

*Supplementary Material*

# In-Situ Sludge Reduction Performance and Mechanism in Sulfidogenic Anoxic–Oxic–Anoxic Membrane Bioreactors

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## Text S1. PARAFAC modeling

The fluorescence spectrometer (F-7000, Hitachi,Tokyo, Japan) was used to determine the three-dimensional excitation-emission matrix (EEM) of the fluorescent organic components in SMP and EPS in each tank of three bioreactors. The operational parameters were set according to Maqbool et al. [1]. Excitation-emission matrix-parallel factor (EEM-PARAFAC) analysis was introduced to elucidate the fluorescence peaks of different compounds [2]. A dataset contained 420 EEMs of SMP and EPS from three bioreactors was used for PARAFAC modeling. A free DOMFlour toolbox of Matlab was used and the detailed analysis procedures could be found elsewhere [3]. The maximum fluorescence intensities ( $F_{\max}$ ) of individual components were in direct proportion to their relative concentrations [4].

**Table S1.** Chemical composition of synthetic wastewater.

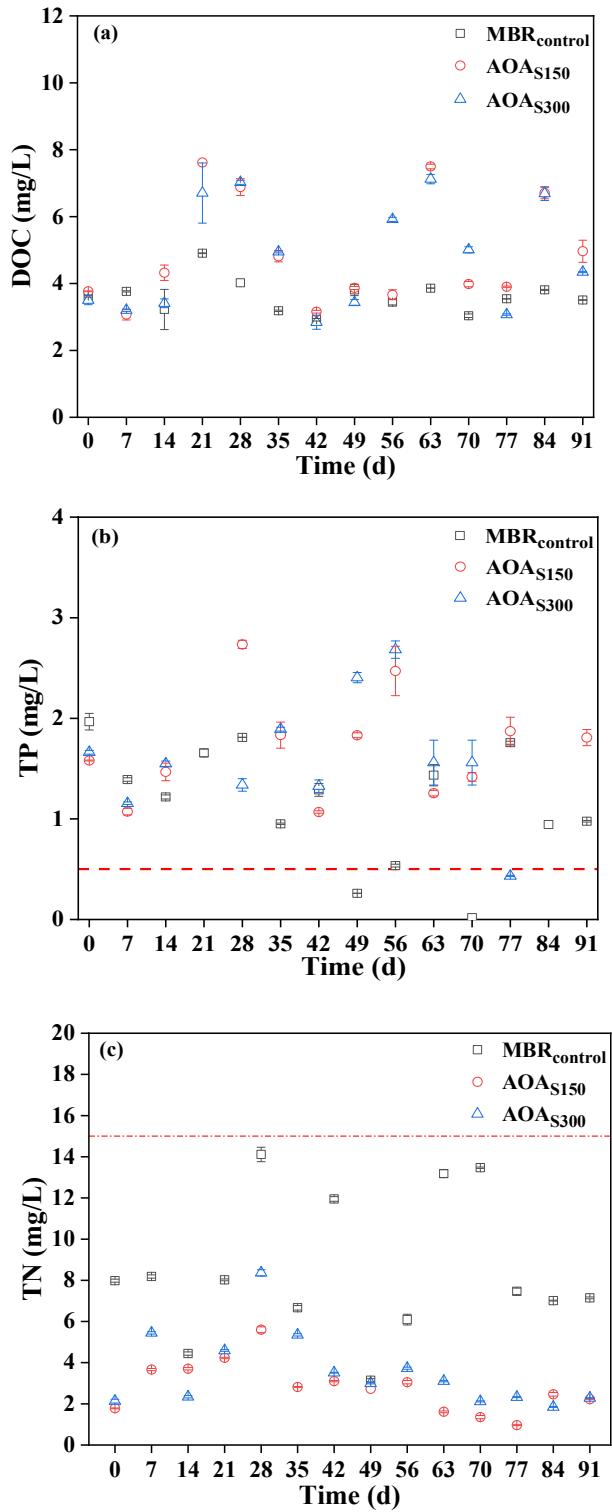
Components	Concentration (mg/L)	
	Control	AOA
Glucose	1000	1185
NH <sub>4</sub> Cl	140	165.9
KH <sub>2</sub> PO <sub>4</sub>	25	29.63
NaNO <sub>3</sub>	40	47.4
MgSO <sub>4</sub> .7H <sub>2</sub> O	5	5.93
FeCl <sub>3</sub>	2.33	2.76
NaHCO <sub>3</sub>	Appropriate to maintain pH = 7.0	Appropriate to maintain pH = 7.0

**Table S2.** The average  $\pm$  standard deviation of basic quality parameters of influent and effluent in three bioreactors.

