

Deciphering the evolution of the functional genes and microbial community of the combined partial nitrification-anammox process with the nitrate build-up and its in-situ restoration

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Figure Captions

Fig. S1. The pH profile in SBR through all the stages. The pH of initial aeration represented the pH value in each SBR cycle when the feeding was finished and aeration initiated.

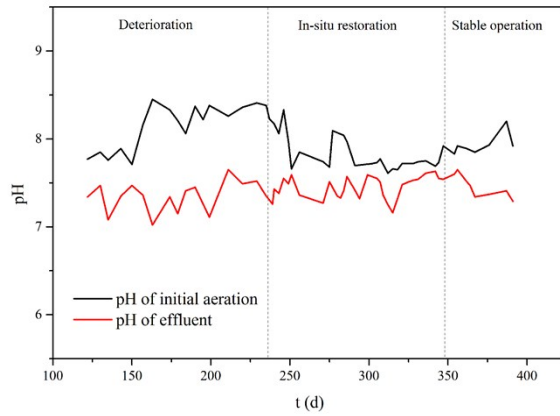


Fig. S2. The evolution of activities of AOB, NOB and anammox and the nitrite accumulation rate during the stages of deterioration, in-situ restoration and recovered stable operation.

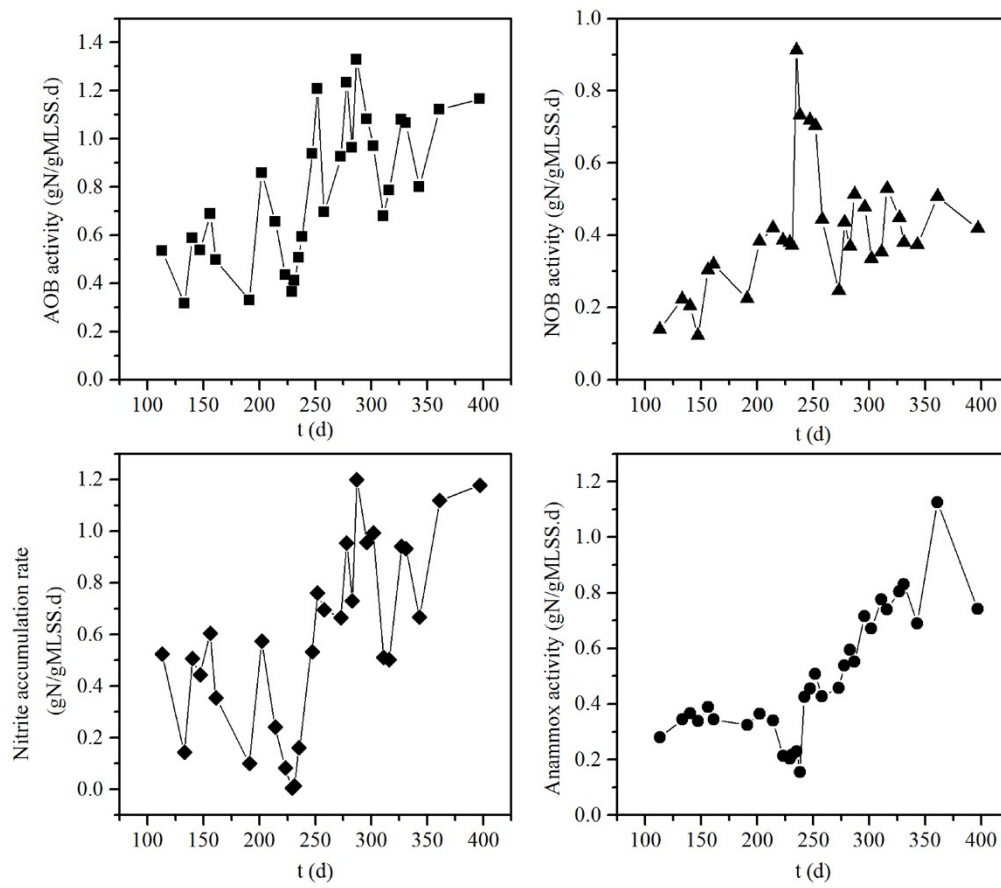


Fig. S3. The simulation of the recovery of NRR by the modified Boltzmann model. The initial of the horizontal axis was corresponding to the start point of the in-situ restoration.

