}$, which demonstrated much consistency with the NZVI FTIR spectra in Fig. 5a. Combined with the results from FTIR and XRD, it indicated that NZVI had been successfully coated by SA where the surface of the coated NZVI was partially oxidized.

3.2. Changes in Cd partitioning

The total concentration of Cd in the sediments was 20.90 ± 0.67 (mg/kg). The concentration of Cd in the sediments was much higher than the effects range low (ERL) value (1.2 mg/kg) recommended by the sediment quality guideline (Burton et al., 2002). However, total Cd concentrations do not necessarily correspond with metal bioavailability in natural systems (Sun et al., 2016). Speciation of the metal ion in the sediment may play a significant role on its bioavailability (Fonti et al., 2015). To highlight and better understand the effect of nanoparticles on the changes of metal distribution and to evaluate the level of their stabilization, fractionation of Cd in the sediment was performed. In general, the mobility and availability of Cd increases in the order of acid-soluble forms> reducible forms> oxidizable forms> residual forms (Soylak, 2015). Changes in metal fraction because of SNZVI applications were
already confirmed after 30 d of incubation (Fig. 6a), and significant changes on distribution of metal forms in the sediment were noted. In all cases, a general increase in the residual fraction was observed with adding SNZVI (NZVI modified with SA of various concentrations from 0, 0.05, 0.1, 0.15, 0.2 wt%). The residual fractions in the sediment were of durable solid phase and not easy to be extracted and residual metal complexes (metals within the structure of the sediment and minerals) have been considered as inert and inaccessible to biota (Obst and Steinbüchel, 2004). It was found that the presence of NZVI, 0.05 wt% SNZVI, 0.1 wt% SNZVI, 0.15 wt% SNZVI, 0.2 wt% SNZVI increased the residual fraction of Cd from initial 5.26, 3.74, 5.0, 4.75, 4.33 mg/kg to 10.84, 11.47, 11.97, 11.46, 11.08 mg/kg and the corresponding residual fraction percentages of Cd increased from 25.17%, 17.90%, 23.99%, 20.72% to 51.89%, 54.90%, 57.28%, 54.87%, 53.02% after 30 days of incubation, respectively (Fig. 6b). Compared to no-amended sediment, the residual fraction percentage of Cd after applying different concentrations of SNZVI was increased from 36.42% to 41.80%. With an increasing concentration of SA from 0.1 wt% to 0.2 wt%, it did not increase further but decreased to some degree. It was presumed that the decrease in negative surface charge with an increasing concentration of SA might be ascribed to the entanglement or cross-linking of the SA molecules on the surface of SNZVI (Dong et al., 2016; Lin et al., 2010). In addition, we tested the fractions of Cd after 90 d incubating. It was found that the corresponding residual fraction percentages of Cd decreased from 51.89%, 54.90%, 57.28%, 54.87%, 53.02% to 49.03%, 50.83%, 53.92%, 51.80%, 50.05% after 90 days
of incubation, respectively (Supplementary Materials, Fig. S3). Few metals were desorbed from Cd in fraction i, ii, iii in sediments treated with SNZVI even after 90 days (Wen et al., 2016). The findings showed that the stabilization of SNZVI was quite durable and using the modified NZVI is a possible solution to alleviate the hazards likely posed to the river and the surrounding environment. Wen et al. (2016) used modified zeolite to immobilize Cd in sediment, and reported that the residual fraction of Cd was significantly increased by 8.3%. Zhang et al. (2010) reported that nano-hydroxyapatite can immobilize Cd in sediment effectively, and the residual fraction of Cd increased from 29.1 (0% addition) to 41.8 (10% addition) after 14 days of remediation. It can greatly weaken the release of metals to the environment by decreasing the active Cd fractions in spite of the unable removal of metals from sediments, the same as the accepted application of zeolites or other materials to the remediation of metal contaminated sediments (Wen et al., 2016; Zhang et al., 2010).

