Synergistic Enhancement in Catalytic Performance of Superparamagnetic Fe₃O₄@Bacillus subtilis as Recyclable Fenton-Like Catalyst

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Abstract: Novel well-defined superparamagnetic Fe₃O₄@B. subtilis composite (Fe₃O₄@B. subtilis SPMC) was synthesized through a facile electrostatic attraction method and used as a recyclable heterogeneous Fenton-like catalyst. With the presence of H₂O₂, Fe₃O₄@B. subtilis SPMC can remove nearly 87% of the doxycycline at the initial concentration of 50 mg L⁻¹, exhibiting enhanced Fenton-like catalytic performance than pristine Fe₃O₄. The mechanism study demonstrates the synergistic effect between Bacillus subtilis adsorption and Fenton-like ability of Fe₃O₄ dominates the enhancement for Fenton-like catalytic efficiency of Fe₃O₄@B. subtilis SPMC. The obtained composite shows excellent recycling ability, reusability, and stability, which pave a new way for future design on highly efficient Fenton-like catalyst for degradation of organic pollutants.

Keywords: Fe₃O₄; Bacillus subtilis; synergistic effect; Fenton-like; recyclable; superparamagnetic

1. Introduction

Magnetite (Fe₃O₄) has attracted growing research interests in various fields, such as Li ion batteries [1], catalysts [2], drug delivery and targeting [3], etc., due to its distinguished physical and chemical properties [4,5]. However, these magnetite Fe₃O₄ nanoparticles (MNP) may aggregate into large clusters because of the anisotropic dipolar interactions, reducing their dispersibility and other specific properties and ultimately diminishing their activity [6]. In addition, another challenge that impedes the practical application of Fe₃O₄ is the production of iron-containing waste sludge, which introduces secondary pollution [7,8]. Hence, to overcome these disadvantages, significant efforts have been made to immobilize the Fe₃O₄ particles onto various support materials, simultaneously preserving their unique magnetic property [9–12].

Lately, microorganisms employed as support materials has been attracting great interest because of their major advantages such as ample resources, environmentally friendliness, and abundant functional groups [13]. Bacillus subtilis is a kind of genus Bacillus and can be easily found in water, soil, air, and decomposing plant matter [13–15]. Thus, owing to its distinguished physicochemical/biological properties, B. subtilis has been selected as an ideal candidate for the synthesis of composite materials for organic pollutants removal [13–16]. However, until now, no one has been focused on the preparation and application of integrated Fe₃O₄@B. subtilis heterogeneous catalyst.

In this study, we present a facile electrostatic attraction method for the coating of Fe₃O₄ nanoparticles onto the surfaces of B. subtilis to form superparamagnetic recyclable Fe₃O₄@B. subtilis composite (Fe₃O₄@B. subtilis SPMC) which can be used as an effective recyclable heterogeneous Fenton-like catalyst.
heterogeneous Fenton-like catalyst. The morphology, crystal structure, functional groups, magnetic property, and Fenton-like catalytic performance of Fe$_3$O$_4$@B. subtilis superparomagnetic composite (Fe$_3$O$_4$@B. subtilis SCP) are systematically characterized and evaluated. The catalytic performance of Fe$_3$O$_4$@B. subtilis SCP is significantly enhanced compared to the pristine Fe$_3$O$_4$. The prepared Fe$_3$O$_4$@B. subtilis SCP exhibits excellent recycling ability due to its superparomagnetic feature. The outstanding reusability was evaluated by successive batches of Doxycycline (DC) degradation, and the good chemical stability was also investigated by X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). The leaching of iron ions from Fe$_3$O$_4$@B. subtilis was evaluated by inductively coupled plasma mass spectrometry (ICP-MS). The mechanism for the enhanced Fenton-like catalytic activity of Fe$_3$O$_4$@B. subtilis SCP is proposed and investigated through photoluminescence (PL) study.

