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Biogenic FeS accelerates reductive dechlorination of carbon tetrachloride by *Shewanella putrefaciens* CN32

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ABSTRACT

Dissimilatory metal reducing bacteria (DMRB) widely exist in the subsurface environment and are involved in various contaminant degradation and element geochemical cycling processes. Recent studies suggest that DMRB can biosynthesize metal nanoparticles during metal reduction, but it is unclear yet how such biogenic nanomaterials would affect their decontamination behaviors. In this study, we found that the dechlorination rates of carbon tetrachloride (CT) by *Shewanella putrefaciens* CN32 was significantly increased by 8 times with the formation of biogenic ferrous sulfide (FeS) nanoparticles. The pasteurized biogenic FeS enabled 5 times faster dechlorination than abiotic FeS that had larger sizes and irregular structure, confirming a significant contribution of the biogenic FeS to CT bioreduction resulting from its good dispersion and relatively high dechlorination activity. This study highlights a potentially important role of biosynthesized nanoparticles in environmental bioremediation.

1. Introduction

Biogenic materials hold potential for various catalytic and environmental remediation applications, due to its advantages such as green synthesis, high reaction efficiency and environmental-benignity [1–3]. A number of biogenic nanomaterials including palladium [4,5], gold [6] and silver particles [7,8] have been reported so far. Dissimilatory metal-reducing bacteria (DMRB), especially *Shewanella* species, are very important synthesizers in this respect. *Shewanella* can reduce the abundant Fe(III) in environment to Fe(II) species. The resulting Fe(II) species could further precipitate with anions such as phosphate and sulfide in the environment to form Fe(II)-bearing biogenic minerals [9,10].

FeS is a critical precursor to the formation of many other stable iron sulfide phases [11] and an important reductant providing a source of Fe(II) and S(-II) species, both of which can act as electron donors for reductive decontamination reactions [12]. Consequently, FeS has been widely applied for remediation of contaminated groundwater and soils [13]. Recently, *Shewanella* was found to simultaneously reduce Fe(III) and sulfur species (such as thiosulfate and element sulfur) to yield FeS nanoparticles [14–16].

Since the *Shewanella* bacteria and abiotic FeS both can reduce contaminants, it is interesting to know how the biogenic FeS nanoparticles in *Shewanella* would affect the decontamination performance of these bacteria. In addition, it is unclear whether the biogenic FeS is more efficient in reductive decontamination than its abiotic counterpart. Here, we used *Shewanella putrefaciens* CN32 as a model bacterium to synthesize FeS nanoparticles, and comparatively evaluated the reactivity of the bacteria, the biogenic materials and abiotic FeS for carbon tetrachloride (CT) reduction. In addition, the underlying mechanisms of the significantly improved dechlorination performance of the strain after FeS biosynthesis were elucidated. This work may facilitate a better understanding on the environmental bioremediation processes and the roles of biosynthesized materials involved.

2. Materials and methods

2.1. Biogenic and abiotic FeS synthesis

S. putrefaciens CN32, obtained from Prof. Han-Qing Yu at University of Science and Technology, was pre-cultured aerobically in

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LB medium until the stationary growth phase [17]. The cultured cells were collected by centrifugation and washed with the basic medium for three times. The resulting concentrated culture was used for the biosynthesis and dechlorination experiments. The FeS biosynthesis experiments were conducted in 125 mL serum bottles with 50 mL defined anaerobic medium [9]. One mM thiosulfate and different concentrations of Fe(III)-citrate were added as electron acceptors and 18 mM lactate as the sole electron donor. PIPES (1,4-piperazinediethanesulfonic acid) (30 mM, pH 7.0) was added as the pH buffer. Each bottle was purged with N_2 gas for 20 min and then sealed with butyl rubber stoppers and aluminum caps, followed by autoclaving at 105 °C for 20 min and then cooled to room temperature. The concentrated cultures were injected into the serum vials to a final cell density of 0.5 at OD_{600}. The serum bottles were incubated on a rotary shaker (180 rpm) at 30 °C. The cells and the formed black precipitate were collected from the bottes after one week of cultivation for the dechloriantion experiments and materials characterization.

The abiotic FeS was synthesized by reacting dissolved Na_2S with $FeSO_4$ solution following the method described previously [12] Black precipitates with poor crystallity were obtained.

2.2. Characterizations of biogenic and abiotic FeS

2.2.1. Morphological analysis

The specific surface area of the synthesized materials was analyzed by Brunauer–Emmett–Teller (BET, Ommishop 100CX, Coulter, USA). The culture suspension after 8 day incubation and the abiotic FeS samples were centrifuged and washed with Milli-Q water for three times and 100% ethanol once. For the BET analysis, the washed biogenic and abiotic samples were vacuum-dried for 12 h and analyzed under room temperature.

