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# Preparing and characterizing $Fe_3O_4$@cellulose nanocomposites for effective isolation of cellulose-decomposing microorganisms

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ABSTRACT

This study developed $Fe_3O_4$@cellulose nanocomposites by co-precipitation synthesis for bacteria capture and isolation. By surface modification with cellulose, the $Fe_3O_4$@cellulose nanocomposites have 20 nm average particle size and 3.3–24.9 emu/g saturation magnetization. Living bacteria could be captured by the $Fe_3O_4$@cellulose nanocomposites and harvested by magnetic field, with high efficiency (95.1%) and stability ( > 99.99%). By metabolizing cellulose and destroying the $Fe_3O_4$@cellulose@bacteria complex, cellulose-decomposing microorganisms lost the magnetism. They were therefore able to be isolated from the inert microbial community and the separation efficiency achieved over 99.2%. This research opened a door to cultivate the uncultivable cellulose-decomposing microorganisms *in situ* and further characterize their ecological functions in natural environment.



## 1. Introduction

With the capability of remote control by magnetic field, magnetic nanoparticles (MNPs) introduce many possibilities in biochemical processes as a novel tool in microbial biotechnology [1]. MNPs surface modification is widely investigated to improve their stability and biocompatibility. Antigen functionalized MNPs achieve high throughput bacteria or cell separation by flow cytometry [2], and chitosan functionalization allows accurate and targeting gene/drug delivery via MNPs by the tagging biological entities [3]. In environmental engineering, MNPs are functionalized with poly-allylamine-hydrochloride to improve biosensor sensitivity [4,5], and remove pathogens for drinking water purification [6,7].

Uncultivable microorganisms account for over 99% of all the species and their functions are important for ecological system [8]. Particularly, cellulose metabolism is a key component of carbon cycle on the planet [9], but the majority of cellulose-decomposing microorganisms remain uncultivable and unknown. The recent progress to cultivate the uncultivable microorganisms with MNPs is the cutting edge for environmental ecology [10], opening a door to reveal the physiological behavior and ecological functions of uncultivable bacteria from complex microbial community. Nevertheless, the macromolecular poly-allylamine-hydrochloride reduces the accessibility of cellulose-decomposing microorganisms to cellulose. New surface functionalization technique can broaden its applicable potential in assessing the fate of various polymers in natural environment.

We developed a novel cellulose functionalization method and prepared the biocompatible $Fe_3O_4$@cellulose nanocomposites. Two different bacterial strains, *Acinetobacter baylyi* and *Aeromonas veronii* (cellulose-decomposing bacterium), were functionalized by $Fe_3O_4$@cellulose nanocomposites and investigated for their magnetism change after cultivation. The successful isolation of *A. veronii* from *Acinetobacter–Aeromonas* community proved the feasibility to cultivate the functional cellulose-decomposing bacteria *in situ*.

## 2. Experimental section

### 2.1. Synthesis of $Fe_3O_4$@cellulose nanocomposites

All the chemicals were analytical grade from Sigma-Aldrich (UK) without specific statement. Cellulose suspension was prepared by dissolving 0.4 g cellulose in 20 mL alkaline solution (NaOH:urea:$H_2O$ = 7:12:81), mixed well and standing at 4 °C overnight [11]. MNPs were synthesized by co-precipitation method [4]. The $Fe_3O_4$@cellulose nanocomposites was subsequently synthesized by gently mixing the MNPs and cellulose suspension (with ratios of 0.60, 0.55, 0.50, 0.45, 0.43, 0.36, 0.30, 0.24, 0.18, 0.12 and 0.06, m/m) for 5 min, captured by permanent magnet for

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5 min, and finally washed by deionized water 2 to 3 times until the pH value was 7.0.

### 2.2. Cellulose-decomposing microorganisms isolation from microbial community

*Acinetobacter baylyi* (no cellulose-decomposing capacity) and *Aeromonas veronii* (cellulose-decomposing bacterium) were used in this study. The artificial microbial community was made by mixing *A. baylyi* and *A. veronii* in water (1:1). To isolate the cellulose-decomposing microorganism from *A. baylyi*, *A. veronii* and microbiota, a hundred microliter of each bacterial suspension (diluted to $1.0 \times 10^8$ CFU/mL) was mixed with 900 μL $Fe_3O_4$@cellulose suspension. After successful functionalization and cultivation for 5 days, the targeting cellulose-decomposing bacteria were harvested from the supernatant (Graphic abstract, details see Supplementary material).

### 2.3. Measurements and data analysis

The morphology of MNPs and $Fe_3O_4$@cellulose nanocomposites were analyzed by transmission electron microscopy (TEM, JEM-2100, 100 kV, Japan). Phase identification was carried out by X-ray diffraction (XRD, D8-Advance, Bruker, UK). The magnetic properties were measured by a vibrating sample magnetometer (VSM, Lake Shore, 7304, USA) at 25 °C and in a magnetic field varying from −1.7 T to +1.7 T. The nanoparticle fingerprint was obtained by InVia Raman microscopy (Renishaw, UK) with a 785-nm excitation laser and 10 s acquisition time. The number of magnetic-free bacteria was determined by quantitative polymerase chain reaction (qPCR, Supplementary material) [12,13].