2. Results and Discussion

2.1. Characterization

The pristine B. subtilis cells (Figure 1a) are rod-shaped with smooth surface and the length and width if which are approximately 1.4 ± 0.2 and 0.6 ± 0.1 µm, respectively (Supplementary Figure S1a,b). The synthesized Fe$_3$O$_4$@B. subtilis SPMC maintain the bacilliform morphology of B. subtilis cells, but with much rougher surfaces, indicating successful coating of Fe$_3$O$_4$ magnetic nanoparticles (Figure 1b). The rougher surfaces may be beneficial for the photocatalytic ability by providing a higher specific surface area (Supplementary Figure S2). Moreover, the slight increase in diameter (length = 1.45 ± 0.5 µm; width = 0.65 ± 0.3 µm (Supplementary Figure S1c,d)) in comparison with B. subtilis cells provides assertive evidence that the Fe$_3$O$_4$ MNPs are attached onto the surfaces of B. subtilis. Furthermore, the magnification image (the insert image in Figure 1b) shows that some residual bare area on the surface of B. subtilis still remain, which might be used as adsorption sites for pollutants. The elemental composition of the prepared samples was studied by Energy Dispersive X-ray Spectroscopy (EDX) analysis. Comparing Figure 2c,d, the detection of Fe element confirmed the anchor of Fe$_3$O$_4$ MNPs on to the B. subtilis surfaces. Simultaneously, two-dimensional X-ray mapping of Fe$_3$O$_4$@B. subtilis (Supplementary Figure S3) clearly shows the dispersions of C, O, and Fe elements on the microorganism surfaces.

![Figure 1. (a,b) Scanning electron microscope (SEM) images and (c,d) EDX spectra for B. subtilis and Fe$_3$O$_4$@B. subtilis.](image-url)
The crystallographic structure of the as-obtained products was determined by XRD analysis (Figure 2a). The broad peak around 2θ = 20° shows that B. subtilis is amorphous (black curve) [13,15]. All the diffraction peaks of Fe₃O₄ MNPs (blue curve) could be readily indexed as face-centered cubic structured Fe₃O₄, which coincides well with the standard data (JCPDS card No. 19-0629) [17]. It is noted that the diffraction peaks corresponding to Fe₃O₄ are also presented in the XRD pattern of Fe₃O₄@B. subtilis SPMC (red curve), confirming the successful coating of Fe₃O₄ nanoparticles onto the surfaces of B. subtilis.

![XRD patterns of B. subtilis, Fe₃O₄, and Fe₃O₄@B. subtilis SPMC; (b) wide scan XPS spectra and (c) high resolution Fe 2p spectra of Fe₃O₄@B. subtilis SPMC; (d) hysteresis loops of Fe₃O₄ and Fe₃O₄@B. subtilis SPMC and its the separation-redispersion process (insert image).](image)

Figure 2. (a) XRD patterns of B. subtilis, Fe₃O₄, and Fe₃O₄@B. subtilis SPMC; (b) wide scan XPS spectra and (c) high resolution Fe 2p spectra of Fe₃O₄@B. subtilis SPMC; (d) hysteresis loops of Fe₃O₄ and Fe₃O₄@B. subtilis SPMC and its separation-redispersion process (insert image).

XPS measurement was employed to characterize the Fe oxidation state in Fe₃O₄@B. subtilis SPMC. The photoelectron lines at binding energies of about 282.1, 397.0, 531.0, and 711.2 eV are attributed to C 1s, N 1s, O 1s, and Fe 2p, respectively (Figure 2b) [18]. In Figure 2c, two main peaks with satellite peaks between 705.0 and 735.0 eV are resolved at 710.9 eV and 724.5 eV, which are assigned to Fe 2p₁/₂ and Fe 2p₃/₂, respectively, and are consistent with the standard Fe₃O₄ XPS spectrum [18,19]. In addition, the elemental concentration of C, N, O and Fe in atomic % calculated according to relative sensitivity factors (RSFs) and spectra intensities were 69.02%, 4.0%, 22.87%, and 4.13%, respectively.

The magnetically controllable aggregation behavior of Fe₃O₄@B. subtilis was investigated by VSM study. Figure 2d shows the magnetic hysteresis loops of Fe₃O₄ and Fe₃O₄@B. subtilis SPMC with an extra magnetic field of 20,000 Oe at 300 K. The negligible coercivity (Hc) of hysteresis loop (71.1 Oe) and consequently no remanence (Mr, 1.23 emu/g) indicate the superparamagnetic nature of the Fe₃O₄@B. subtilis SPMC [20], exhibiting excellent redispersion stability (Figure 2d, inset). The saturation magnetization of the Fe₃O₄@B. subtilis SPMC (16.8 emu g⁻¹) is lower than the pristine Fe₃O₄ because of the non-magnetic properties of B. subtilis. In their homogeneous dispersion, Fe₃O₄@B. subtilis SPMC show quick movement under the extra magnetic field and re-disperse quickly with a slight shake.
after removing the magnetic field, suggesting the prepared Fe3O4@B. subtilis SPMC presents excellent magnetic responsivity and redispersibility [21].