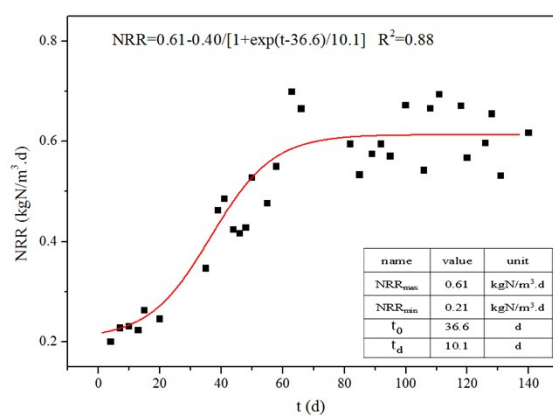


Fig. S4. The fitting curve of the *nrxA* gene abundance during the in-situ restoration and the recovered stable operation. The initial of the horizontal axis was corresponding to the day 230 with the maximum *nrxA* abundance in the whole operation of CPNA process. The vertical axis represented the gene copy concentration (copies gMLSS⁻¹).

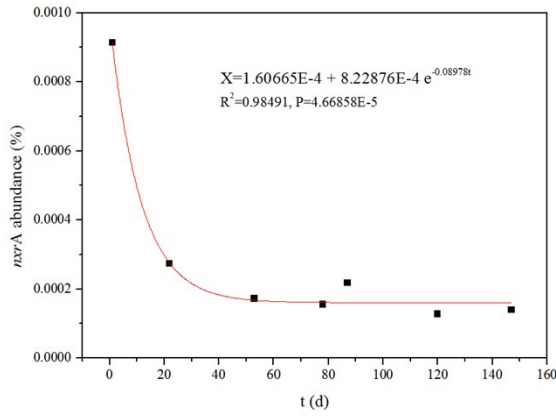


Fig. S5. The fitting curves of the exponential growth of the functional genes during the second in-situ restoration stage with NH_2OH dosing and SRT control and the recovered stable operation. The initial of the horizontal axis was corresponding to the start point of the second stage of in-situ restoration. The vertical axis represented the gene copy concentration (copies gMLSS^{-1}).