The reasons that can be accounted for metal stabilization in sediment using modified NZVI: Firstly, during NZVI preparation, iron oxidation produces surface hydroxides in proximity to FeOOH (Sun et al., 2006). The immobilization of Cd$^{2+}$ by SNZVI appears to involve a diffusion of metal ions to SNZVI particles and surface complexation of Cd$^{2+}$ with iron hydroxides. The surface reactions of Cd immobilization by NZVI may be described by the following equations (Zhang et al., 2014):

$$\text{FeOH} + \text{Cd}^{2+} + \text{H}_2\text{O} \rightarrow \text{FeOCdOH} + 2\text{H}^+ \quad (2)$$

In addition, each monomeric unit of SA contains one carboxylate and two hydroxyl
groups. And the hydroxyl and Cd combine to form Cd(OH)$^+$, which is adsorbed onto SNZVI or the structure of FeOOH (Zhang et al., 2014). Previous studies showed that Cd was stoichiometrically coprecipitated with Fe(III) (oxyhydr)oxides (Muehe et al., 2013).

### 3.3. Enzyme activities

Fig. 7a shows the changes of urease activities with different SNZVI concentrations in sediments during incubation time. It increased with SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%) and varied as the incubation proceeded. After 30 d of incubation, this enzyme activity increased 3.80, 4.03, 4.73, 4.42, 4.34 times higher than that of the unamended sediment with NZVI, 0.05 wt% SNZVI, 0.1 wt% SNZVI, 0.15 wt% SNZVI, 0.2 wt% SNZVI treatments, respectively. It was observed that the urease activities in the sediment increased significantly after 10 days of incubation. Moreover, urease activity was higher in various SA treatments than in the control of no adding SA. The aging phenomenon was observed, because the adsorption of Cd to SNZVI changed the fraction of Cd into residual forms, and thus decreased the bioavailability of Cd. Previous research has shown that enzyme activity increased with available contents of heavy metals decreasing (Wang et al., 2007).

The sensitivities of catalase to different levels of SNZVI in sediments were shown in Fig. 7b. The catalase activity increased slightly with the increasing of incubation periods. This enzyme was higher in various SNZVI treatments compared to the control. In the presence of 0.1 wt% SNZVI, the enzymes activities were slightly higher than those in other concentrations on day 30, and the catalase activity was 1.78
times higher than that of the unamended sediment.

Dehydrogenase activity decreased significantly with SNZVI of different concentrations in the amended samples in Fig. 7c1. This enzyme activity of the control group was significantly lower than in treatments and the image of control was not shown in Fig. 7c1, because it is not visible beside the other results. The inhibition of dehydrogenase by SNZVI indicated that it may not be very useful for the evaluation of sediments recovery under Cd pollution. Although it showed a downward trend, the dehydrogenase activity was still higher than that of the original sediment. Exclusive of dehydrogenase, the activities of the other two enzymes tested were enhanced with the increase of incubation time. For this reason, according to several previous studies (Menon et al., 2005; Stępniewski et al., 2000), we concluded that dehydrogenase might be inhibited by ferric ions formed from SNZVI whereas no obvious inhibition was found against other enzymes. Generally, applications of SNZVI increased sediment enzymatic activities. Compared with unamended sediment, urease, catalase, and dehydrogenase activities under SNZVI treatments were enhanced by 3.8-4.73, 1.29-1.78 and 134.32-297.51 times, respectively. It was clearly observed from Fig. 7a2, Fig. 7b2, and Fig. 7c2 that the activities of the enzymes tested increased multiples compared with the control sediment after 30 d incubation, respectively. Statistical analysis of data by one-way ANOVA and two-way ANOVA showed urease, catalase, dehydrogenase that indicated significant differences (P < 0.05 or P < 0.01) in incubation time and different SNZVI concentrations (Table 2). Significant interaction effects of both time and concentrations on the activities of the enzymes tested were
observed statistically (Table 2).