For the SEM characterization, the biogenic FeS culture was withdrawn from the serum bottle, centrifuged and then fixed by 5% glutaraldehyde for 12 h at 4 °C, subsequently dehydrated with ethanol [30,50, 70, 80, 95, and 100% (v/v) ethanol, each for 15 min]. The abiotic FeS samples were centrifuged and washed for three times with Milli-Q water and once with 100% ethanol. Then the biogenic and abiotic samples were diluted and loaded on a carbon-coated silicon grid, which was mounted on a standard aluminum stub by a double-coated carbon conductive Table. The samples were then observed by SEM (Quanta 400 FEG).

For TEM characterization (HT7700, Japan), the samples were subjected to the same pretreatment as for SEM characterization, then diluted and mounted on a carbon-coated copper grid for observation.

2.2.2. X-ray Powder Diffraction (XRD)

The biogenic FeS particles and abiotic FeS were identified on X-ray diffractometer (Bruker D8 Advance) with Cu Ka radiation (λ = 1.54178 Å). The particles were centrifuged, washed and vacuum-dried. The samples were scanned between 20° to 70° 2θ. XRD patterns of samples were analyzed by comparing to those in the Joint Committee on JCPDS diffraction data files (JADE 6).

2.2.3. X-ray photoelectron spectroscopy (XPS)

Biogenic and abiotic samples were centrifuged and washed 3 times with Milli-Q water and once with 100% ethanol. The washed samples were vacuum dried for 12 h before XPS analysis (ESCALAB 250, Thermo-VG Scientific, USA)

2.3. Fe(II) detection and CT dechlorination

To obtain biogenic FeS with different composition, 1 mM thiosulfate and different concentrations of Fe(III)-citrate (from 1 to 10 mM) were added into the bacterial culture medium. The groups with Fe(III) dosage of 1, 2, 5 and 10 mM were denoted as Fe(1), Fe(2), Fe(5) and Fe(10), respectively. For comparison, the groups without thiosulfate and without bacteria were set as the S-free control and abiotic control, respectively. To measure the total Fe(II) content, 0.5 mL mixed sample was withdrawn at given time intervals using a sterile syringe and acidified for 12 h using 0.5 mL HCl (1 M). When the Fe(II) concentration became stable, dechlorination process was initiated by spiking specific amount of CT. At given time intervals, 50 μL gas samples were withdrawn using a gastight syringe (100 μL, SGE) to determine the CT concentration. Under each experimental condition four parallel serum bottles were used, two for the Fe(II) measurement and the other two for CT quantification.

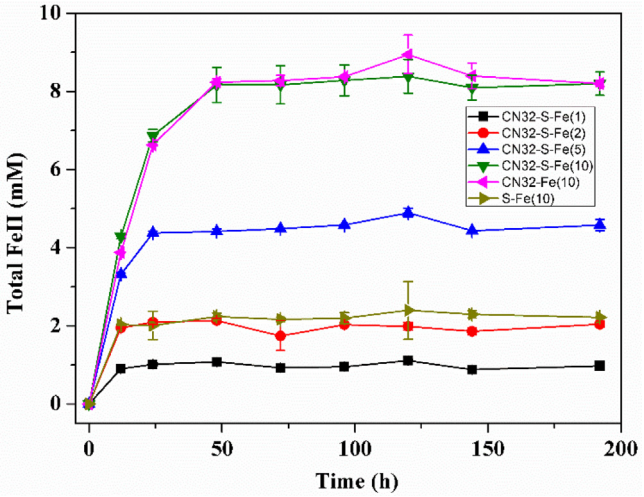

Fig. 1. Total Fe(II) produced from the reduction of Fe(III)-citrate by *Shewanella putrefaciens* CN32. Fe(1)-F(10) refer to different Fe(III) dosage from 1 to 10 mM. S refers to 1 mM thiosulfate.

2.4. Analytical method

Fe(II) was measured by the 1,10-Phenanthroline spectrophotometry method at 510 nm using a UV–vis spectrometer (Shimadzu UV-1800). To measure the aqueous-phase Fe(II), samples were filtered through 0.45 μm PTFE membrane to remove particles before acidification with 1 M HCl. The solid-phase Fe(II) was defined as the sum of the structural Fe(II) in FeS and sorbed Fe(II) on the surface of FeS. CT samples were quantified by an gas chromatograph (GC, Agilent 6890) equipped with an electron capture detector (ECD) [18].

3. Results and discussion

3.1. Biogenic FeS synthesis

The Fe(III)-citrate reduction by *S. putrefaciens* CN32 can be reflected by the increase of Fe(II) concentration during the incubation, which process was stabilized within a period ranging from 10 to 50 h depending on the Fe(III) dosage. Increasing the Fe(III) dosage led to higher Fe(II) level, indicating a good Fe(III) bioreduction capacity of *Shewanella* (Fig. 1). The presence of thiosulfate did not affect the Fe(III) reduction, mainly because thiosulfate reduction was thermodynamically less competitive compared with Fe(III) reduction (Reaction (1) and (2)). In addition, thiosulfate reduction as an intracellular reaction was also kinetically restricted due to limited mass transfer [19]. This reaction superiority of Fe(III) over thiosulfate reduction was also evidenced by the fact that black precipitate occurred only after Fe(II) concentration became stable. Therefore, in our reaction system Fe(III) was reduced first followed

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