

SUPPORTING INFORMATION (SI)

Metagenomics analysis provide insights into physiological characterization of ANAMMOX communities under humic substances

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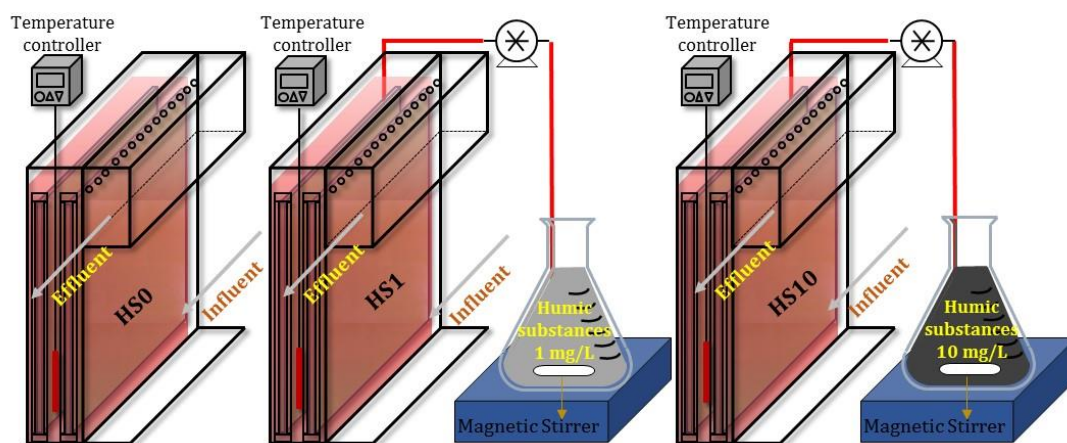


Figure S1 Schematic diagram of the lab-scale anammox bioreactors. HS0, HS1 and HS10 indicated 0, 1 and 10 mg $\cdot$ L⁻¹ humic substance, respectively.

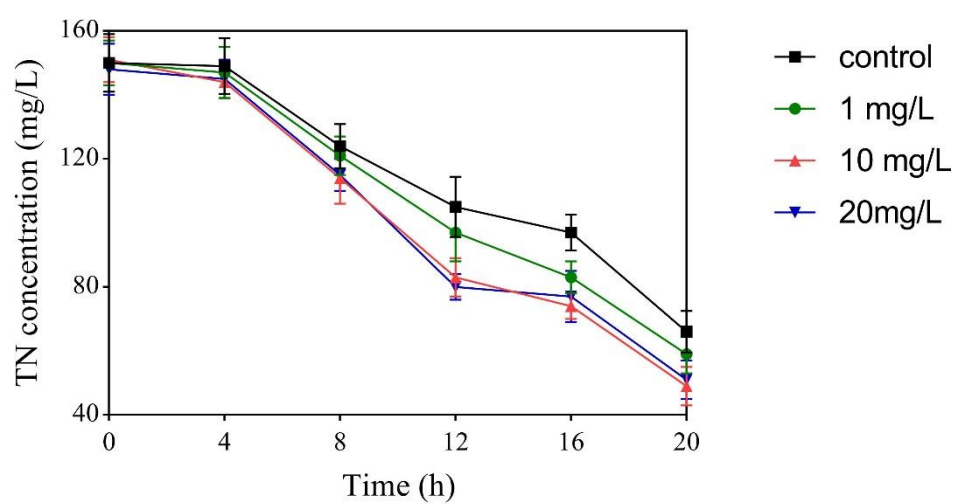


Figure S2 The short-term effects of HS on the nitrogen removal for synthetic wastewater. Error bars represent standard deviations of triplicate testes.