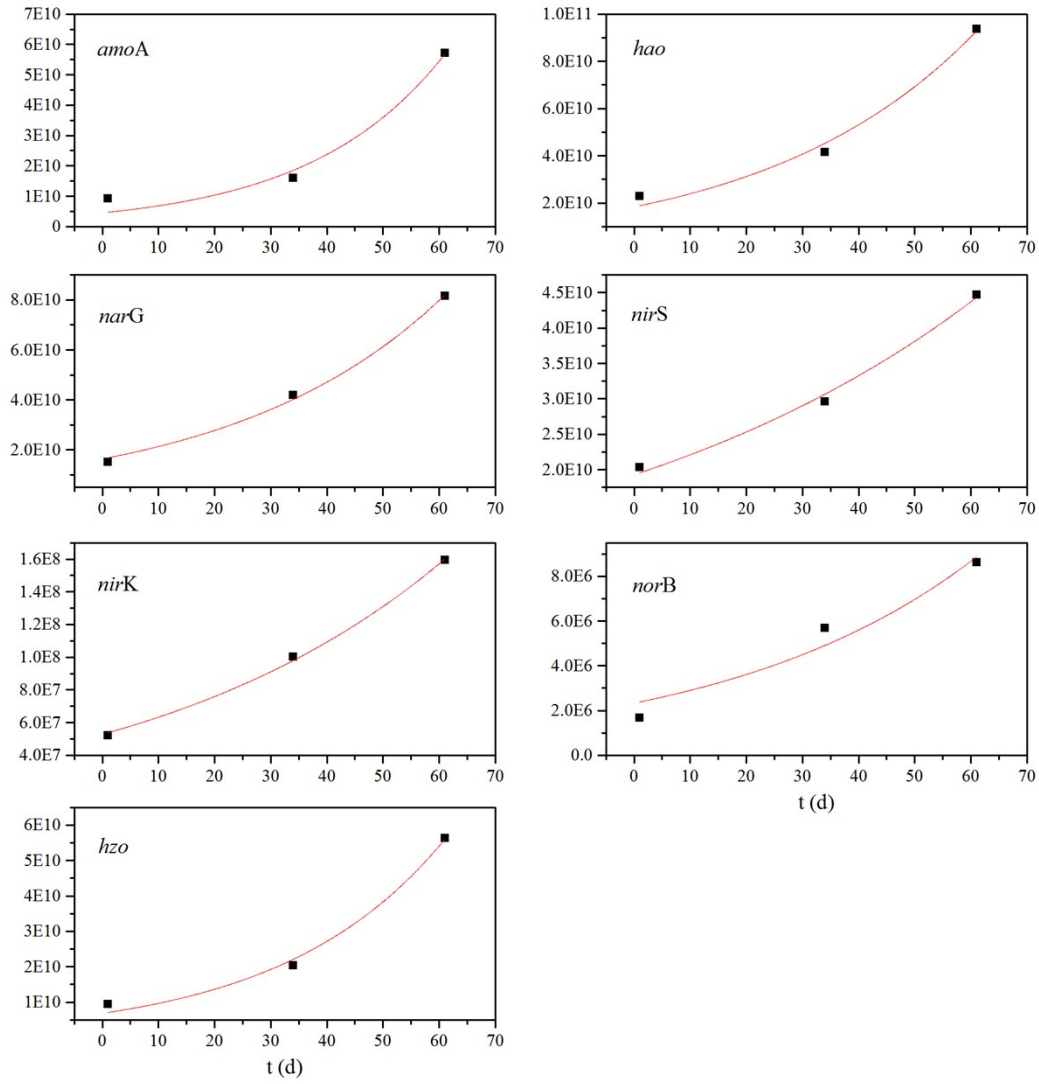


Fig. S6. Rarefaction curves for each sample.

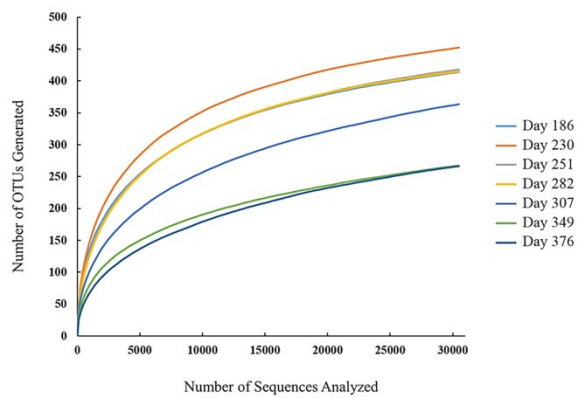


Fig. S7. Evolution of microbial community at phylum level.

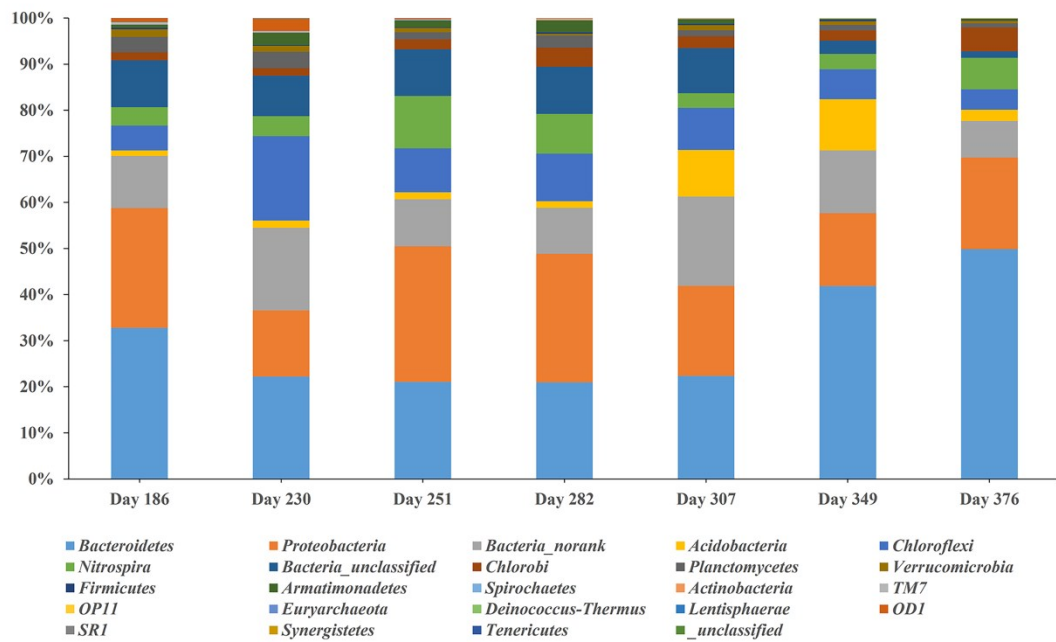


Table S1 The primers sequences, expected amplicon size, and annealing temperature for each target gene used in this study.

