An efficient magnetically modified microbial cell biocomposite for carbazole biodegradation

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Abstract
Magnetic modification of microbial cells enables to prepare smart biocomposites in bioremediation. In this study, we constructed an efficient biocomposite by assembling Fe₃O₄ nanoparticles onto the surface of Sphingomonas sp. XLDN2-5 cells. The average particle size of Fe₃O₄ nanoparticles was about 20 nm with 45.5 emu g⁻¹ saturation magnetization. The morphology of Sphingomonas sp. XLDN2-5 cells before and after Fe₃O₄ nanoparticle loading was verified by scanning electron microscopy and transmission electronic microscopy. Compared with free cells, the microbial cell/Fe₃O₄ biocomposite had the same biodegradation activity but exhibited remarkable reusability. The degradation activity of the microbial cell/Fe₃O₄ biocomposite increased gradually during recycling processes. Additionally, the microbial cell/Fe₃O₄ biocomposite could be easily separated and recycled by an external magnetic field due to the super-paramagnetic properties of Fe₃O₄ nanoparticle coating. These results indicated that magnetically modified microbial cells provide a promising technique for improving biocatalysts used in the biodegradation of hazardous compounds.

Keywords: Carbazole; Immobilization; Nanoparticles; Biodegradation; Reusability

Background
As types of toxic and mutagenic common nitrogen compounds, carbazole and its derivatives readily undergo radical chemistry to generate the more poisonous hydroxynitrocabazoles [1-4]. Soil, river sediments, and ground water polluted by carbazole have become a great threat to the environment. Therefore, it is necessary to establish effective methods to clear up carbazole and its derivatives.

Nanoscale iron particles represent a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental cleanup problems [5]. Due to biocompatibility, large surface areas, high surface reactivity, and super-paramagnetic properties, nanoscale iron particles provide enormous flexibility for environmental applications [6-8]. Research has shown that nanoscale iron particles are very effective for the transformation and detoxification of a wide variety of common environmental contaminants, such as hazardous organic compound [9-11] and heavy metal ions [8,12].

The use of immobilized microorganisms rather than free cells in biodegradation can be advantageous to enhance the stability of the biocatalyst and to facilitate its recovery and reuse. Entrapment method as a traditional method is widely used in the immobilization of microorganisms [13]. In our previous study, Sphingomonas sp. XLDN2-5 as a carbazole-degrading strain was entrapped in the mixture of Fe₃O₄ nanoparticles and gellan gum using modified traditional entrapment method [7]. However, the mass-transfer problems of limited diffusion and steric hindrance reduced microbial cell access to substrate [14]. Therefore, we constructed an efficient biocomposite by assembling Fe₃O₄ nanoparticles onto the surface of Sphingomonas sp. XLDN2-5 cells in this study. The resulting microbial cell/Fe₃O₄ biocomposite exhibited good biodegradation activity and reusability.

Methods
Analytical grade carbazole was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and commercially available.

Sphingomonas sp. XLDN2-5, which can use carbazole as the sole source of carbon, nitrogen, and energy, was cultivated in the mineral salts medium (MSM) as previously described [15]. Cells were harvested in the exponential
phase (the optical density was about 0.68 to 0.70 at 620 nm) by centrifugation at 12,000 rpm for 10 min. The pellet was washed thrice with sodium chloride solution (0.9%, w/v) and then resuspended in sodium chloride solution (0.9%, w/v).

Fe₃O₄ nanoparticles were prepared as previously described [7]. Fe₃O₄ powder (1.0 g) was put into 100 ml distilled water to form the Fe₃O₄ particle suspension. After ultrasonic disruption (25 KHz, 10 min; BUG25-06, Branson, MO, USA) of the suspension, the Fe₃O₄ nanoparticles were well dispersed in distilled water to form a stable suspension.

Fe₃O₄ particle suspension (1%, w/v) and cell suspension were mixed with the ratio of cell wet weight to Fe₃O₄ of 1 (w/w). Microbial cells and Fe₃O₄ nanoparticles were fully mixed by vortexing, then the mixture was incubated at 30°C for 2 h in a dark shaker to obtain microbial cell/Fe₃O₄ biocomposites.

