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Reduction of hexavalent chromium by *Pannonibacter phragmitetus* LSSE-09 coated with polyethylenimine-functionalized magnetic nanoparticles under alkaline conditions

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ABSTRACT

A novel cell separation and immobilization method for Cr (VI)-reduction under alkaline conditions was developed by using superparamagnetic Fe_3O_4 nanoparticles (NPs). The Fe_3O_4 NPs were synthesized by coprecipitation followed by modification with sodium citrate and polyethyleneimine (PEI). The surface-modified NPs were monodispersed and the particle size was about 15 nm with a saturation magnetization of 62.3 emu/g and an isoelectric point (pI) of 11.5 at room temperature. PEI-modified Fe_3O_4 NPs possess positive zeta potential at pH below 11.5, presumable because of the high density of amine groups in the long chains of PEI molecules on the surface. At initial pH 9.0, *Pannonibacter phragmitetus* LSSE-09 cells were immobilized by PEI-modified NPs via electrostatic attraction and then separated with an external magnetic field. Compared to free cells, the coated cells not only had the same Cr (VI)-reduction activity but could also be easily separated from reaction mixtures by magnetic force. In addition, the magnetically immobilized cells retained high specific Cr (VI)-reduction activity over six batch cycles. The results suggest that the magnetic cell separation technology has potential application for Cr (VI) detoxification in alkaline wastewater.

1. Introduction

Chromium (Cr) is widely used by electroplating, leather tanning, water cooling, metal finishing, etc. [1,2]. Its widespread use has led to large quantities of this element being released into environments [3]. The effluents from these industries contain both hexavalent chromium, Cr (VI), and trivalent chromium, Cr (III). It is important to note that Cr (VI) is toxic and carcinogenic to humans but Cr (III) exhibits only a little toxicity [4]. Hence, Cr (VI) containing wastewaters have become a well recognized bio-hazard in water pollution control [5]. Environmental regulations of Environmental Protection Agency (EPA) on Cr (VI) concentration in drinking water (<50 $\mu g\,L^{-1}$) have prompted extensive research on Cr (VI) removal [6]. Conventional methods for the detoxification of Cr (VI)-contaminated wastewaters include chemical reduction followed by hydroxide precipitation, membrane separation and adsorption technology [1,7]. To overcome the drawbacks such as high cost for Cr (VI) reduction, poor removal efficiency and costly safe disposal of toxic sludge [8], microbial reduction of Cr (VI) to Cr (III) which could be economic and eco-friendly have attracted worldwide attention recently [5,9].

Many researchers have focused their studies on the bioconversion of Cr (VI) by free bacterial cells [10–14]. However, methods using free cells have been considered undesirable because of the loss of activity resulting from Cr (VI) toxicity [15], and difficult solid–liquid separation [16]. Compared to free cells, immobilized cells which have enhanced stability, improved catalytic efficiency and facilitated recovery property have been used in bioconversions, biotransformation and biosynthesis processes [17,18]. Several biopolymers such as alginate, agarose, carrageenan, cellulose and chitosan are widely used as entrapment matrices for cell immobilization as they are non-toxic, efficient and inexpensive [17–19]. On the other hand, magnetic immobilization and separation technology which show lower diffusional limitations and steric hindrance provide a quick, easy and convenient alternative over traditional cell-immobilizing methods [18,20]. Fe_3O_4 nanoparticles (NPs) are often modified by functional chemicals including

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chitosan [19,21], ammonium oleateon [18,22] and carbohydrates [23] to possess specific properties such as optimal particle surface charge and functional groups. Cell immobilization is then achieved by the magnetic nanoparticles under neutral conditions via electrostatic attraction [21] and hydrophobic interaction [18,22]. It is convenient to concentrate and reuse the dispersed coated-cells from a suspension by a magnetic method. However, until recently the information about the utilization of Fe_3O_4 NPs for cell immobilization is still insufficient, especially for those specific cases under alkaline conditions.

One functional polymer that has widely attracted attention for Fe_3O_4 NPs-coating is polyethyleneimine (PEI) [24,25]. PEI is a water-soluble polycation consisting of primary, secondary, and tertiary amine functional groups [26]. It has been used for the design of DNA delivery vehicles [27,28] and as a functional coating to enhance the performance of heavy metals adsorption [29–32]. It is known that electrostatic interaction between adsorbent and adsorbate plays an important role in the process of adsorption [29]. Since the surface charge of bacteria is negative in aqueous systems [33], adsorbents with positive surface charges are favorable for bacteria sorption. PEI is the organic macromolecule with the highest cationic-charge density potential, and has a high buffer capacity over a very broad pH as well [34]. Several researchers have reported that PEI-modified adsorbents exhibited positive zeta potential under alkaline conditions [29,35,36]. From the electrostatic interaction point of view, it is possible to use PEI-modified NPs as a tool for negative-charged bacterial cells immobilization under alkaline conditions.