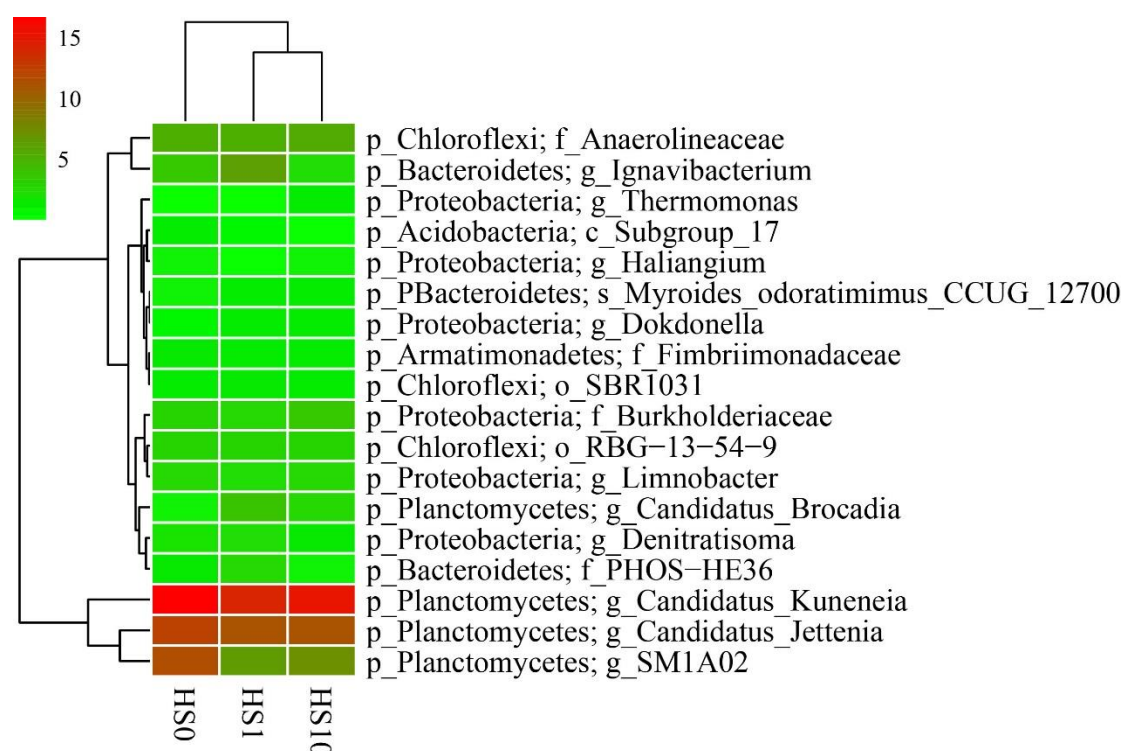


Figure S3 The top 15 abundant genera in each sample were selected (a total of 18 genera for all the 3 samples) for generating a heatmap. HS0, HS1 and HS10 indicated 0, 1 and 10 mg · L⁻¹ humic substance, respectively.

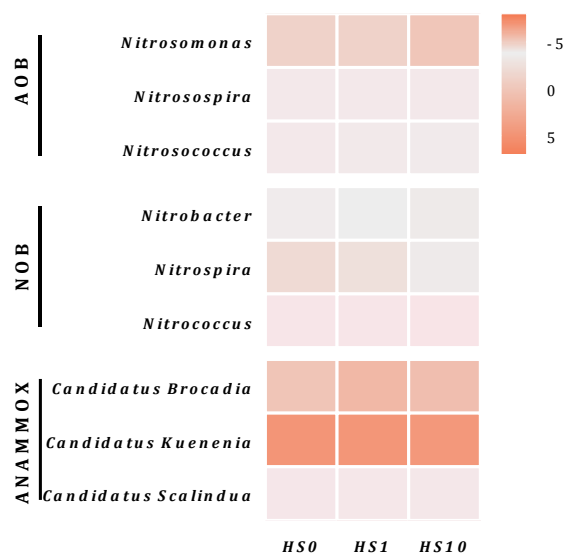


Figure S4 Abundances of AOB, NOB and ANAMMOX microorganisms in the three samples as determined from metagenome sequences. HS0, HS1 and HS10 indicated 0, 1 and 10 mg · L⁻¹ humic substance, respectively.

