

Supplementary materials for

**Adding exogenous protein relieves the toxicity of nanoparticles to anammox
granular sludge by adsorption and the formation of eco-corona**

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Text 1 Methods of determining specific anammox activity (SAA).

A glass serum bottle(160 mL) was used. And the liquid volume was kept at 120 mL, which contained 5 mL anammox granular sludge, 5 mL basal mineral medium, 200 mg L⁻¹ total nitrogen and MgO NPs. The mixed solution was removed oxygen by argon gas aeration and put in a constant temperature shaker (35 °C, 180 rpm). 1 mL supernate was withdrew from bottle to determine the consumed substrate concentration every hour until the substrate was totally consumed.

Table S1 Primers used for gene quantifications in this study.

Category	Target gene	Primer	Sequence (5'-3')	Annealing Temperature (°C)
Functional genes	<i>hdh</i>	<i>hdh</i> -F	GGTGGTTTGAGGGGTCCAA	55
		<i>hdh</i> -R	TATGGCGACCTCTGTGCATC	
	<i>nirS</i>	<i>nirS</i> -F	G TSAACG TSAAGGARACSGG	56
		<i>nirS</i> -R	GASTTCGGSTGSGTCTTGA	
	<i>hzsA</i>	1597F	WTYGGKTATCARTATGTAG	55
		1857R	AAABGGYGAATCATARTGGC	
	<i>nirK</i>	<i>nirK</i> -F	TCATGGTGCTGCCGCGKGACGG	70
		<i>nirK</i> -R	GAACTTGCCGGTKGCCAGAC	
	<i>norZ</i>	<i>norZ</i> -F	CGKTGTTTCMTCGACAGCCAG	53
		<i>norZ</i> -R	CGSACCTTSTTGCCSTYGCG	
	<i>norB</i>	<i>norB</i> -F	ACACAAATCACTGCCGCCCA	65
		<i>norB</i> -R	TGCAGTACACCGGCAAAGGT	

Table S2 Composition of the synthetic wastewater

Composition	Component	Concentration
Substrate (mg L ⁻¹) ^a	NaNO ₂	70/140/210/280
	(NH ₄) ₂ SO ₄	70/140/210/280
Inorganic solution (mg L ⁻¹)	NaH ₂ PO ₄	10
	CaCl ₂ •2H ₂ O	5.6
	MgSO ₄ •7H ₂ O	300
	NaHCO ₃	1250
Trace elements (mL L ⁻¹)	Trace elements I	1.25
	Trace elements II	1.25
Trace elements I (g L ⁻¹)	EDTA	5
	FeSO ₄ •7H ₂ O	9.14
	H ₃ BO ₄	0.014
	MnCl ₂ •4H ₂ O	0.99
	CuSO ₄ •5H ₂ O	0.25
	ZnSO ₄ •7H ₂ O	0.43
Trace elements II (g L ⁻¹)	EDTA	15
	CoCl ₂ •6H ₂ O	0.24
	NaMnO ₄ •H ₂ O	0.22
	NiCl ₂ •6H ₂ O	0.21

^a The concentrations of substrates were adjusted depending on the operational performance of anammox during the experimental period.

Table S3 Relative abundance of microbial composition at genus level in different phases