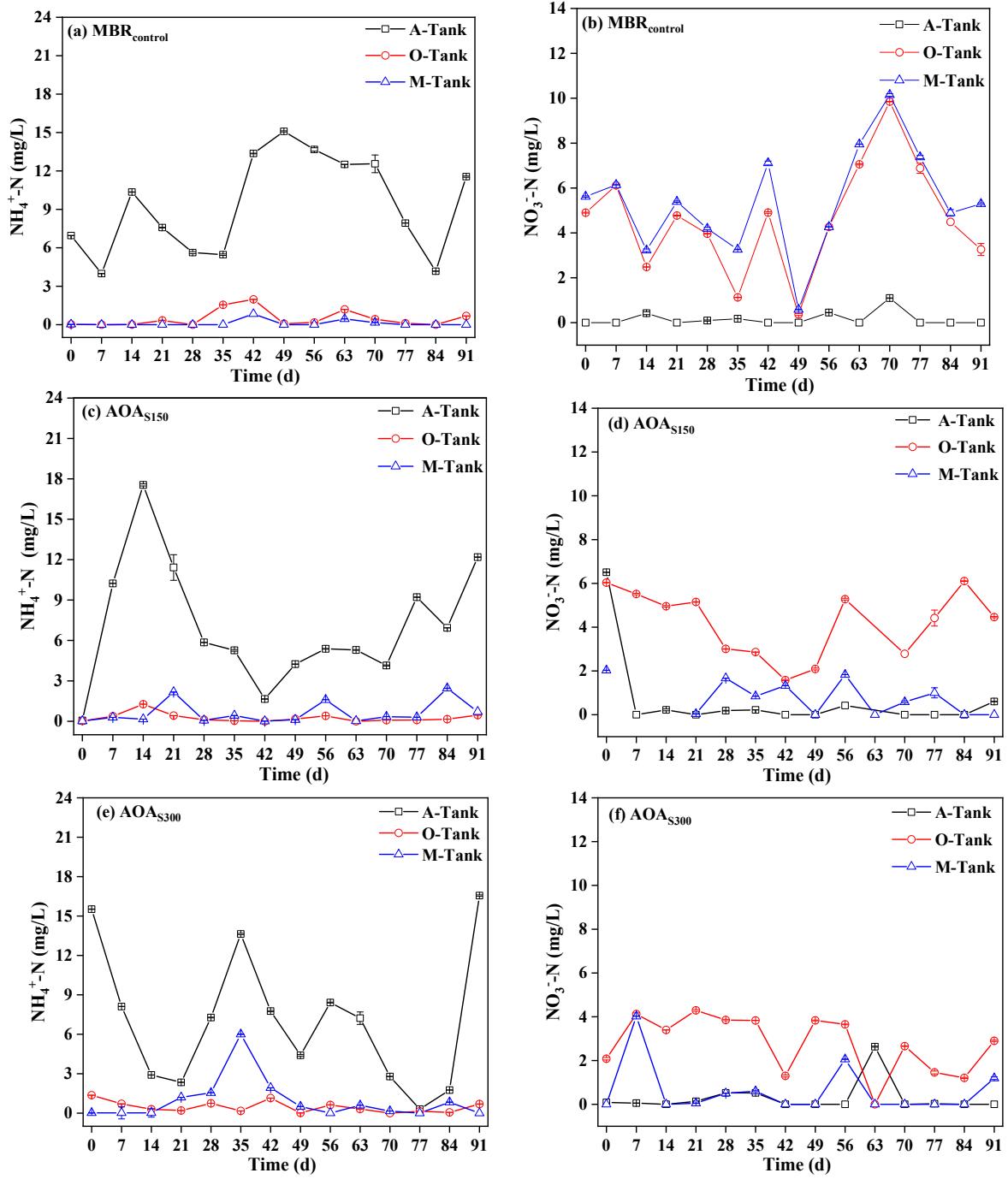
Items	Influent (mg/L)		Effluent (mg/L)			Removal Efficiency (%)		
	MBR <sub>control</sub>	AOA	MBR <sub>control</sub>	AOA <sub>S150</sub>	AOA <sub>S300</sub>	MBR <sub>control</sub>	AOA <sub>S150</sub>	AOA <sub>S300</sub>
TOC	389.98 $\pm$ 5.94	441.2 $\pm$ 10.64	3.62 $\pm$ 0.48	4.87 $\pm$ 1.55	4.8 $\pm$ 1.56	> 99	> 98	> 98
TN	45.20 $\pm$ 2.72	52.88 $\pm$ 6.30	8.49 $\pm$ 3.27	2.82 $\pm$ 1.17	3.59 $\pm$ 1.76	> 81	> 94	> 93
TP	6.13 $\pm$ 0.07	7.34 $\pm$ 0.19	1.16 $\pm$ 0.56	1.70 $\pm$ 0.49	1.60 $\pm$ 0.57	> 81	> 76	> 78

