Supplementary Information

Distinguishing the roles of different extracellular polymeric substances fractions of periphytic biofilm in defending Fe_2O_3 nanoparticles toxicity

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1. Characterizations of IONPs and periphytic biofilm

1.1 Materials and methods

The morphology of the IONPs was determined by drying the NP suspension on a copper grid overnight and imaging the sample with transmission electron microscopy (TEM) (HT-7700, Hitachi, Japan). The hydrodynamic diameters of IONPs before use and after being immersed in WC medium for 2 h were determined by a Zetasizer (90PLUS PALS, Nano Brook, USA). The phase composition and crystal structure of the NPs was determined by X-ray diffraction (XRD) analysis with a D/max-2500V/PC powder X-ray diffractometer (X'Pert PRO, Philips, Netherlands). Raman spectrometer (Nexus, Nicolet, USA) was employed to confirm the crystallinity of IONPs.

To characterize its composition, the periphyton in stationary phase was observed by confocal scanning laser microscopy (CSLM) (LSM 710, Zeiss, Germany). Surface morphology of periphyton were observed by scanning electron microscopy (SEM) (SU3500, Hitachi, Japan). 16S rDNA high-throughput sequencing by Illumina MiSeq was employed to characterize the bacterial composition in the periphyton. The method and process of high-throughput sequencing was according to our previous study.[1]

1.2 Characterizations

Results showed that most of the IONPs were spherical, with relatively uniform size with a diameter of 62.8 ± 9.8 nm (Figure S1 a). The average hydrodynamic diameters

of IONPs (5 mg L^{-1}) before use and after being immersed in WC medium were similar, about 75.5 ± 11.3 nm (Figure S1 b). This was close to the diameter determined by TEM, indicating that the aggregation of IONPs (5 mg L^{-1}) in WC medium was not obvious and the IONPs solutions were stable. A previous study on the toxicity of IONPs (> 5 mg L^{-1}) to algae showed that dissolution of the IONPs in the culture medium was undetectable (< 0.081 mg/L) and the contribution of NP dissolution to toxicity was negligible under prolonged exposure.[2] Therefore, the dissolution of IONPs in the culture medium was not the dominant mechanism for the toxicity and was not examined in the present study. XRD (Figure S1 c) shows that all the diffraction peaks of the IONPs clearly indicated a pure rhombohedral phase [space group: *R*-3c (167)] of a-Fe_2O_3 (JCPDS No. 89-0597, a = 5.039 Å, c = 13.77 Å). According to the comparison of standard Raman spectra of Fe_2O_3 crystallized structure, namely two A_{1g} modes (225 and 498 cm^{-1}) and five E_g modes (247, 293, 299, 412 and 613 cm^{-1}), IONPs in this study exhibit all these standard spectral features (Figure S1 d). This implies that the IONPs were α-Fe_2O_3, crystallized NPs.

In the present study, according to the SEM and CLSM images (Figure S2), microorganisms including algae, cyanobacteria and bacteria could be observed in the periphytic biofilm and were encapsulated by abundant EPS. High-throughput sequencing (Figure S3) showed that twenty-one main bacterial classes were detected in the periphyton including *Cyanobacteria*, *Bacilli*, *Gemmatimonadetes*,

Sphingobacteria, *Alphaproteobacteria*, *Planctomycetes* and *Spirochaetes*. Therefore, the periphytic biofilm employed in this study had abundant EPS and rich species composition.

