

Insights into the Responses of the Partial Denitrification Process to Elevated Perfluorooctanoic Acid Stress: Performance, EPS Characteristic and Microbial Community

Shaoqing Zhang ^{1,†}, Hang Zhang ^{2,†}, Yucheng Feng ², Naga Raju Maddela ³, Shugeng Li ^{4,5} and Liqiu Zhang ^{2,4,*}

¹ School of Chemistry and Civil Engineering, Shaoguan University, Shaoguan 512005, China; zsqli617@163.com

² School of Civil Engineering, Guangzhou University, Guangzhou 510006, China; h15122376276@163.com (H.Z.); 13536202075@163.com (Y.F.)

³ Departamento de Ciencias Biológicas, Facultad de Ciencias de la Salud, Universidad Técnica de Manabí, Portoviejo 130105, Ecuador; raju.maddela@utm.edu.ec

⁴ Key Laboratory for Water Quality and Conservation of the Pearl River Delta, Ministry of Education, Guangzhou 510006, China; lishugeng@gzhu.edu.cn

⁵ School of Environmental Science and Engineering, Guangzhou University, Guangzhou 510006, China

* Correspondence: zllqiu@gzhu.edu.cn

† These authors contributed equally to this work.

Table S1. Summary of the reactor performances throughout the whole experimental period

Phase	Influent concentration (mg/L)		Effluent concentration (mg/L)			NAR (%)	NTR (%)	PFOA removal efficiency (%)
	NO ₃ ⁻ -N	PFOA	NO ₃ ⁻ -N	NO ₂ ⁻ -N	PFOA			
Phase I (days 0-25)	48.83±1.35	—	5.68±1.30	30.76±1.43	—	71.28±1.41	88.35±2.71	—
Phase II (days 26-50)	48.93±1.27	0.1	5.10±0.99	31.17±0.99	0.03±0.01	71.19±2.55	89.83±1.92*	67.42±3.39
Phase III (days 51-75)	50.00±1.48	0.5	3.84±1.00	32.06±0.86	0.24±0.04	69.52±3.13*	92.33±1.96***	51.06±8.36
Phase IV (days 76-100)	49.58±1.46	2	5.98±1.55	30.02±1.44	1.12±0.17	68.97±3.42**	87.89±3.33	43.77±8.28
Phase V (days 101-125)	48.71±1.33	5	6.23±1.21	28.91±1.28	3.92±0.15	68.11±3.11***	87.21±2.47	21.50±3.02
Phase VI (days 126-150)	49.54±1.45	10	5.21±1.11	30.11±1.25	9.34±0.52	67.94±2.25***	89.49±2.15	6.56±5.25
Phase VII (days 151-175)	49.20±1.18	20	5.20±1.32	30.19±1.44	20.23±1.7 5	68.66±2.90***	89.41±2.80	—

Note: NAR: nitrite accumulation rate. NTR: nitrate transformation ratio. The data are expressed as mean ± standard deviation (SD). Asterisks indicate the statistical significance as compared with Phase I. The statistical significance was determined by a two-tailed independent sample t-test: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Table S2. The effects of PFOA on nitrogen removal performances

No.	PFOA dosage	Effects	References
1	<0.5	Severe inhibition on nitrite accumulation; significant increase in nitrate reduction	This study
	0.5-20	Severe inhibition on nitrite accumulation; no significant effect on nitrate reduction	
2	1	Severe inhibition on TN and ammonium removal; nitrate and nitrite reduction were inhibited by 13.1% and 5.8%, respectively	[1]
3	< 20	No significant effect	[2]
	> 20	Severe inhibition on TN and ammonium removal and nitrate and nitrite reduction	
4	5	No significant effect	[3]
	10	No significant effect	
	20	No significant effect	
5	0.05	No significant effect	[4]
	0.5	Decrease in TN removal efficiency by 8.32%	
6	0.5	Increase in TN removal efficiency by 24.6%	[5]

Table S3. EEM spectrum information of EPS under different PFOA stresses

		Spectral peak information (Ex/Em, intensity)													
		Phase I		Phase II		Phase III		Phase VI		Phase V		Phase VI		Phase VII	
		0 mg/L		0.1 mg/L		0.5 mg/L		2 mg/L		5 mg/L		10 mg/L		20 mg/L	
Peak	Related substances	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity	Ex/Em	Intensity
A	tryptophan protein-like	290/347	35017	290/348	187433	285/345	218654	285/346	175717	285/343	175700	285/343	256545	285/343	204070
B	humic acid-like aromatic	370/457	7729	365/456	43592	360/453	49463	360/453	36301	355/450	33777	355/447	36056	350/445	25219
C	protein II-like	230/331	7733	225/329	44596	225/330	50159	225/320	31608	225/306	52287	225/306	70307	225/306	155109

Table S4. Relative percentage of a protein secondary structure in the amide I region at different PFOA concentrations