2.2. Fenton-Like Catalytic Degradation of Doxycycline

To test the catalytic performance of the as-prepared Fe3O4@B. subtilis SPMC, Fenton-like reactions were conducted for DC degradation. Comparable dosages of Fe3O4@B. subtilis, B. subtilis and Fe3O4 were used. Direct oxidation (blank) by H2O2 without any particles was also performed as a reference. Solution pH values were not controlled during the degradation process because they decreased very slightly (ΔpH < 0.5).

As depicted in Figure 3a, direct oxidation of DC molecules (blank) by H2O2 is negligible. B. subtilis shows very low removal rate for DC within 30 min. When the Fe3O4@B. subtilis SPMC were used as catalyst, DC was nearly degraded completely in 30 min, showing a superior high catalytic activity in the Fenton-like system. Contrastively, under the same reaction condition, only 68.5% of DC was degraded by the as-synthesized Fe3O4 MNPs. The pseudo-first-order reaction was used to describe the kinetics of these catalytic reactions, and the rate constants k for DC degradation are calculated according to the regression curves of −ln(C/C0) vs. time (t) (Figure 3b). The Fe3O4@B. subtilis SPMC shows a highest k value of 0.129 min⁻¹, followed by Fe3O4 (0.0374 min⁻¹) and B. subtilis (0.00222 min⁻¹), indicating the faster catalytic degradation kinetics and stronger catalytic ability of Fe3O4@B. subtilis SPMC.

![Figure 3](image.png)

**Figure 3.** (a) Degradation and adsorptive (inset) performances for DC over as-prepared samples (B. subtilis, 0.35 g L⁻¹; Fe3O4, 0.125 g L⁻¹; Fe3O4@B. subtilis SPMC, 0.5 g L⁻¹): initial concentration of DC, 25 mg L⁻¹; H2O2 (20 mmol), room temperature, agitation speed 150 rpm; (b) −ln(C/C0) as a function of time for DC degradation.

The enhanced catalytic activity of Fe3O4@B. subtilis SPMC compared to pristine B. subtilis and Fe3O4 MNPs can be attributed to the synergistic effect between adsorption by the B. subtilis bodies and Fenton-like oxidation by the Fe3O4 particles. The isoelectric point of Fe3O4@B. subtilis SPMC was determined to be 4.1 according to the zeta potentials curves (Supplementary Figure S4). Thus, the negative charge of Fe3O4@B. subtilis SPMC surfaces can absorb cationic form of DC via electrostatic interaction at neutral pH, resulting in a higher reactant concentration around the composite surfaces. In turn, the adsorption sites on the B. subtilis surface can be refreshed because of the decomposition of the DC molecules by Fe²⁺/Fe³⁺/H₂O₂ Fenton-like system.

2.3. Effect of H₂O₂ Dosage on Degradation of DC

The degradation efficiency of DC increased with increasing H₂O₂ dosage from 5.0 to 20.0 mmol, and then slightly decreased beyond 20.0 mmol (Figure 4a). The decreased DC removal under higher H₂O₂ dosage may be because that excessive H₂O₂ can induce OH⁻ radicals scavenging effect (HO⁻ + H₂O₂ → HOO⁻ + H₂O) [15,22]. It is noteworthy that the generation of another radical HOO⁻,
whose oxidation ability is much lower than that of the HO• radicals, shows much less contribution to DC degradation [23]. Therefore, the initial H₂O₂ dosage was selected as 20.0 mmol to achieve the highest removal of DC in the present work.

Figure 4. (a) Effect of H₂O₂ dosage on removal efficiency of DC by Fe₃O₄@B. subtilis SPMC (catalyst dosage 0.5 g L⁻¹, initial concentration of DC 25 mg L⁻¹, netural pH, room temperature); (b) The cyclic utilization of Fe₃O₄@B. subtilis SPMC for degrading DC (catalyst dosage 0.5 g L⁻¹, initial concentration of DC 25 mg L⁻¹, H₂O₂ 20 mmol, netural pH, room temperature).