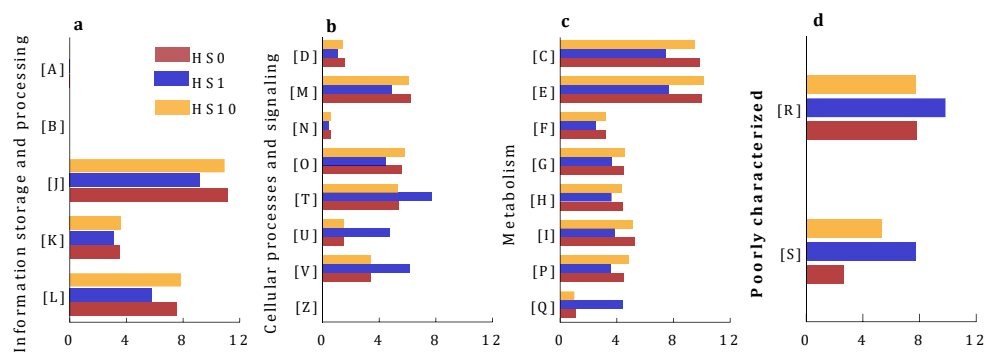


Figure S5 Microbial gene abundances, grouped using the Clusters of Orthologous Genes (COG) hierarchy of functions. HS0, HS1, HS10 indicated 0, 1, 10 mg L⁻¹ humic substances, respectively.

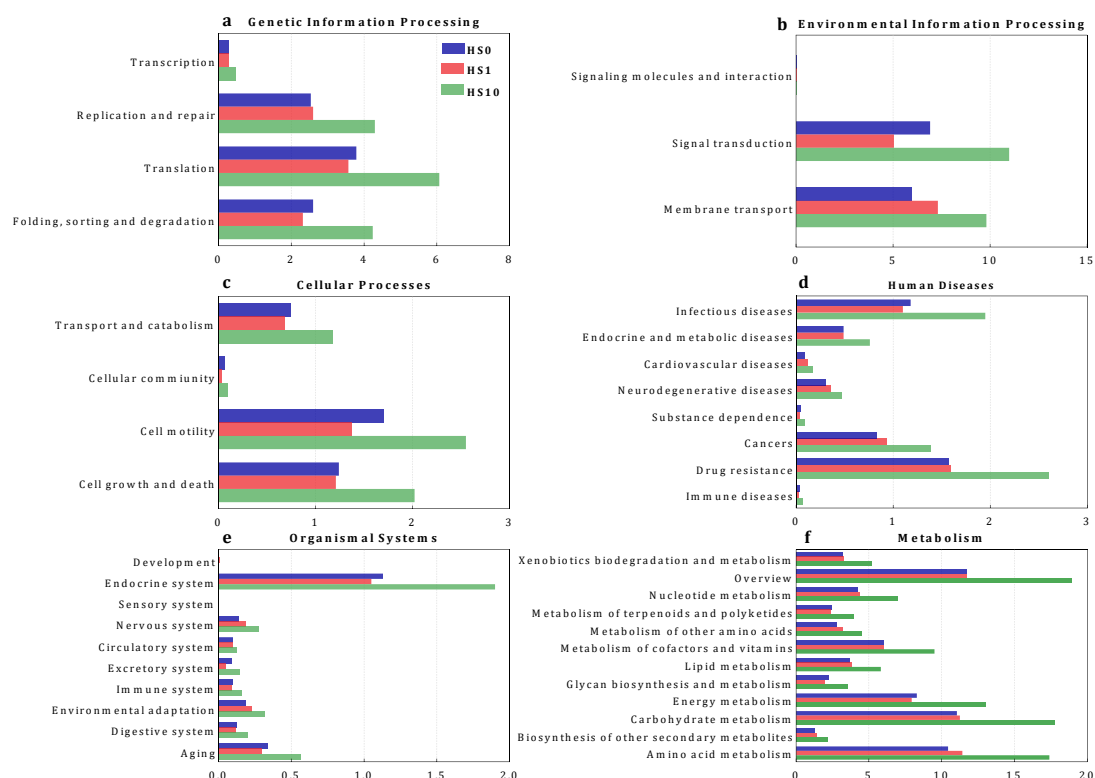


Figure S6 Functional categories of Kyoto Encyclopedia of Genes and Genomes (KEGG) category profiles of the three samples. HS0, HS1, HS10 indicated 0, 1, 10 mg L⁻¹ humic substances, respectively.