**Table S3.** Sequencing of bacterial 16S rRNA gene along with alpha diversity of microbial taxa in three bioreactors.

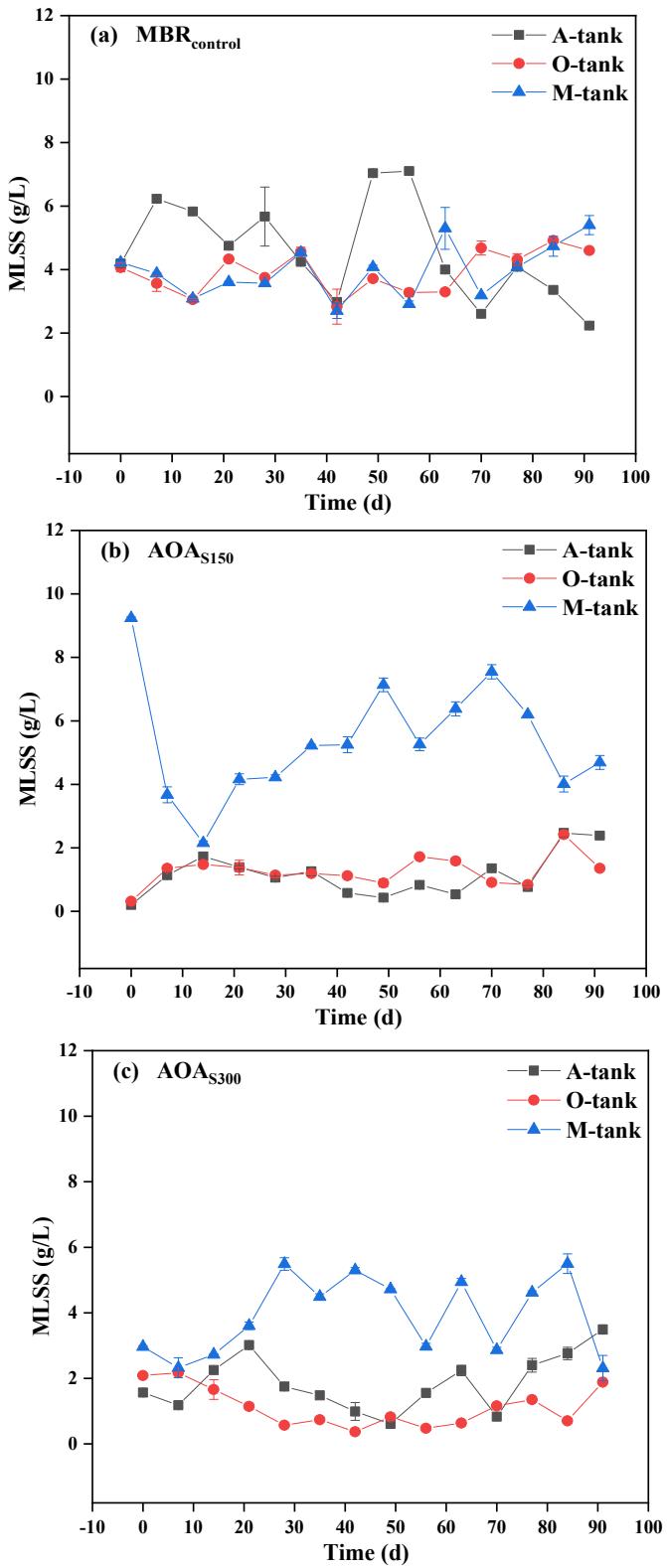
Sample description	Sequencing results		Species diversity
	Effective tags	OTUs	Simpson
MBR <sub>control</sub> -A-1d-a <sub>1</sub>	56402	1720	0.018
MBR <sub>control</sub> -O-1d-o <sub>1</sub>	65627	1449	0.11
MBR <sub>control</sub> -M-1d-m <sub>1</sub>	58914	1520	0.116
MBR <sub>control</sub> -A-45d-a <sub>2</sub>	58448	1627	0.057
MBR <sub>control</sub> -O-45d-o <sub>2</sub>	67622	1489	0.0694
MBR <sub>control</sub> -M-45d-m <sub>2</sub>	56745	1268	0.0544
MBR <sub>control</sub> -A-90d-a <sub>3</sub>	61203	1232	0.133
MBR <sub>control</sub> -O-90d-o <sub>3</sub>	59066	1225	0.114
MBR <sub>control</sub> -M-90d-m <sub>3</sub>	61120	1258	0.115
AOAs <sub>150</sub> -A-1d-a <sub>4</sub>	59282	1395	0.064
AOAs <sub>150</sub> -O-1d-o <sub>4</sub>	63154	1195	0.0953
AOAs <sub>150</sub> -M-1d-m <sub>4</sub>	60434	1370	0.0596
AOAs <sub>150</sub> -A-45d-a <sub>5</sub>	63338	1080	0.18
AOAs <sub>150</sub> -O-45d-o <sub>5</sub>	68505	1039	0.29
AOAs <sub>150</sub> -M-45d-m <sub>5</sub>	64844	1194	0.211
AOAs <sub>150</sub> -A-90d-a <sub>6</sub>	50333	1236	0.0588
AOAs <sub>150</sub> -O-90d-o <sub>6</sub>	61612	1185	0.0836
AOAs <sub>150</sub> -M-90d-m <sub>6</sub>	62850	1186	0.0913
AOAs <sub>300</sub> -A-1d-a <sub>7</sub>	58914	1520	0.094
AOAs <sub>300</sub> -O-1d-o <sub>7</sub>	59902	1288	0.0568
AOAs <sub>300</sub> -M-1d-m <sub>7</sub>	59713	1575	0.0472
AOAs <sub>300</sub> -A-45d-a <sub>8</sub>	56745	1268	0.0784
AOAs <sub>300</sub> -O-45d-o <sub>8</sub>	68153	1121	0.0924
AOAs <sub>300</sub> -M-45d-m <sub>8</sub>	58512	1169	0.12
AOAs <sub>300</sub> -A-90d-a <sub>9</sub>	61120	1258	0.11
AOAs <sub>300</sub> -O-90d-o <sub>9</sub>	57553	1240	0.115
AOAs <sub>300</sub> -M-90d-m <sub>9</sub>	58938	1132	0.123