OTU ID	R01	R02	R03	R04	R11	R12	R13	R14
norank_f_norank_o_SBR1031	22.20%	24.39%	25.10%	16.90%	22.20%	22.45%	14.82%	15.11%
norank_f_A4b	10.26%	11.20%	10.98%	9.62%	10.26%	7.44%	9.62%	9.44%
OLB14	0.53%	0.38%	2.78%	1.61%	0.53%	0.70%	0.39%	0.36%
norank_f_AKYG1722	1.36%	0.15%	1.34%	0.94%	1.36%	0.14%	0.90%	0.76%
norank_f_Caldilineaceae	1.10%	0.64%	0.83%	0.76%	1.10%	0.51%	0.75%	1.28%
norank_f_JG30-KF-CM45	0.95%	0.14%	1.09%	0.82%	0.95%	0.08%	0.79%	0.81%
OLB13	1.09%	2.26%	1.91%	4.16%	1.09%	3.58%	2.52%	1.72%
Candidatus_Kuenenia	24.15%	16.39%	21.76%	23.09%	24.15%	27.81%	23.49%	29.78%
SM1A02	3.43%	4.97%	2.10%	2.55%	3.43%	4.55%	2.64%	3.92%
Denitratisoma	4.29%	8.27%	4.90%	9.41%	4.29%	6.37%	15.58%	11.89%
norank_f_norank_o_JG36-GS-52	1.16%	1.15%	2.12%	2.14%	1.16%	1.28%	1.05%	1.24%
Arenimonas	0.14%	0.25%	1.06%	1.18%	0.14%	0.41%	1.20%	0.86%
Xanthobacteraceae	0.67%	0.34%	0.71%	0.95%	0.67%	0.29%	1.57%	1.61%
Limnobacter	6.59%	4.44%	7.63%	6.29%	6.59%	4.73%	4.83%	4.04%
Kapabacteriales	1.25%	1.03%	0.81%	1.07%	1.25%	0.92%	0.73%	0.80%
AKYH767	0.97%	0.72%	0.39%	0.76%	0.97%	0.73%	1.20%	0.76%
norank_f_norank_o_norank_c_SJA-28	0.78%	1.54%	0.80%	1.20%	0.78%	1.06%	0.49%	0.70%
norank_f_norank_o_norank_c_Dojkabacteria	1.97%	2.53%	2.15%	3.34%	1.97%	1.94%	2.55%	2.01%
Subgroup_10	1.63%	1.72%	1.31%	0.95%	1.63%	1.15%	1.16%	0.89%
Haliangium	0.72%	3.63%	0.28%	0.39%	0.72%	1.92%	0.76%	0.57%
norank_f_norank_o_Fimbriimonadales	1.06%	0.53%	0.35%	1.28%	1.06%	0.64%	1.24%	1.10%
others	13.70%	13.30%	9.60%	10.59%	13.70%	11.28%	11.71%	10.35%

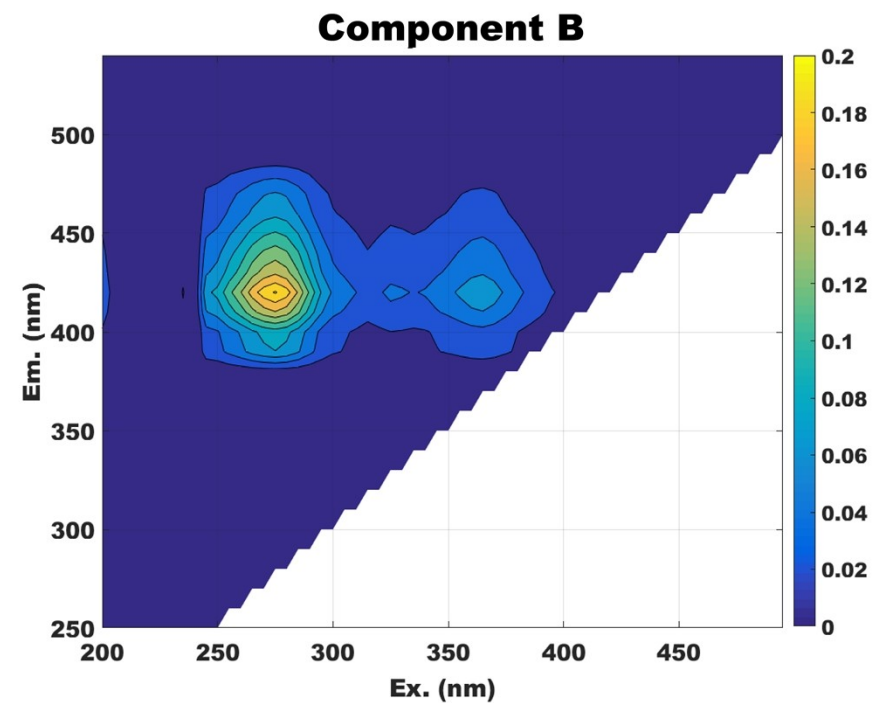
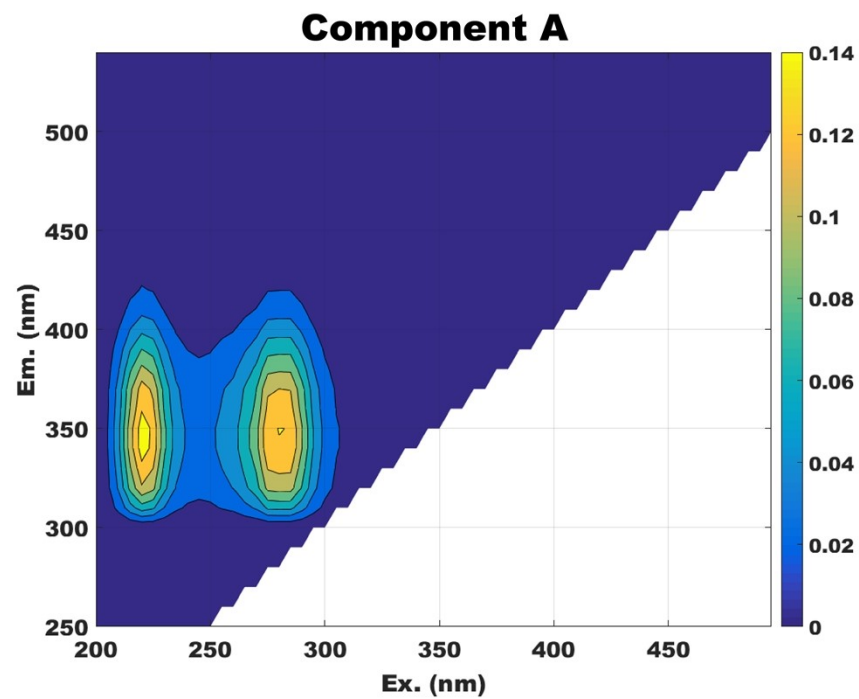