All biodegradation experiments were carried out in 100-ml flasks containing 10-ml MSM at 30°C on a reciprocal shaker at 180 rpm. In each experiment, 3,500 μg of carbazole was added to MSM, and the microbial cell/Fe₃O₄ biocomposites made by 2 ml mixture of Fe₃O₄ particle suspension and cell suspension served as biocatalysts. Additionally, the same amount of cells was conducted in the batch biodegradation experiment. All the subsequent experiments contained the same amount of carbazole and biocatalysts as above.

In the recycle experiments, after each batch of biodegradation, the microbial cell/Fe₃O₄ biocomposites were

Figure 1 The nature of Fe₃O₄ nanoparticles. A is the TEM image of Fe₃O₄ (magnification × 100,000); B is the magnetic curve for Fe₃O₄ nanoparticles. (σₛ, saturation magnetization; emu, electromagnetic unit; Oe, Oersted).
collected using a magnetic field, and then were washed thrice with MSM to remove the free cells. After the MSM was drained, 10 ml of fresh MSM containing carbazole was added to repeat the cycle. All experiments were performed in triplicate.

After each batch of biodegradation, the biodegradation mixture was added 20 ml ethanol, followed by centrifugation (12,000 rpm for 20 min) and filtration. Residual contents of carbazole were determined using High-performance liquid chromatography (HPLC). HPLC was performed with an Agilent 1100 series (Hewlett-Packard) instrument equipped with a reversed-phase C18 column (4.6 mm × 150 mm, Hewlett-Packard). The mobile phase was a mixture of methanol and deionized water (90:10, v/v) at a flow rate of 0.5 ml min⁻¹, and carbazole was monitored at 254 nm with a variable-wavelength detector.

The size and morphology of magnetic nanoparticles and microbial cell/Fe₃O₄ biocomposite were determined by transmission electronic microscopy (TEM; JEM-100cx II, JEOL, Akishima-shi, Japan). The sample was prepared by evaporating a drop of properly diluted microbial cell/Fe₃O₄ biocomposite or nanoparticle suspension on a carbon copper grid. The morphology of free cells was determined using a scanning electron microscope (SEM; S-570, Hitachi, Chiyoda-ku, Japan). Magnetization curves for the magnetic immobilized cells were obtained with a vibrating sample magnetometer (MicroMag 2900/3900, Princeton Measurements Corp., Westerville, OH, USA).

Results and discussion

Characteristics of microbial cell/Fe₃O₄ biocomposites

Among nanoparticles, nanoscale magnetite (Fe₃O₄) is of great interest as an immobilization carrier because of its biocompatibility, stability, large surface area, and super-paramagnetic properties [16,17]. In this study, an efficient microbial cell/Fe₃O₄ biocomposite was constructed by assembling Fe₃O₄ nanoparticles onto the surface of Sphingomonas sp. XLDN2-5 cells. Figure 1 showed the TEM images of Fe₃O₄ nanoparticles and their saturation magnetization. The average particle diameter of Fe₃O₄ nanoparticles was about 20 nm (Figure 1A), and their saturation magnetization was 45.5 emu·g⁻¹ (Figure 1B), which provided the nanoparticles with super-paramagnetic properties.

Figure 2 shows the microbial cells of Sphingomonas sp. XLDN2-5 before and after Fe₃O₄ nanoparticle loading. The Fe₃O₄ nanoparticles were efficiently assembled on the surface of the microbial cell because of the large specific surface area and the high surface energy of the nanoparticles as shown in Figure 2B. It was clear that the size of the sorbent was much smaller than that of microbial cell, which was about a few micrometers as shown in Figure 2A. Due to the super-paramagnetic properties of Fe₃O₄ nanoparticle coating, the microbial cell/Fe₃O₄ biocomposite could be easily separated and recycled by external magnetic field as shown in Figure 3. When a magnet was touched to the side of a vial containing a suspension of microbial cell/Fe₃O₄ biocomposite (Figure 3A), the cells aggregated in the region where the magnet touched the vial (Figure 3B), which can be used with high efficiency in difficult-to-handle samples [14].