Table S1 Species and abundance of ANAMMOX bacteria detected by metagenomic data

Species	Relative abundance (%)		
	<i>HS0</i>	<i>HS1</i>	<i>HS10</i>
<i>Candidatus</i> Brocadia fulgida	0	0	0
<i>Candidatus</i> Brocadia sinica	0.90	2.17	1.39
<i>Candidatus</i> Kuenenia stuttgartiensis	22.42	21.24	18.20
<i>Candidatus</i> Scalindua brodae	0.01	0.02	0.02

Note: HS0, HS1, HS10 indicated 0, 1, 10 mg ·L⁻¹ humic substances, respectively.

Table S2 Abundance comparison of AOB, NOB and ANAMMOX based on metagenomic sequencing in three samples

Nitrification organisms	Genus	HS0 (%)	HS1 (%)	HS10 (%)
AOB	<i>Nitrosomonas</i>	0.38	0.37	0.82
	<i>Nitrospira</i>	0.02	0.02	0.02
	<i>Nitrosococcus</i>	0.02	0.02	0.03
	<i>Nitrosovibrio</i>	0	0	0
	Total	0.42	0.41	0.87
NOB	<i>Nitrobacter</i>	0.04	0.05	0.07
	<i>Nitrospira</i>	0.22	0.15	0.07
	<i>Nitrococcus</i>	0.01	0.01	0.01
	<i>Nitrobacteria</i>	0	0	0
	<i>Nitrolancetus</i>	0	0	0
	Total	0.26	0.21	0.16
ANAMMOX	<i>Candidatus Anammoximicrobium</i>	0	0	0
	<i>Candidatus Anammoxoglobus</i>	0	0	0
	<i>Candidatus Brocadia</i>	0.90	2.17	1.39
	<i>Candidatus Jettenia</i>	0	0	0
	<i>Candidatus Kuenenia</i>	22.84	21.68	18.51
	<i>Candidatus Scalindua</i>	0.01	0.02	0.02
	Total	23.75	23.87	19.92

Note: HS0, HS1, HS10 indicated 0, 1, 10 mg · L⁻¹ humic substances, respectively.

Table S3 Clusters of Orthologous Genes (COG) annotation statistics

Function Category/Sample name	Class ID	HS0 (num)	HS0 (%)	HS1 (num)	HS1 (%)	HS10 (num)	HS10 (%)
RNA processing and modification	A	251	0.03	253	0.02	290	0.03
Chromatin structure and dynamics	B	13	0.00	25	0.00	15	0.00
Energy production and conversion	C	90,685	9.85	87,675	7.45	88,113	9.53
Cell cycle control, cell division, chromosome partitioning	D	14,475	1.57	13,297	1.13	13,357	1.44
Amino acid transport and metabolism	E	92,314	10.02	89,987	7.64	93,805	10.14
Nucleotide transport and metabolism	F	29,835	3.24	29,939	2.54	30,026	3.25
Carbohydrate transport and metabolism	G	41,397	4.49	42,740	3.63	42,468	4.59
Coenzyme transport and metabolism	H	40,786	4.43	42,779	3.63	40,420	4.37
Lipid transport and metabolism	I	48,310	5.24	45,109	3.83	47,185	5.10
Translation, ribosomal structure and biogenesis	J	102,476	11.13	108,358	9.20	101,201	10.94
Transcription	K	32,489	3.53	36,303	3.08	33,650	3.64
Replication, recombination and repair	L	69,634	7.56	68,421	5.81	72,699	7.86
Cell wall/membrane/envelope biogenesis	M	57,854	6.28	58,049	4.93	56,091	6.07
Cell motility	N	5,361	0.58	5,100	0.43	5,254	0.57
Posttranslational modification, protein turnover, chaperones	O	51,888	5.63	52,588	4.47	53,806	5.82
Inorganic ion transport and metabolism	P	41,526	4.51	42,192	3.58	44,743	4.84
Secondary metabolites biosynthesis, transport and catabolism	Q	9,821	1.07	51,761	4.40	9,386	1.01
General function prediction only	R	71,849	7.80	115,822	9.84	71,409	7.72
Function unknown	S	24,500	2.66	67,414	5.73	25,641	2.77
Signal transduction mechanisms	T	50,004	5.43	90,922	7.72	49,328	5.33
Intracellular trafficking, secretion, and vesicular transport	U	13,913	1.51	55,727	4.73	14,180	1.53
Defense mechanisms	V	31,666	3.44	72,919	6.19	31,712	3.43
Cytoskeleton	Z	21	0.00	1	0.00	17	0.00