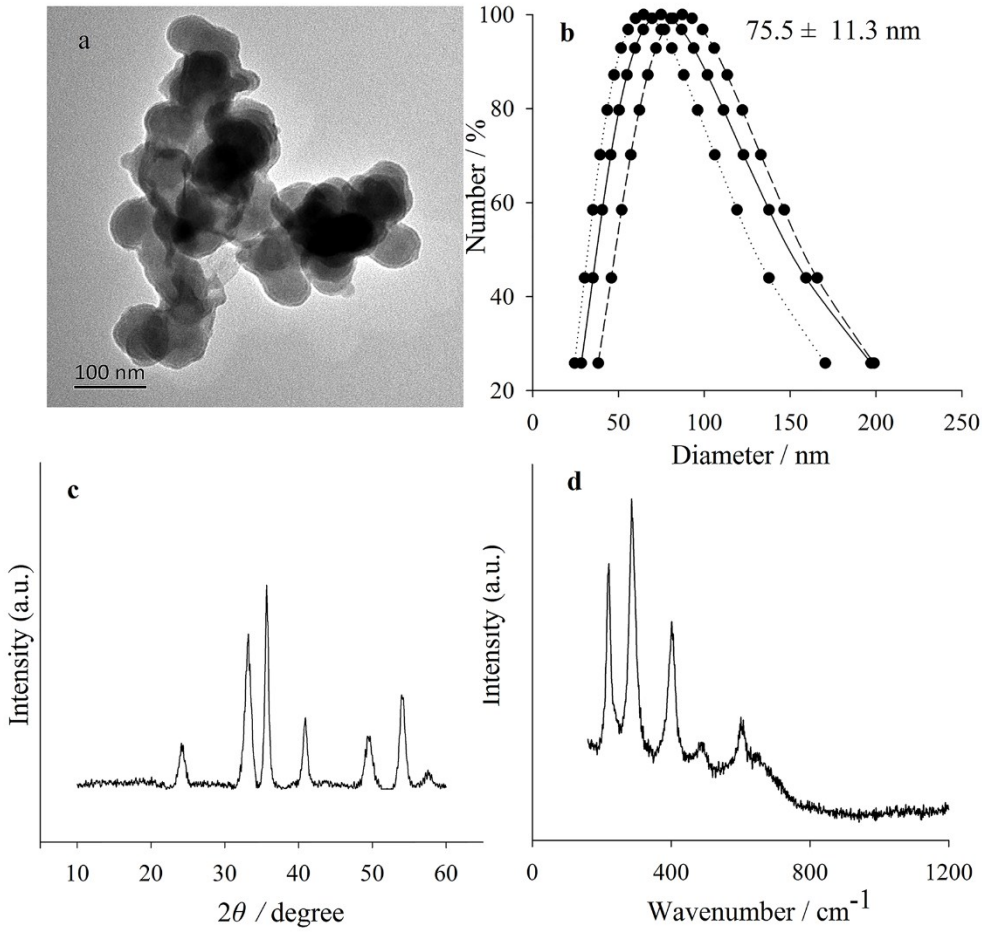

Figure S1. (a) TEM image of IONPs. (b) Hydrodynamic diameter of IONPs. (c) XRD patterns of IONPs. (d) Raman spectra of IONPs.

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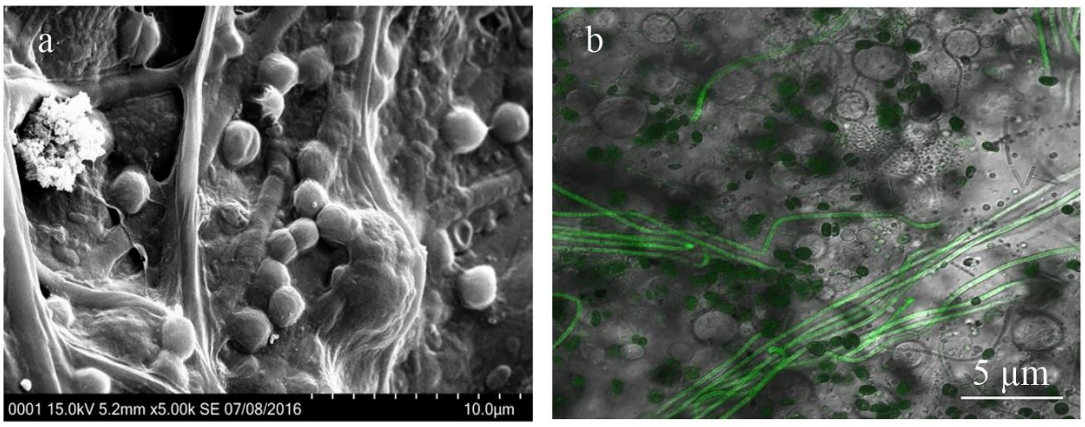

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77 **Figure S2.** (a) SEM and (b) CLSM images of periphytic biofilm before the exposure

78 experiment.