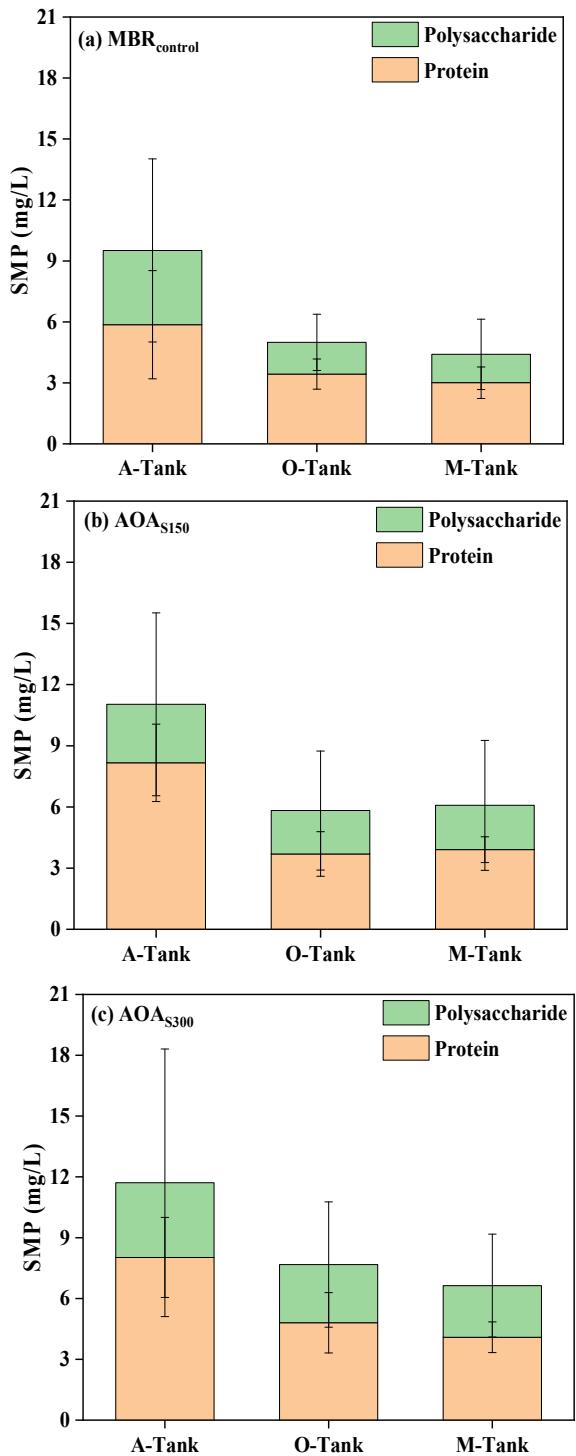
**Figure S1.** The temporal variations in basic quality parameters (a) DOC, (b) TN, and (c) TP in effluents from three bioreactors.