Fig. S1 The identified fluorescent components of EPS by perform parallel factor (PARAFAC)

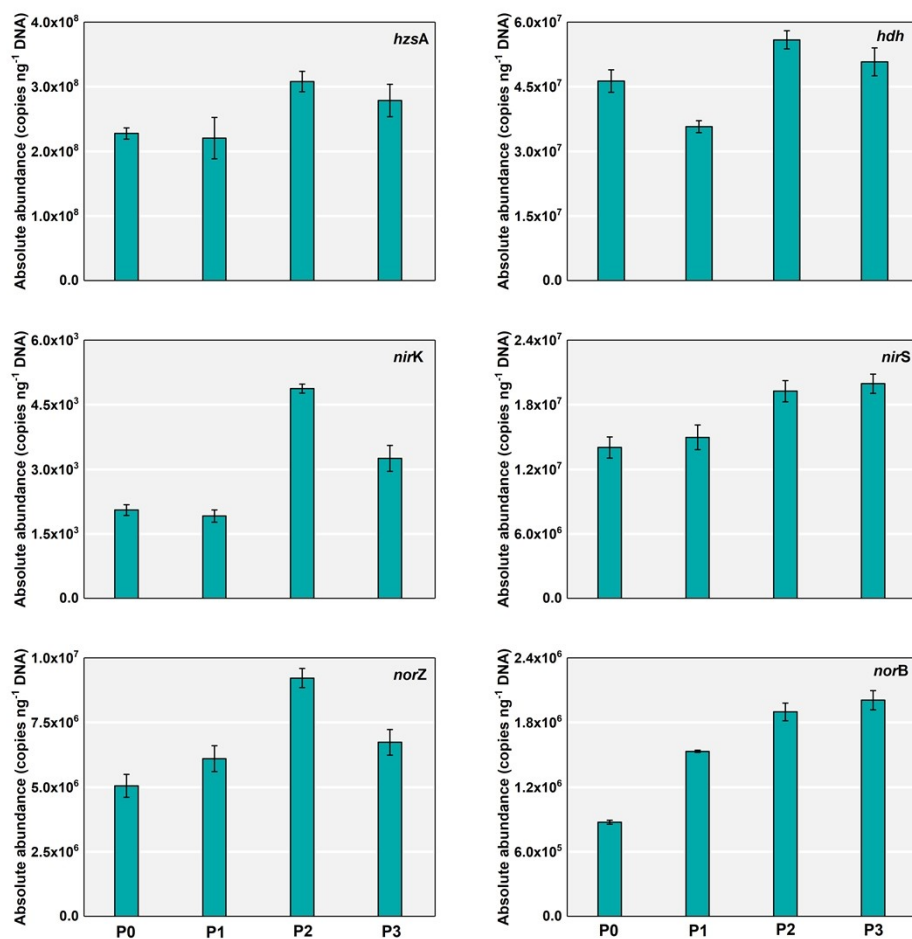


Fig. S2 Nitrogen cycle genes involved anammox (*hzsA* and *hdh*) and denitrification (*nirS*, *nirK*, *norZ* and *norB*) process in two reactors