Note: HS0, HS1, HS10 indicated 0, 1, 10 mg L⁻¹ humic substances, respectively.

Table S4 Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation statistics

Sample name	HS0 (num)	HS0 (%)	HS1 (num)	HS1 (%)	HS10 (num)	HS10 (%)
Aging	415	0.34	198	0.30	480	0.57
Endocrine system	1,391	1.13	696	1.05	1,602	1.90
Transcription	347	0.28	192	0.29	409	0.49
Energy metabolism	10,181	8.30	5,251	7.94	10,929	12.99
Substance dependence	61	0.05	27	0.04	79	0.09
Sensory system	3	0.00	3	0.00	2	0.00
Nervous system	175	0.14	123	0.19	235	0.28
Circulatory system	126	0.10	67	0.10	109	0.13
Signaling molecules and interaction	9	0.01	5	0.01	11	0.01
Nucleotide metabolism	5,204	4.24	2,882	4.36	5,852	6.96
Metabolism of terpenoids and polyketides	2,984	2.43	1,555	2.35	3,342	3.97
Amino acid metabolism	12,812	10.44	7,557	11.43	14,643	17.40
Cellular commiunity	88	0.07	26	0.04	86	0.10
Immune diseases	48	0.04	22	0.03	59	0.07
Transport and catabolism	917	0.75	447	0.68	994	1.18
Translation	4,648	3.79	2,359	3.57	5,109	6.07
Infectious diseases	1,449	1.18	730	1.10	1,637	1.95
Cell motility	2,099	1.71	914	1.38	2,149	2.55
Replication and repair	3,113	2.54	1,719	2.60	3,615	4.30
Endocrine and metabolic diseases	599	0.49	325	0.49	643	0.76
Development	-	-	4	0.01	-	-
Drug resistance	1,923	1.57	1,054	1.59	2,191	2.60
Digestive system	154	0.13	82	0.12	168	0.20
Cell growth and death	1,523	1.24	803	1.21	1,702	2.02
Signal transduction	8,481	6.91	3,340	5.05	9,249	10.99
Cardiovascular diseases	108	0.09	77	0.12	143	0.17
Xenobiotics biodegradation and metabolism	3,934	3.21	2,150	3.25	4,389	5.22
Environmental adaptation	238	0.19	150	0.23	273	0.32
Cancers	1,019	0.83	614	0.93	1,166	1.39
Carbohydrate metabolism	13,563	11.05	7,436	11.25	14,920	17.73
Biosynthesis of other secondary metabolites	1,583	1.29	951	1.44	1,841	2.19
Glycan biosynthesis and metabolism	2,776	2.26	1,296	1.96	2,951	3.51
Lipid metabolism	4,491	3.66	2,549	3.85	4,907	5.83
Excretory system	115	0.09	35	0.05	124	0.15
Overview	14,409	11.74	7,755	11.73	15,938	18.94
Metabolism of other amino acids	3,386	2.76	2,109	3.19	3,812	4.53
Metabolism of cofactors and vitamins	7,336	5.98	3,958	5.99	8,005	9.51
Membrane transport	7,322	5.97	4,831	7.31	8,256	9.81
Neurodegenerative diseases	363	0.30	237	0.36	399	0.47
Folding, sorting and degradation	3,189	2.60	1,536	2.32	3,565	4.24
Immune system	123	0.10	58	0.09	136	0.16

Note: HS0, HS1, HS10 indicated 0, 1, 10 mg · L⁻¹ humic substances, respectively.