2.4. Iron Ion Leaching

To exclude the possibility that the observed catalytic activity of Fe₃O₄@B. subtilis SPMC in Fenton-like system is caused by the leaching ions, similar batch reactors with solutions (100 mL) containing Fe₃O₄@B. subtilis SPMC (0.5 g L⁻¹) at a neutral pH were mechanically stirred for 30 min at room temperature. Then, the Fe₃O₄@B. subtilis SPMC were removed by centrifugation to obtain leaching solution. 0.1 mL of the above supernatant was diluted with 4.9 mL HNO₃ (5%) to analyze the dissolved ions with ICP-MS. The result shows a Fe ions concentration of 0.612 ppm, which correspond to about 0.68% of the total Fe content in Fe₃O₄@B. subtilis SPMC. The remaining part of the leaching solution was employed to the homogeneous Fenton-like degradation of DC, and was initiated by the addition of fresh H₂O₂. By adding 2.5 mg DC and 20 mmol H₂O₂, only 7.5% of DC was removed within 30 min, which was much less than the removal of 98.1% in the Fe₃O₄@B. subtilis SPMC-H₂O₂ Fenton-like system at neutral pH (Figure 4a). Since the homogeneous degradation is negligible, the DC degradation at neutral pH mainly originates from the heterogeneous Fe₃O₄@B. subtilis SPMC-H₂O₂ Fenton-like system instead of the leached ions.

2.5. Stability and Reusability

The reusability and stability of Fe₃O₄@B. subtilis SPMC was evaluated by successive batches of DC degradation. After each run, Fe₃O₄@B. subtilis SPMC was re-collected, rinsed with DI water, and tested again. Fe₃O₄@B. subtilis SPMC can be reused for at least five consecutive runs, and the reused ones nearly retained the catalytic activity of the fresh catalyst (Figure 4b). The reusability of Fe₃O₄ was not investigated in this work because of two reasons: (1) the removal efficiency of DC, used for evaluation of reusability of Fe₃O₄@B. subtilis in Figure 4b, can be attributed the synergistic effect of adsorption by B. subtilis support and degradation by generated radicals, which is more complicated than bare Fe₃O₄; (2) Fe₃O₄ MNPs may present higher removal efficiency of DC after six cycles than that of Fe₃O₄@B. subtilis because they may leach much more iron ions for involving the Fenton-like process. However, in turn, more leaching ions means worse stability. Therefore, few publications have reported on the comparison between the repeatability of Fe₃O₄ and Fe₃O₄@support.

The good chemical stability of Fe₃O₄@B. subtilis SPMC was further confirmed by conducting XRD and XPS measurements after reaction. No considerable changes in XRD patterns (Figure 5a) were
observed after being reused for five cycles, indicating the stable chemical and crystalline structure of the Fe₃O₄@B. subtilis SPMC. XPS measurement (Figure 5b) demonstrates that the at % of C, N, O, and Fe are almost the same as those before reaction. These results indicate that Fe₃O₄@B. subtilis SPMC are durable and can be reused without great loss of catalytic activity in a long term Fenton-like reaction system at a neutral pH.

![XRD patterns of Fe₃O₄@B. subtilis SPMC before and after Fenton-like reaction](image1)

![Wide scan XPS spectra of Fe₃O₄@B. subtilis SPMC after Fenton-like reaction](image2)

![Fluorescence spectral changes observed during Fenton-like process with Fe₃O₄@B. subtilis SPMC suspended in a 5 × 10⁻⁴ M basic solution of TA (λₑₓc = 315 nm)](image3)

Figure 5. (a) XRD patterns of Fe₃O₄@B. subtilis SPMC before and after Fenton-like reaction; (b) wide scan XPS spectra of Fe₃O₄@B. subtilis SPMC after Fenton-like reaction; (c) Fluorescence spectral changes observed during Fenton-like process with Fe₃O₄@B. subtilis SPMC suspended in a 5 × 10⁻⁴ M basic solution of TA (λₑₓc = 315 nm).