3.4. PCR-DGGE for bacterial community structure

PCR-DGGE was used to investigate the structural diversity of bacterial communities under SNZVI treatments of different concentrations on day 30. DNA was extracted from the Cd-contaminated samples and the DGGE patterns of PCR-amplified 16S rRNA were shown in Fig. 8. In these samples, the PCR-DGGE patterns indicated a greater complexity of banding pattern about bacterial community structure at 0.1% wt SA than at other concentrations, resulting in a high number of different bacterial taxa emerging. While the bacterial DGGE profiles of 16S rRNA gene fragments from different SA-treated sediments were generally similar, indicating that the microbes with those bands were stable and little influenced by SA. However, there were a few bands emerged after SNZVI treatments. The DGGE profile showed that the structure of bacterial community was changed after 30 d of incubation, particularly at 0.1% wt SA concentration. Similarity dendrograms and phylogenetic analysis showed by the image analysis of DGGE combined with the Dice similarity coefficient indicated that PCR-DGGE patterns of SNZVI treated samples could be well distinguished from the control group (Fig. 8), indicating that many common microbial members were still presented in each treatment. The DGGE gel profiles were further visualized by the Shannon-Wiener diversity index (H), which provided a direct indication of the apparent diversity of a microbial community (Fig. 9). The experimental groups showed more abundance and diversity of bacteria. The Shannon diversity of the bacteria reached the peak in sample 4.
In an attempt to explain the effects of different concentrations of SNZVI on the indigenous microorganisms, we performed a principal component analysis (PCA) of the results from the sediment samples. The results were shown in Fig. 10. The sample (1, 2, 3, 4, 5, 6) represents the different concentrations of SA (0, 0.05, 0.1, 0.15, 0.2 wt%). The cumulative contribution rate of the two principal components (52.1 and 28.1 % for PC1 and PC2, respectively) reached 80.2%. The PCA results clearly indicated that the bacterial community structure has changed obviously in the sediment after the SNZVI application, and the change was determined by the SA concentrations. On one hand, it was noticed that the sediment samples were divided into 4 groups. PC1 exhibited positive correlation with the sample 1, 2 and 5 and PC2 exhibited positive correlation with the sample 1 and 3. But PC1 and PC2 had exhibited negative correlation with the sample 4 and 6. On the other hand, the bands were mostly concentrated in the 2nd and 3rd quadrant and the sample 3, 4 and 6 were also in these two quadrants, indicating the high correlation between these samples and these bands, and showing that these bands represented the bacterial species were main species in these samples.

Microbial populations have complex interactions, such as association and competition (Diao and Yao., 2009). Effects of heavy metals were not only observed on the microbial species, but also on the microbial populations in the sediment (Němeček et al., 2014). SA was such a biodegradable polymer that certain bacteria were able to hydrolyze it (Obst and Steinbüchel, 2004), improving the bioavailability of carbon and nitrogen. Increase in bacterial abundance shows that the
Microorganisms can use SA as a nutrient source since the system was carbon or nitrogen limited. But the degradation of polymers by bacteria often requires the molecule to be drawn into the cell membrane (Kawai, 2010). Since nanoparticles larger than 10 nm may not be internalized by bacteria with intact membranes (Neal, 2008), it is not probable that biodegradable polymers can be transformed when covalently bound to a nanoparticle. Although polymers are biodegradable (Kaplan et al., 1979), the time scales are particularly slow (on the order of 1% degradation over 80 days). Previous studies have shown that addition of polyaspartate coated NZVI did not decrease the count of total bacteria and also found that the polymer coating can be bioavailable when bonded to a nanoparticle (Kirschling et al., 2011).

4. Conclusions

In this study, characterization with SEM, DLS, FTIR and XRD analyses demonstrated that the presence of SA led to a decrease in aggregation of iron nanoparticles and a small number of iron oxides formed on the surface of SNZVI. The findings have shown that the addition of SNZVI was effective in immobilizing Cd in polluted sediments, resulting in an increased (or a bigger) residual fraction of Cd and a decrease of the bioavailability of Cd. Moreover, the increase of enzymes activities (urease, catalase, and dehydrogenase) and bacterial community diversity indicated the recovery of metabolic function to some extent by adding SNZVI of different concentrations. Additionally, these results could probably provide a reference for risk assessments of using NZVI particles for sediment remediation and of using surface coatings on these nanoparticles.
However, as noted in our study, this nanotechnology still has its limitations. It is possible that Cd become remobilized due to long-term processes or changes of the environmental conditions change (Calderon and Fullana, 2015). And the heavy metals existing in inactive form still remain in sediment. In addition, further studies are needed to reveal the potential effects of SNZVI application on other metals in contaminated sediments.

Acknowledgements

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Supplementary data

This file contains additional Fig. S1-S3 and Table S1.