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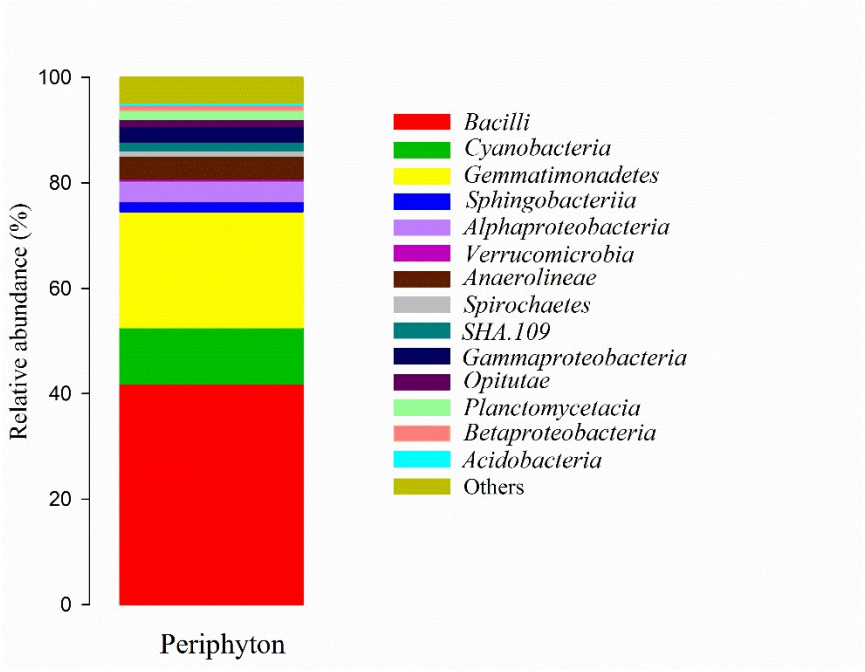
80 

81 **Figure S3**. Microbial community composition of the periphytic biofilm at the class

82 level.