2.6. Degradation Mechanism

The dramatic Fenton-like catalytic activity of the as synthesized Fe₃O₄@B. subtilis SPMC motivated us to further investigate the mechanism of the Fenton-like degradation process. Although the Fenton-like reaction mechanism has not been fully understood, it is generally accepted that a series of reactive radical species (RRS)—such as HOO·, hydroxyl radical (OH·), superoxide radical (O₂⁻), or singlet oxygen (¹O₂)—are supposed to be involved in the Fenton-like process [24–26]. However, which one plays the most important role in the Fenton-like process is still unclear, and is quite different from various systems. Luo et al. suggested that OH· radicals make a major contribution in the Fenton-like degradation of organic dyes at weak acidic conditions [27]. Li and co-workers demonstrated that the singlet oxygen ¹O₂ produced from HOO· and HO· directly participates in the degradation of organic pollutants [25]. Therefore, on the basis of all the information obtained above and observations in the literatures [28–30], we propose that the mechanism of the H₂O₂ activation by Fe₃O₄@B. subtilis SPMC under neutral condition may involve the initial formation of complex intermediates between ≡Fe²⁺, ≡Fe³⁺, and H₂O₂, being marked as H₂O₂≡Fe²⁺ Equation (1) and H₂O₂≡Fe³⁺ Equation (2), where ≡Fe²⁺ and ≡Fe³⁺ stands for Fe(II) and Fe(III) sites on the surface of Fe₃O₄@B. subtilis SPMC. The intermediate H₂O₂≡Fe³⁺ can convert to ≡Fe²⁺ species and HO₂⁻ (Equation (3)). The generated HO₂⁻ can further react with ≡Fe³⁺ to produce ≡Fe²⁺ species and O₂.
Equation (4), giving an explanation of those bubbles generated in the reaction process. All the formed≡FeII species Equations (3) and (4) and the initially generated≡FeIII species Equation (1) activate H2O2 to generate hydroxyl radicals HO· Equation (5), which is a very strong oxidative radical to degrade DC molecules Equation (6).

\[
\begin{align*}
H_2O_2^+ & \equiv Fe^{II} \rightarrow H_2O_2 \equiv Fe^{II} \\
H_2O_2^+ & \equiv Fe^{III} \rightarrow H_2O_2 \equiv Fe^{III} \\
\equiv & Fe^{III} + H_2O_2 \rightarrow \equiv Fe^{II} + HO_2^- + H^+ \\
\equiv & Fe^{III} + HO_2 \rightarrow \equiv Fe^{II} + O_2 + H^+ \\
H_2O_2^+ & \equiv Fe^{II} \rightarrow \equiv Fe^{III} + HO^- + OH^- \\
HO^- + DC & \rightarrow CO_2 + H_2O
\end{align*}
\]

Based on our hypothesis, HO· radicals make a great contribution to DC degradation by Fe3O4@B. subtilis SPMC. Thus, the formation of HO· was confirmed by the PL spectra using terephthalic acid (TA) as a probe molecule (Supplementary Figure S5). Figure 5 shows the fluorescence spectral changes observed during Fenton-like process with Fe3O4@B. subtilis suspended in a basic TA solution. As can be seen, with reaction time prolongs the PL intensity of 2-hydroxy terephthalic acid around 428 nm gradually increased, demonstrating the production of the hydroxyl radicals. Therefore, we can conclude that the enhanced degradation performance of DC by Fe3O4@B. subtilis under neutral pH can be due to the synergistic effect between the excellent adsorption ability and hydroxyl radicals generated from Fenton-like activation of H2O2 by Fe3O4@B. subtilis SPMC.

3. Experimental

3.1. Materials

B. subtilis powder was purchased from Guangzhou lvhui biological Company. All chemical reagents including FeCl3·6H2O, N2H4·H2O, CH2O, H2SO4, and Doxycycline were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA) and used directly without further purification. The chemical structure of doxycycline is presented in Figure 6. Absolute ethanol and double-distilled water were used through this work.

![Figure 6. Chemical structure of Doxycycline.](image-url)

3.2. Synthesis of Fe3O4 MNPs

Fe3O4 MNPs were synthesized through a hydrothermal method using FeCl3·6H2O as a single iron source [31]. Specifically, 1.20 g of FeCl3·6H2O was placed in a dry beaker, and then 5 mL of N2H4·H2O was added into the beaker dropwise with continuous stirring. After that, 2 mL of CH2O and 33 mL deionized water were added into the baker, stirred for 10 min, and then the mixture was transferred to a 50 mL Teflon-lined stainless-steel autoclave and heated at 120 °C for 5 h. After cooling down, the black precipitate was collected with a magnet and washed with water and absolute ethanol for three times and then dried at 80 °C for 10 h.
3.3. Preparation of Fe₃O₄@B. subtilis Superparamagnetic Composite (SPMC)