Secondary structures	Wavenumber (cm ⁻¹)	TB-At (%)						
		Phase I	Phase II	Phase III	Phase IV	Phase V	Phase VI	Phase VII
Aggregated strands	1600-1625	21.42	13.16	17.48	10.58	13.69	20.88	13.17
β-Sheet	1625-1640	25.65	22.29	22.61	22.08	21.39	24.65	20.90
Random coil	1640-1650	0.00	0.00	0.00	28.47	0.00	0.00	0.00
α-Helix	1650-1660	26.05	25.42	24.67	0.00	27.22	25.10	27.05
3-Turn helix	1660-1670	16.67	23.06	20.92	22.20	22.22	18.14	23.71
Antiparallel β- structures/Aggregated strands	1670-1700	10.21	16.08	14.33	16.67	15.49	11.24	15.17

Table S5. Species diversity indices of sludge samples at different PFOA concentrations

	sobs	ace	chao	shannon	simpson	coverage
Phase I	817±26.87	990.85±26.16	980.74±11.47	3.88±0.05	0.09±0.00	0.99±0.00
Phase II	698±7.07*	860.44±24.81*	853.71±17.61*	4.03±0.02	0.07±0.00*	0.99±0.00
Phase III	710±0.00*	891.88±30.89	901.13±57.23	4.10±0.01*	0.05±0.00***	0.99±0.00
Phase IV	704.5±47.38	881.18±41.92	882.63±60.27	4.07±0.26	0.06±0.01	0.99±0.00
Phase V	669±28.28*	820.21±55.60	827.63±57.89	4.10±0.05	0.05±0.00**	0.99±0.00
Phase VI	598±1.41**	710.79±10.78**	717.05±15.69**	4.05±0.09	0.05±0.01*	0.99±0.00
Phase VII	586.5±27.58*	713.41±12.97**	714.66±32.14**	3.92±0.06	0.05±0.00**	0.99±0.00

Note: The data are expressed as mean ± standard deviation (SD). Asterisks indicate the statistical significance as compared with Phase I. The statistical significance was determined by a two-tailed independent sample t-test: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

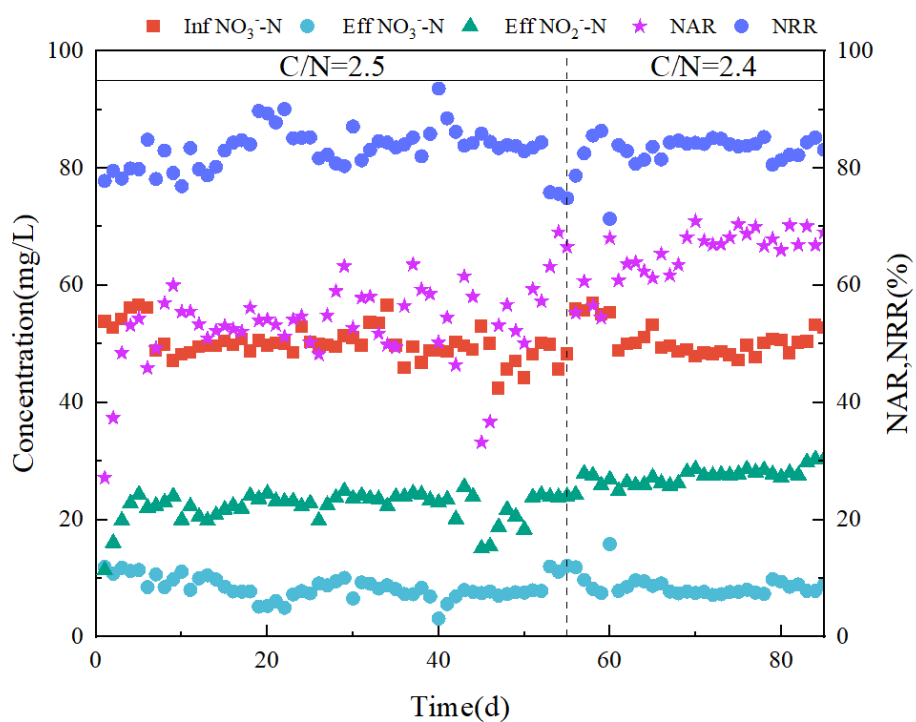


Figure. S1. Changes in the performance of partial denitrification during PD domestication process. The corresponding PFOA concentrations of phase I, II, III, IV, V, VI, and VII were 0, 0.1, 0.5, 2, 5, 10, and 20 mg/L, respectively.