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Table 1 - The main characteristics of the sediment.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± standard deviation (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (cm) 0-20 cm</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.79 ± 0.03</td>
</tr>
<tr>
<td>Organic carbon (g/kg)</td>
<td>7.88 ± 0.90</td>
</tr>
<tr>
<td>Organic matter (g/kg)</td>
<td>13.57 ± 1.54</td>
</tr>
<tr>
<td>Water (%)</td>
<td>55.75 ± 0.55</td>
</tr>
<tr>
<td>CEC (cmol/kg)</td>
<td>13.62 ± 3.58</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>58.72 ± 0.79</td>
</tr>
<tr>
<td>Total nitrogen (g/kg)</td>
<td>2.16 ± 0.04</td>
</tr>
<tr>
<td>Total phosphorus (g/kg)</td>
<td>0.17 ± 0.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Element</th>
<th>Total content (mg/kg)</th>
<th>Acid-soluble</th>
<th>Reducible</th>
<th>Oxidizable</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>167.10 ± 1.33</td>
<td>4.11%</td>
<td>15.41%</td>
<td>9.45%</td>
<td>71.03%</td>
</tr>
<tr>
<td>Cu</td>
<td>69.35 ± 1.60</td>
<td>5.20%</td>
<td>6.39%</td>
<td>19.69%</td>
<td>68.72%</td>
</tr>
<tr>
<td>Cr</td>
<td>159.90 ± 0.92</td>
<td>13.09%</td>
<td>4.17%</td>
<td>10.33%</td>
<td>72.41%</td>
</tr>
<tr>
<td>Cd</td>
<td>20.90 ± 0.67</td>
<td>49.23%</td>
<td>27.07%</td>
<td>8.21%</td>
<td>15.49%</td>
</tr>
</tbody>
</table>
Table 2 - ANOVA analysis of enzyme activities in Cd polluted sediment with different SNZVI concentrations and incubation time as two main effects.

<table>
<thead>
<tr>
<th>Facor</th>
<th>One-way ANOVA</th>
<th>Two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urease</td>
<td>Catalase</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.033*</td>
<td>0.004**</td>
</tr>
<tr>
<td>incubation time</td>
<td>0.00**</td>
<td>0.00**</td>
</tr>
</tbody>
</table>

* Significant differences at the 0.05 level (p < 0.05).

** Significant differences at the 0.01 level (p < 0.01).
Fig. 1 - Molecular structures of sodium alginate (SA) and the schematic diagram of SA modified NZVI.
Fig. 2 - Schematics of the preparation of sodium alginate (SA) modified NZVI and their application for adsorption of Cd$^{2+}$. 
Fig. 3 - SEM images of laboratory synthesized iron particles with and without a modify material. a. NZVI; b. 0.1 wt% SNZVI.
Fig. 4 - XRD patterns of samples. a. NZVI; b. 0.1 wt% SNZVI. F = ferrous oxide, M = magnetite/maghemite, L = lepidocrocite.
Fig. 5 - FTIR spectra of sample. a. NZVI; b. 0.1 wt% SNZVI.
Fig. 6 - Fractioning of Cd in sediment with the addition of SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%) and varied as the incubation proceeded. (Concentration of NZVI or SNZVI: 0.5 g/L). a. SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%); b. SNZVI (0.1 wt%).
Fig. 7 - Enzyme activities under different treatments of SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt% ) and enzyme activities increased multiples at 30 days. a1. Urease; a2. Urease increased multiples (0.1 wt% SNZVI); b1. Catalase; b2. Catalase increased multiples (0.1 wt% SNZVI); c1. Dehydrogenase; c2. Dehydrogenase increased multiples (0.1 wt% SNZVI). Error bars indicate standard deviation (n = 3).
Fig. 8 - Similarity dendrograms and cluster analysis of banding patterns generated by PCR-DGGE of 16S rRNA fragments from SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%) treated samples. a. similarity dendrograms; b. cluster analysis.
Fig. 9 - The diversity index $H$ rooted in DGGE profiles of amplified bacterial 16S rRNA genes. The sample (2, 3, 4, 5, 6) represents the different concentrations of SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%), the sample 1 as control group.
Fig. 10 - Loading plot. Eigenvectors calculated by PCA using the response variables i) relative abundance of microbial population and ii) different samples: SNZVI (0, 0.05, 0.1, 0.15, 0.2 wt%) and control group. Color circular represented 6 samples taken from two systems. The angles between arrows indicate correlations between two variables.
Highlights

- SNZVI applied to the remediation of Cd contaminated river sediments can affect Cd mobility.
- We investigated the relativity between Cd mobility and changes in enzyme activities as well as bacterial community diversity.
- The maximum residual percentage of Cd increases from 15.49% to 57.28% after 30 days of incubation at 0.1 wt% SA.
- SNZVI and NZVI could increase bacterial taxa and improve bacterial abundance.