Fe₃O₄@B. subtilis SPMC were fabricated based on an electrostatic attraction method (Supplementary Figure S6). Typically, dried magnetic Fe₃O₄ MNPs and 1.0 g of B. subtilis dry cell powder were dispersed in 100 mL of distilled water separately and the pH values was adjusted to 5.0 by adding 1 M H₂SO₄. The isoelectric points of Fe₃O₄ MNPs and B. subtilis were determined to be pH 2.2 and 6.6, respectively. Therefore, according to the zeta potential-pH curve (Supplementary Figure S4), at an experimental pH of 5.0, B. subtilis is negatively charged and Fe₃O₄ MNP is positively charged. Next, the two suspensions were sonicated for 20 min to facilitate deaggregation and then centrifuged. After that, the Fe₃O₄ MNPs and B. subtilis were re-dispersed in 100 mL distilled water separately and mixed under continuous mechanical stirring for 1 h at room temperature. The mixture was then left for 3.0 h at room temperature to form the Fe₃O₄@B. subtilis SPMC. The resulting particles were centrifuged, washed by ethanol and water for three times. The actual Fe content (18%, i.e., Fe₃O₄ content of 25%) in Fe₃O₄@B. subtilis SPMC was detected using ICP-MS. For the ICP-MS measurements, 10 mg Fe₃O₄@B. subtilis SPMC were first dissolved with 5 mL 5% concentrated HNO₃, and then diluted 50 times using HNO₃ (5%).

3.4. Characterizations

Scanning electron microscopy (SEM) images were recorded using a microscope (6300F, JEOL Ltd., Tokyo, Japan). The elemental composition was carried out by energy dispersive spectroscopy (EDX) analysis and energy dispersive X-ray (EDX) mapping. XRD patterns were acquired over a diffraction angle range (2θ) 5–80° using an X’Pert X-ray diffraction spectrometer with a Cu Kα X-ray source (MiniFlex 600, Rigaku, Tokyo, Japan). XPS spectra were obtained on an electron spectrometer (ESCALab220i-XL, VG Scientific, Waltham, MA, USA) using 300 W Al-Kα radiation. Magnetization measurements at room temperature were obtained using a vibrating sample magnetometer (Lake Shore Cryotronics, Inc., Carson, CA, USA).

3.5. Catalytic Tests

The catalytic performance of Fe₃O₄@B. subtilis SPMC was tested by the degradation of DC in semi-batch operation mode at neutral pH. 0.05 g catalysts were dispersed into 100 mL aqueous solution of DC (25 mg L⁻¹) at room temperature. The suspensions were mechanically stirred in dark for 30 min to achieve the adsorption/desorption equilibrium. The DC concentrations after equilibration were measured and taken as the initial concentration (C₀). Then, the degradation reaction was initiated by the addition of H₂O₂ under mechanical stirring conditions at room temperature. At regular time intervals, about 5 mL of solution were taken out and immediately centrifuged at 10,000 rpm for 3 min, and the supernatant was determined by using a UV–vis spectrophotometer. Each experiment was conducted in triplicate.

3.6. Detection of Radical Species

Terephthalic acid (TA) was used to detect OH·. Briefly, 0.1 g Fe₃O₄@B. subtilis SPMC was dispersed in a 100 mL of the TA (5 × 10⁻⁴ mol L⁻¹) aqueous with an addition of 20.0 mmol H₂O₂ at room temperature and without any DC. The intensity of the PL signal at 428 nm was investigated on a fluorescence spectrophotometer (Shimadzu RF-5301PC, Tokyo, Japan) at an excitation wavelength of 315 nm.

4. Conclusions

In this study, novel superparamagnetic Fe₃O₄@B. subtilis Fenton-like catalyst was synthesized through a facile electrostatic attraction process. The obtained Fe₃O₄@B. subtilis exhibits significantly enhanced Fenton-like catalytic ability than pristine Fe₃O₄ and excellent recycling ability, reusability, and stability. The detailed doxycycline degradation mechanism was explored by employing
photoluminescence technology. The result clearly demonstrates that the synergistic effect of Bacillus subtilis adsorption and Fenton-like ability of Fe₃O₄ dominates the enhancement of Fenton-like catalytic efficiency of Fe₃O₄@B. subtilis.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/7/11/349/s1,
Figure S1: Size histogram of B. subtilis (a,b) and Fe₃O₄@B. subtilis (c,d), Figure S2: N2 adsorption-desorption isotherm and pore size distribution (inset) of (a) the bare B. subtilis and (b) Fe₀₃–C/H₂O₂ and (c) Fe₀₃@B. subtilis, Figure S3: Selected zones of Fe₀₃@B. subtilis SPMC (a) and corresponding X-ray mapping, for C (b), O (c), and Fe elements (d), Figure S4: Zeta potential of B. subtilis, Fe₀₃ and Fe₀₃@B. subtilis suspensions as a function of pH, Figure S5: Formation of hydroxyl products as the result of reaction between terephthalic acid (TA) and OH–, Figure S6: Schematic illustration of the synthesis process of Fe₀₃@B. subtilis.

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