Target genes	Primer	Sequences (5'-3')	Amplicon size(bp)	Annealing temp. (°C)	Reference
<i>amoA</i>	amoA-F	GGGGTTTCTACTGGTGGT	491	60	1
	amoA-R	CCCCTCKGSAAAGCCTTCTTC			
<i>hao</i>	Hao1F	TGAGCCAGTCCAACGTGCAT	70	55	2
	Hao1R	AAGGCAACAACCCTGCCTCA			
<i>nxrA</i>	nxrA-F	CAGACCGACGTGTGCGAAAG	322	58	3
	nxrA-R	TCYACAAGGAACGGAAGGTC			
<i>narG</i>	1960m2F	TAYGTSGGGCAGGARAAACTG	110	50	4
	2050m2R	CGTAGAAGAAGCTGGTGCTGTT			
<i>nirS</i>	cd3AF	G TSAACG TSAAGGARACSSG	425	58	5
	R3cd	GASTTCGGRTGSGTCTTGA			
<i>nirK</i>	nirK-1F	GGMATGGTKCCSTGGCA	515	56	6
	nirK-5R	GCCTCGATCAGRTRRTGG			
<i>norB</i>	cnorB2F	GACAAGNNNTACTGGTGGT	389	55	7
	cnorB6R	GAANCCCCANACNCCNGC			
<i>nosZ</i>	nosZ2F	CGCRACGGCAASAAGGTSMSSTG	264	55	8
	nosZ2R	CAKRTGCAKSGCRTGGCAGAA			
<i>hzo</i>	hzocl1F1	TGYAAGACYTG YCAYTGG	470	55	9
	hzocl1R2	ACTCCAGATRTGCTGACC			
16S rRNA	1369F	CGGTGAATACGTTTCYCGG	128	55	10
	1492R	GGWTACCTTGTTACGACTT			

Table S2 Pearson correlation analysis of top 10 genera and N-transformation genes along with the nitrate build-up and in-situ restoration.

	<i>amoA</i>	<i>hao</i>	<i>nxA</i>	<i>narG</i>	<i>nirS</i>	<i>nirK</i>	<i>norB</i>	<i>nosZ</i>	<i>hzo</i>
<i>Gp4</i>	-0.353	-0.280	-0.523	-0.092	-0.125	-0.665	-0.323	-0.217	-0.050
<i>Gp3</i>	0.301	0.266	0.523	-0.320	0.121	0.780*	0.283	0.535	-0.238
<i>Chthonomonas</i>	-0.306	-0.406	0.755*	0.002	-0.575	0.291	-0.199	0.590	-0.525
<i>Armatimonadetes gp5</i>	0.616	0.791*	-0.329	-0.317	0.768*	0.612	-0.158	0.464	0.202
<i>Wandonia</i>	-0.150	-0.176	-0.292	-0.470	-0.063	-0.361	-0.114	0.171	-0.208
<i>Lewinella</i>	0.056	0.075	0.303	-0.681	0.116	0.457	0.791*	0.074	-0.363
<i>Haliscomenobacter</i>	0.025	-0.004	-0.566	0.517	0.149	-0.746	-0.382	-0.390	0.588
<i>Litorilinea</i>	0.271	0.426	0.131	-0.549	0.501	0.589	0.091	0.712	-0.166
<i>Ignavibacterium</i>	0.615	0.430	-0.179	-0.290	-0.160	0.802*	-0.353	0.189	-0.239
<i>Nitrospira</i>	0.790*	0.582	-0.287	0.005	0.001	0.739	-0.268	-0.096	0.100
<i>Parcubacteria genera incertae sedis</i>	-0.513	-0.450	0.826*	-0.196	-0.244	0.061	0.024	0.834*	-0.492
<i>Phycisphaera</i>	-0.809*	-0.724	0.867*	-0.243	-0.468	-0.100	0.491	0.277	-0.641
<i>Candidatus Kuenenia</i>	-0.520	-0.483	-0.379	-0.095	-0.342	-0.722	-0.212	-0.313	-0.188
<i>Nitrosomonas</i>	0.933**	0.766*	-0.611	0.215	0.350	0.449	-0.191	-0.387	0.541
<i>Comamonas</i>	0.228	0.188	0.274	-0.433	0.108	0.550	0.769*	-0.125	-0.189
<i>Chondromyces</i>	-0.228	-0.260	-0.289	-0.445	-0.162	-0.420	-0.177	0.150	-0.259
<i>Byssovorax</i>	0.393	0.148	-0.356	0.411	-0.236	0.022	-0.033	-0.775*	0.316
<i>Bacteriovorax</i>	-0.230	-0.222	0.047	-0.431	-0.235	0.063	0.709	-0.541	-0.373
<i>Arcobacter</i>	0.540	0.325	-0.213	-0.272	-0.290	0.713	-0.201	-0.095	-0.266
<i>Subdivision3 genera incertae sedis</i>	-0.423	-0.599	0.691	-0.362	-0.827*	0.243	0.160	0.270	-0.806*
<i>Opitutus</i>	-0.454	-0.484	-0.392	-0.315	-0.154	-0.373	0.922**	-0.288	-0.208

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

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