Biodegradation activity and reusability of microbial cell/Fe₃O₄ biocomposites

With the purpose of understanding the biodegradation activity of the microbial cell/Fe₃O₄ biocomposite, the biodegradation rates of free cells and microbial cell/Fe₃O₄ biocomposite were tested at 30°C, respectively. Figure 4A showed that the microbial cell/Fe₃O₄ biocomposites had the same biodegradation activity as free Sphingomonas sp. XLDN2-5 cells. These results indicated that the Fe₃O₄ nanoparticle coating did not have a negative effect on the biodegradation activity of Sphingomonas sp. XLDN2-5. The reason may be that the coating layer of nanoparticles...
does not change the hydrophilicity of the cell surface due
to biocompatibility of Fe_{3}O_{4} nanoparticles [10,18], which
are very important for the immobilization of microbial
cells. Additionally, the effect of the coating layer on mass
transfer is negligible because the structure of the coating
layer is looser than that of the cell wall [11]. Thus, the
microbial cell/Fe_{3}O_{4} biocomposite could produce a sys-
tem not limited by diffusional limitations [19].

In an industrial bioremediation process, the recycle of
the biocatalysts could be an important factor that deter-
mines the effectiveness of degradation for a long time.
The carbazole biodegradation activities of microbial cell/

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![Figure 3](image)

**Figure 3** Digital photo of microbial cell/Fe_{3}O_{4} biocomposite suspension before (A) and after collection (B) using a magnetic field.

![Figure 4](image)

**Figure 4** The carbazole biodegradation by free cells and microbial cell/Fe_{3}O_{4} biocomposites. A is for carbazole biodegradation. B is for the reuse of microbial cell/Fe_{3}O_{4} biocomposites.
Fe₃O₄ biocomposite were tested repeatedly. Each test was performed until the carbazole was consumed completely. At the end of each test, the microbial cell/Fe₃O₄ biocomposites were collected by application of a magnetic field and then reused in another test. As shown in Figure 4B, from the first to the sixth cycle, 3,500 µg carbazole was completely consumed by microbial cell/Fe₃O₄ biocomposite in 9 h; from the seventh to the tenth cycle, the same amount of carbazole was completely consumed in only 2 h. It was clear that the biodegradation activity of microbial cell/Fe₃O₄ biocomposites increased gradually during the recycling processes, which may be due to that more microbial cells was immobilized by Fe₃O₄ nanoparticles with the microbial cell growth and reproduction. Additionally, carbazole can be quickly transferred to the biocatalyst surface where nonsorbed were located and resulted in the increase of biodegradation rate [10,14]. These results are different from other researchers’ report which stated that the desulfurization activity of microbial cells coated by magnetite nanoparticles decreased gradually after a few test cycles [11].

Conclusions
In conclusion, the microbial cell/Fe₃O₄ biocomposite was evaluated as a novel aspect of the industrialization of microbial cell immobilization. Moreover, magnetic (Fe₃O₄) nanoparticles have a large specific surface and super-paramagnetic properties, which not only reduced the mass transfer resistance of traditional immobilization method, but also facilitated the recovery of immobilized cells in the reuse process. Additionally, the recycle experiments demonstrated that the biodegradation activity of microbial cell/Fe₃O₄ biocomposites increased gradually during the recycling processes. These results indicated that magnetically modified microbial cells provide a promising technique for improving biocatalysts used in the biodegradation of hazardous organic compounds.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
YL and XD designed the biodegradation experiments and carried out the characterization. CW and XL participated in Fe₃O₄ nanoparticles and microbial cell/Fe₃O₄ biocomposite fabrication. XW and PX made substantial contributions to the conception and design of this paper. XW and YL wrote the paper. All authors read and approved the final manuscript.

Acknowledgements
This work was supported by grants from the National Natural Science Foundation of China (21177074), Excellent Middle-Aged and Youth Scientist Award Foundation of Shandong Province (BS2010SW016), and New Teacher Foundation of Ministry of Education of China (20090131120005).

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Received: 2 September 2013 Accepted: 4 December 2013 Published: 11 December 2013

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doi:10.1186/1556-276X-8-522
Cite this article as: Li et al.: An efficient magnetically modified microbial cell biocomposite for carbazole biodegradation. Nanoscale Research Letters 2013 8:522.