As we previously reported, *Pannonibacter phragmitetus* LSSE-09 was isolated from the industrial sludge. This Gram-negative bacteria showed a strong potential to reduce Cr (VI) to Cr (III) aerobically and anaerobically under alkaline conditions [10]. We have evaluated the capability of strain LSSE-09 encapsulated in alginate capsules for reducing Cr (VI) along with organo-Cr (III) removal [37]. However, due to the lower diffusional limitations and steric hindrance, the Cr (VI)-reduction rate of encapsulated cells was lower than that of free cells. Herein, we attempted to develop a simple and effective technique for Cr (VI) reduction under alkaline conditions, by integrating the advantages of magnetic separation and cell immobilization. To the best of our knowledge, there have not been any reports about the utilization of Fe_3O_4 NPs-immobilized cells under alkaline conditions, especially for Cr (VI) reduction. In the present work, Fe_3O_4 NPs were modified with PEI to produce polycationic magnetic NPs, which successfully recovered the cells of strain LSSE-09 from the biological systems. The magnetically immobilized cells exhibited good catalytic activity under alkaline conditions over several batch cycles.

2. Materials and methods

2.1. Materials

Iron (II) chloride ($FeCl_2{\cdot}4H_2O$, analytical reagent grade), iron (III) chloride ($FeCl_3{\cdot}6H_2O$, analytical reagent grade), and concentrated ammonium hydroxide (25%) were purchased from Sinopharm Chemical Regent Beijing Co., Ltd. Poly (ethyleneimine) (PEI, 10172 Da), sodium citrate ($C_6H_5O_7Na_3{\cdot}2H_2O$, analytical reagent grade) were purchased from Sigma–Aldrich.

2.2. Preparation of surface modified magnetic NPs

The PEI-modified magnetic Fe_3O_4 NPs were prepared by a co-precipitation method with minor modification [38]. Firstly, 8.67 mmol of $FeCl_3{\cdot}6H_2O$ and 4.34 mmol of $FeCl_2{\cdot}4H_2O$ were dissolved in 100 mL deionized water. The mixture was stirred for 30 min under a nitrogen gas atmosphere at 85 °C. Then 25 mL of concentrated ammonium hydroxide (25%) was added to the solution. After the solution turned black, 0.26 mmol of sodium citrate was quickly added into the system. After 5 min stirring, 50 mL of the prepared PEI aqueous solution containing 0.26 mmol PEI was added, and the reactions continued for 2 h. Finally, the precipitate was separated by magnet and washed three times with de-ionized water. The resulting nanoparticles were suspended in water at 4 °C for storage. The concentration of magnetic nanoparticles was expressed in terms of dry weight per unit volume of suspension medium.

2.3. Preparation NPs-coated bacterial strain

P. phragmitetus LSSE-09 cells were inoculated into 100 mL LB medium, cultured aerobically at 37 °C and 150 rpm for 12 h. The cells were harvested by centrifugation (4000 × *g*) for 15 min at 4 °C, washed twice with de-ionized water and then suspended in 50 mM pH 9.0 Tris–HCl buffers. The dry weight of cells was approximate 1.95 g L^{-1}. A volume of 0.5 mL of PEI-modified NPs (10 g L^{-1}) was added and mixed thoroughly. The microbial cells were coated by adsorbing the magnetic NPs. For magnetic separation, a permanent magnet was placed at the side of the vessel. After several minutes (1–2 min), the coated cells were concentrated and separated from the suspension medium by decantation (Fig. 8).

2.4. Characterization

2.4.1. TEM measurement

The PEI-modified NPs and the NPs-coated cells were characterized by JEM-2010 transmission electron microscopy coupled with an EDS (Oxford) system. Samples were prepared by placing a drop of aqueous solution containing NPs and NPs-coated cells onto a Formvar-covered copper grid, and dried in the air.

2.4.2. FTIR measurement

FTIR spectra of PEI, bare Fe_3O_4 nanoparticles, and PEI conjugated Fe_3O_4 nanoparticles were recorded on a Bruker Vecter 22 FTIR spectrometer. Lyophilized nanoparticles was dispersed in KBr and then pelletized before measurements.

2.4.3. TGA measurement

The number of PEI incorporated into magnetite nanoparticles was determined by thermogravimetric analysis (Netzsch STA 449 C). Samples were heated from 30 to 800 °C at a heating rate of 10 °C min^{-1} in air.

2.4.4. Magnetization measurement

The magnetization measurement of magnetic nanoparticles was carried out with a vibrating sample magnetometer at room temperature (VSM, Model 4 HF VSM of ADE Technologies, Inc.).

2.4.5. Zeta potential measurement

The zeta potential of PEI-modified NPs and NPs-coated cells at different pH values was observed using Zeta PALS (Brookhaven Instruments Co., USA). The pH of the solution was adjusted with 0.01 M NaOH or 0.01 M HCl. After 1 h of stabilization with self-sealing rubber septum, the final solution pH was recorded, and the supernatant with small PEI-modified NPs or NPs-coated cells was then used to conduct zeta potential measurements.

2.5. Microbial reduction of Cr (VI)

2.5.1. Cr (VI) reduction

Cr (VI)-reduction experiments were performed anaerobically at 37 °C and 150 rpm under alkaline conditions (initial pH 9.0).