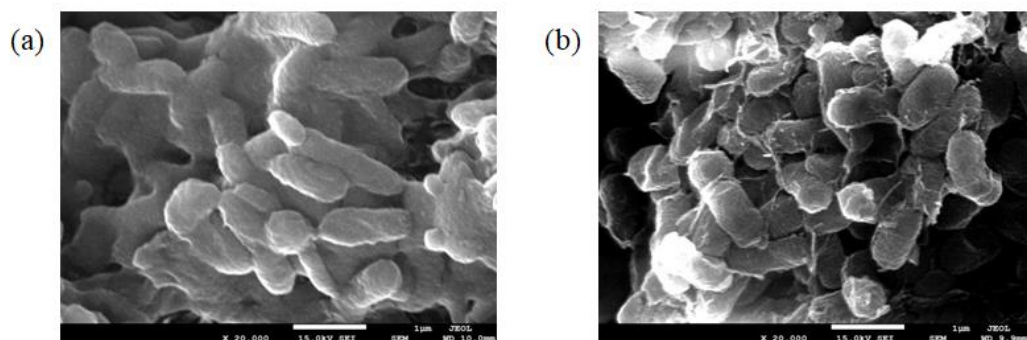


Figure. S2. SEM image of the sludge before and after PFOA stress: (a) phase I x 20000; (b) phase VII x 20000.

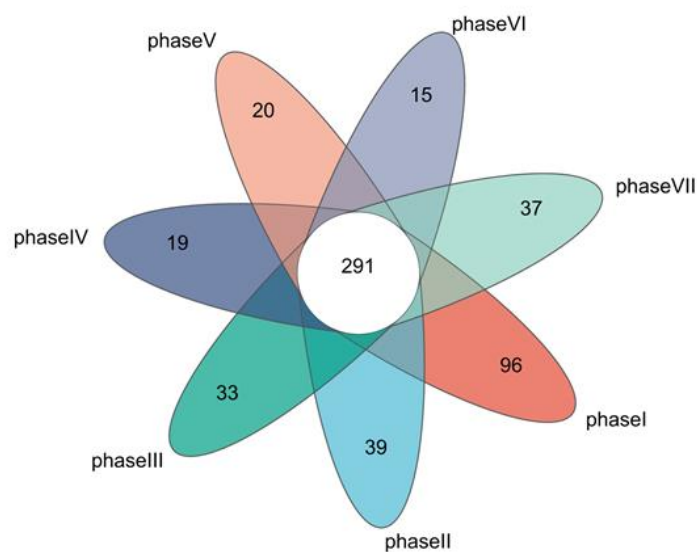


Figure. S3. Venn diagram showing the unique and shared OTUs in different phases. The corresponding PFOA concentrations of Phase I, II, III, IV, V, VI, and VII were 0, 0.1, 0.5, 2, 5, 10, and 20 mg/L, respectively.

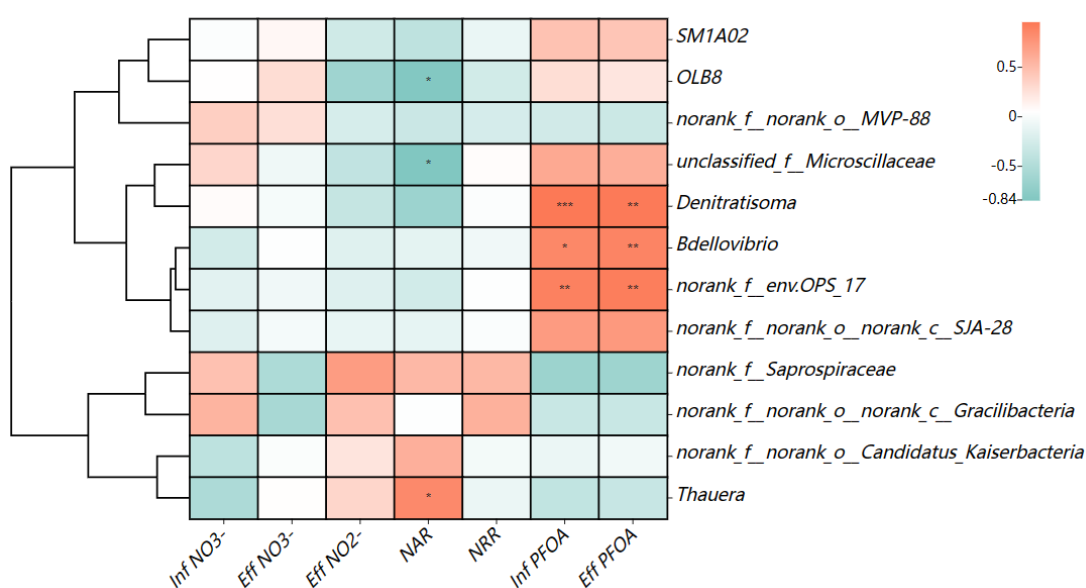


Figure. S4. Spearman's rank correlations between performance parameters and the major genera. The corresponding PFOA concentrations of phase I, II, III, IV, V, VI, and VII were 0, 0.1, 0.5, 2, 5, 10, and 20 mg/L, respectively.