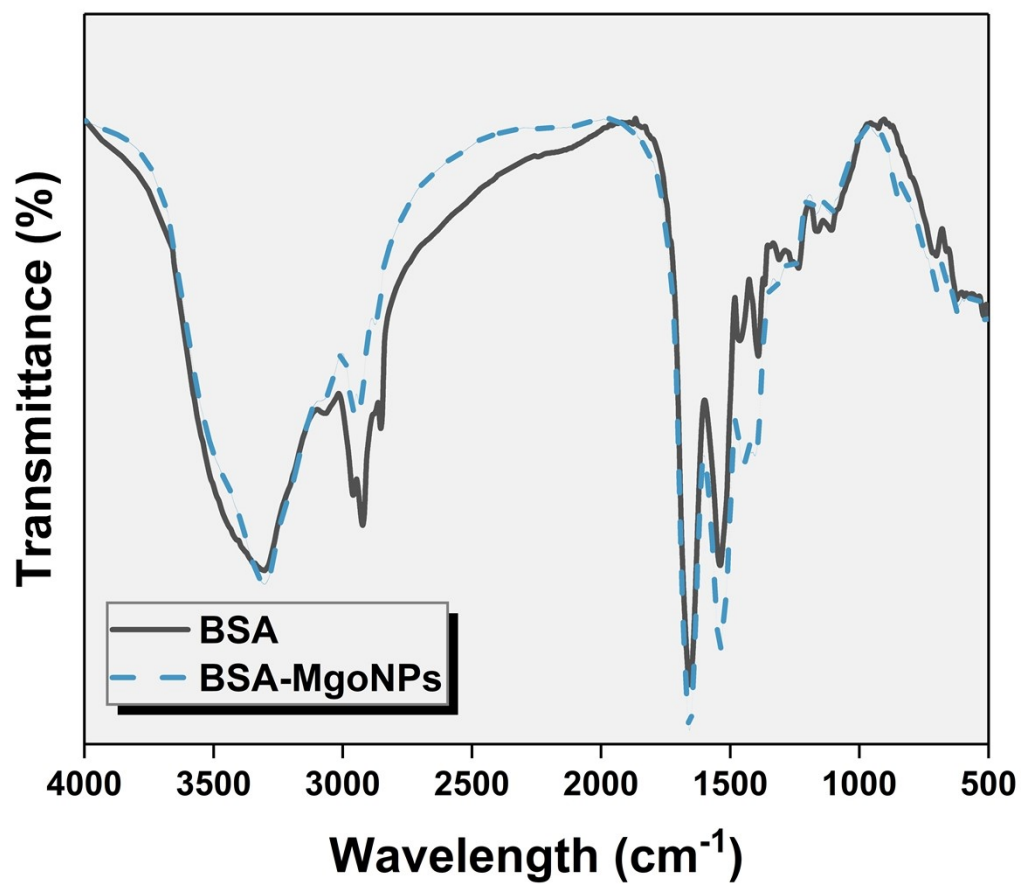


Fig. S3 FTIR absorbance spectra of the freeze-dried BSA and BSA-MgO NPs complex

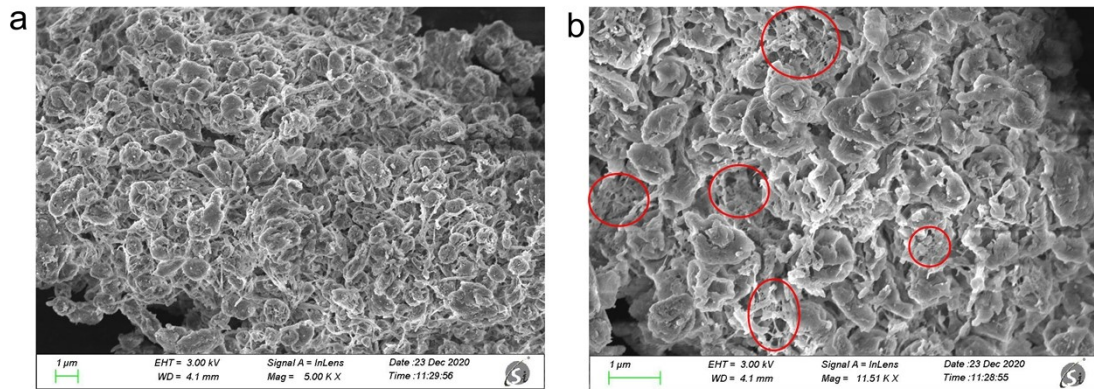


Fig. S4 Scanning electron microscope characterization of original anammox granular sludge (a) and explored by MgO NPs