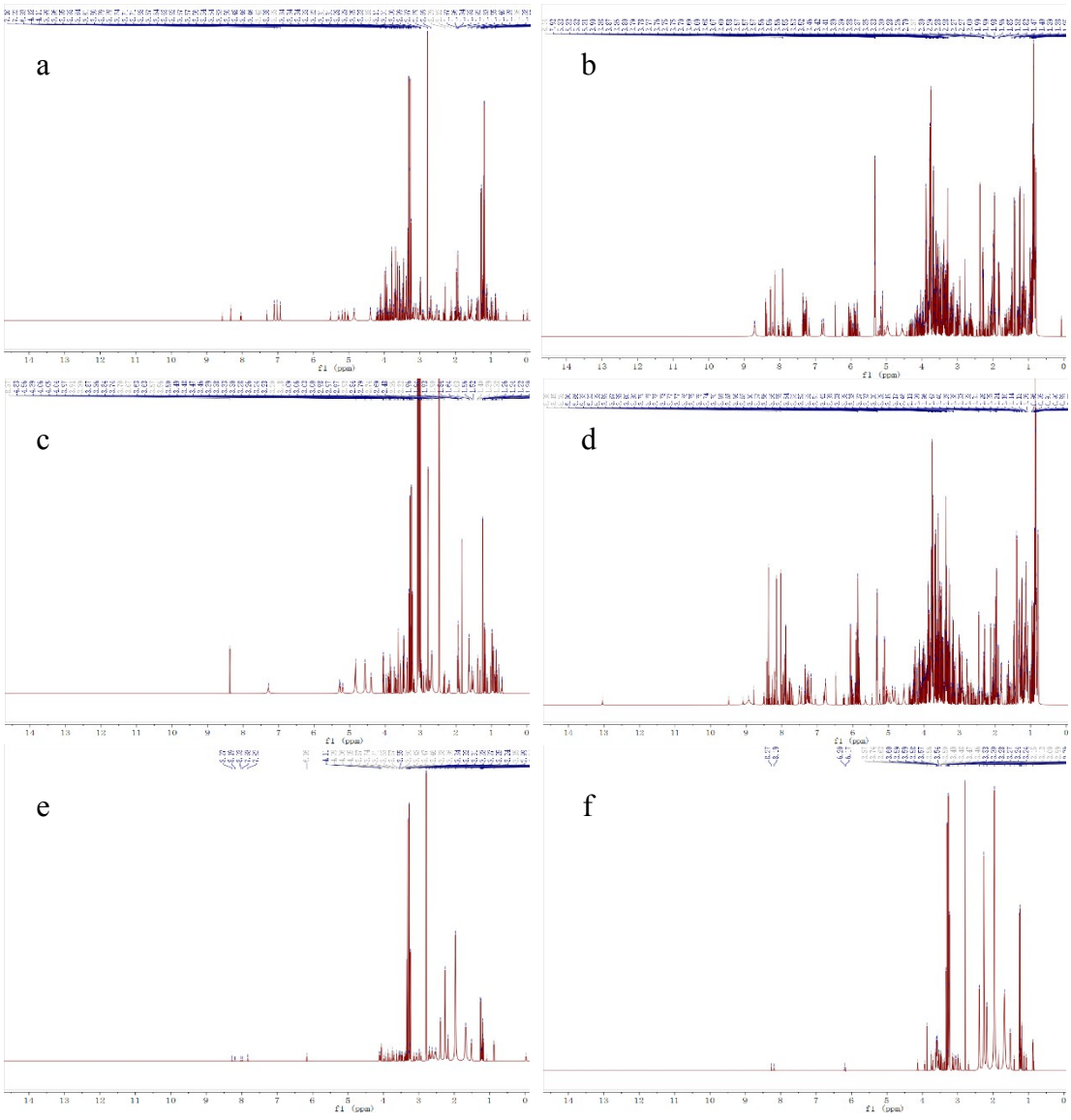


Figure S4. ^{1}H NMR spectra of (a) soluble EPS (SE) in CK, (b) SE in IONPs treatment, (c) loosely bound EPS (LE) in CK, (d) LE in IONPs treatment, (e) tightly bound EPS (TE) in CK, and (f) TE in IONPs treatment.

90 Table S1. Element proportion of energy dispersive spectrometer (EDS) in Figure 4(b).

Element	Weight %	Atomic %
C	51.17	59.76
N	3.17	3.17
O	38.93	34.13
Na	0.93	0.57
Mg	0.30	0.17
Si	0.19	0.09
P	0.64	0.29
S	1.67	0.73
Cl	0.43	0.17
K	1.85	0.66
Ca	0.73	0.25
Fe	0.00	0.00
Total	100.00	100.00

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93 Table S2. Element proportion of energy dispersive spectrometer (EDS) in Figure 4(d)

Element	Weight %	Atomic %
C	36.59	48.05
N	1.81	2.04
O	36.84	36.32
Mg	8.33	5.40
Al	1.35	0.79
Si	10.06	5.65
P	0.65	0.33
K	0.55	0.22
Ca	1.11	0.44
Fe	2.71	0.77
Total	100.00	100.00

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96 Table S3. Element proportion of energy dispersive spectrometer (EDS) in Figure 4(f)

Element	Weight %	Atomic %
C	47.43	55.81
N	11.34	11.44
O	33.47	29.57
Na	0.01	0.01
Mg	0.98	0.57
P	1.36	0.62
S	2.19	0.96
K	0.51	0.18
Ca	1.44	0.51
Fe	1.28	0.32
Total	100.00	100.00

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99 Table S4. Element proportion of energy dispersive spectrometer (EDS) in Figure 4(h)

Element	Weight %	Atomic %
C	59.54	66.54
N	9.40	9.01
O	27.64	23.19
Na	0.00	0.00
Mg	0.59	0.32
P	0.33	0.14
S	0.57	0.24
K	0.07	0.02
Ca	0.95	0.32
Fe	0.92	0.22
Total	100.00	100.00

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103 Table S5. Element proportion of energy dispersive spectrometer (EDS) in Figure 5(d)

Element	Weight %	Atomic %
C	19.93	29.43
N	2.52	3.20
O	40.28	44.66
Na	1.15	0.89
Mg	7.49	5.46
Al	5.36	3.52
Si	16.40	10.36
P	0.20	0.12
K	1.15	0.52
Ca	0.66	0.29
Ti	0.16	0.06
Fe	4.69	1.49
Total	100.00	100.00

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Table S6. Element proportion of energy dispersive spectrometer (EDS) in Figure 5(c).

Element	Weight %	Atomic %
C	51.10	58.87
N	10.04	9.92
O	33.70	29.15
Na	0.62	0.37
Al	0.19	0.10
Si	0.21	0.10
P	0.14	0.06
S	1.33	0.57
K	0.30	0.11
Ca	1.62	0.56
Fe	0.77	0.19
Total	100.00	100.00

References

1. H. D. Shangguan, J. Z. Liu, Y. Zhu, Z. G. Tong and Y. H. Wu, *Bioresour. Technol.*, 2015, **193**, 456-462.
2. C. Lei, L. Zhang, K. Yang, L. Zhu and D. Lin, *Enviro. Pollut.*, 2016, **218**, 505-512.