## 3. Results and discussion

From the TEM morphology (Fig. 1A), raw MNPs showed a round shape and had strong self-aggregation attributing to the large surface-to-volume ratio and the expressed surface energy [14]. The XRD pattern (Fig. 1C) identified the diffraction peaks of MNPs as $2\theta = 30.0°$, 35.4°, 43.2°, 53.6°, 57.1° and 62.7°, indexed to (220), (311), (400), (422), (511) and (440) lattice planes [15]. The mean size of MNPs was calculated as 8 nm by Scherer equation ($D = \kappa\lambda/\beta\cos\theta$). $Fe_3O_4$@cellulose nanocomposites had bigger size (20 nm, Fig. 1B) but with less aggregation since polymer functionalization could improve their stability by steric repulsion [4].

The Raman spectra (Fig. 1D) showed that the characteristic peaks of $Fe_3O_4$@cellulose nanocomposites fitted well with those of MNPs (magnetite at 678 $cm^{-1}$) and cellulose (υ(C–O–C) asym at 1094 $cm^{-1}$ and 1120 $cm^{-1}$, υ(C–O–C) at 906 $cm^{-1}$, δ($CH_3$) at 1380 $cm^{-1}$ and δ($CH_3$) asym at 1460 $cm^{-1}$) [16]. All the magnetization curves behaved S shape, and raw MNPs had the highest the saturation magnetization (43.4 emu/g, Fig. 1E). The saturation magnetization of $Fe_3O_4$@cellulose was positively related to the ratio of MNPs to cellulose, as 24.9 emu/g for 0.6:1 (MNPs:cellulose), 11.4 emu/g for 0.4:1 and 3.3 emu/g for 0.12:1, respectively.

$Fe_3O_4$@cellulose nanocomposites could effectively capture bacteria via electrostatic adsorption. The ratio of MNPs to cellulose affected the bacteria capture efficiency (Fig. 2A). When the MNPs: cellulose ratio was above 0.1, the bacteria capture efficiency was above 90%, whereas it declined to 84.3% at the ratio of 0.06. The optimized ratio was set as 0.4 to achieve both high capture efficiency and sufficient cellulose for bacterial growth.

The capture efficiency was above 90% when the bacterial amount was less than $4.0 \times 10^{14}$ CFU/g $Fe_3O_4$@cellulose (Fig. 2B). Langmuir isotherm equation (Eq. (1)) can describe the adsorption isotherm of $Fe_3O_4$@cellulose nanocomposites and fitted well with the experimental data (Fig. 2B).

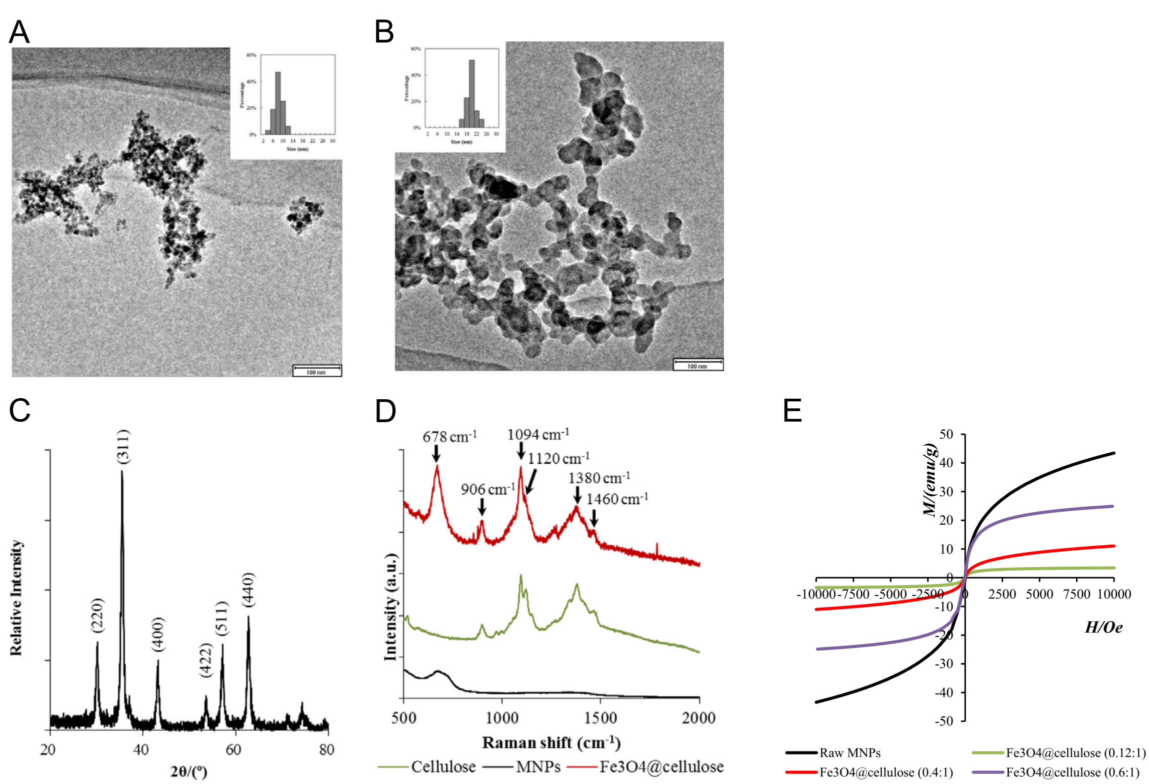


**Fig. 1.** TEM images of MNPs (A) and $Fe_3O_4$@cellulose nanocomposites (B). The XRD pattern of $Fe_3O_4$@cellulose nanocomposites (C). Raman microscopy of cellulose, MNPs and $Fe_3O_4$@cellulose (D). The magnetization curve of synthesized MNPs and $Fe_3O_4$@cellulose (E).