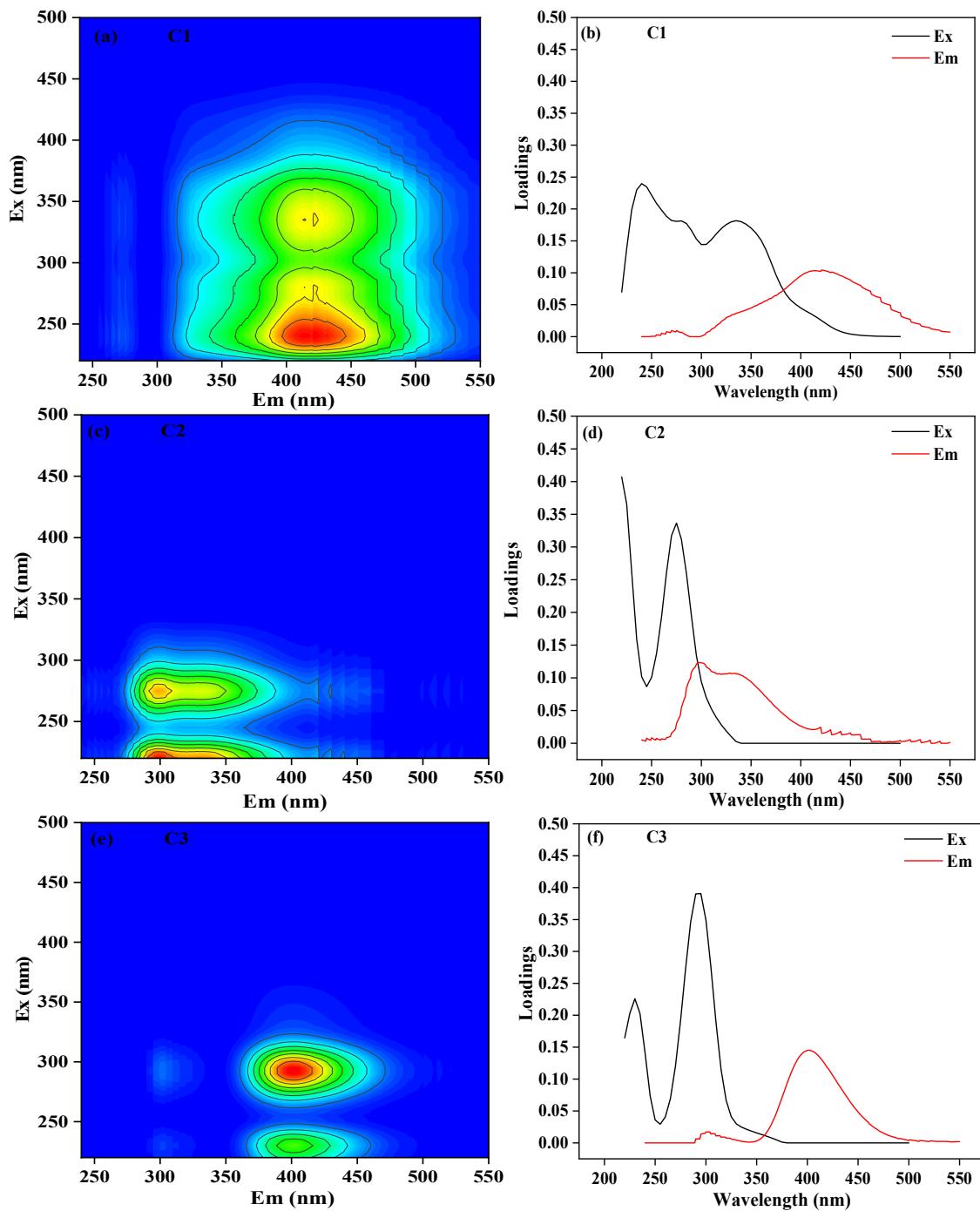
**Figure S2.** The temporal variations in  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N concentrations in different tanks of three bioreactors.



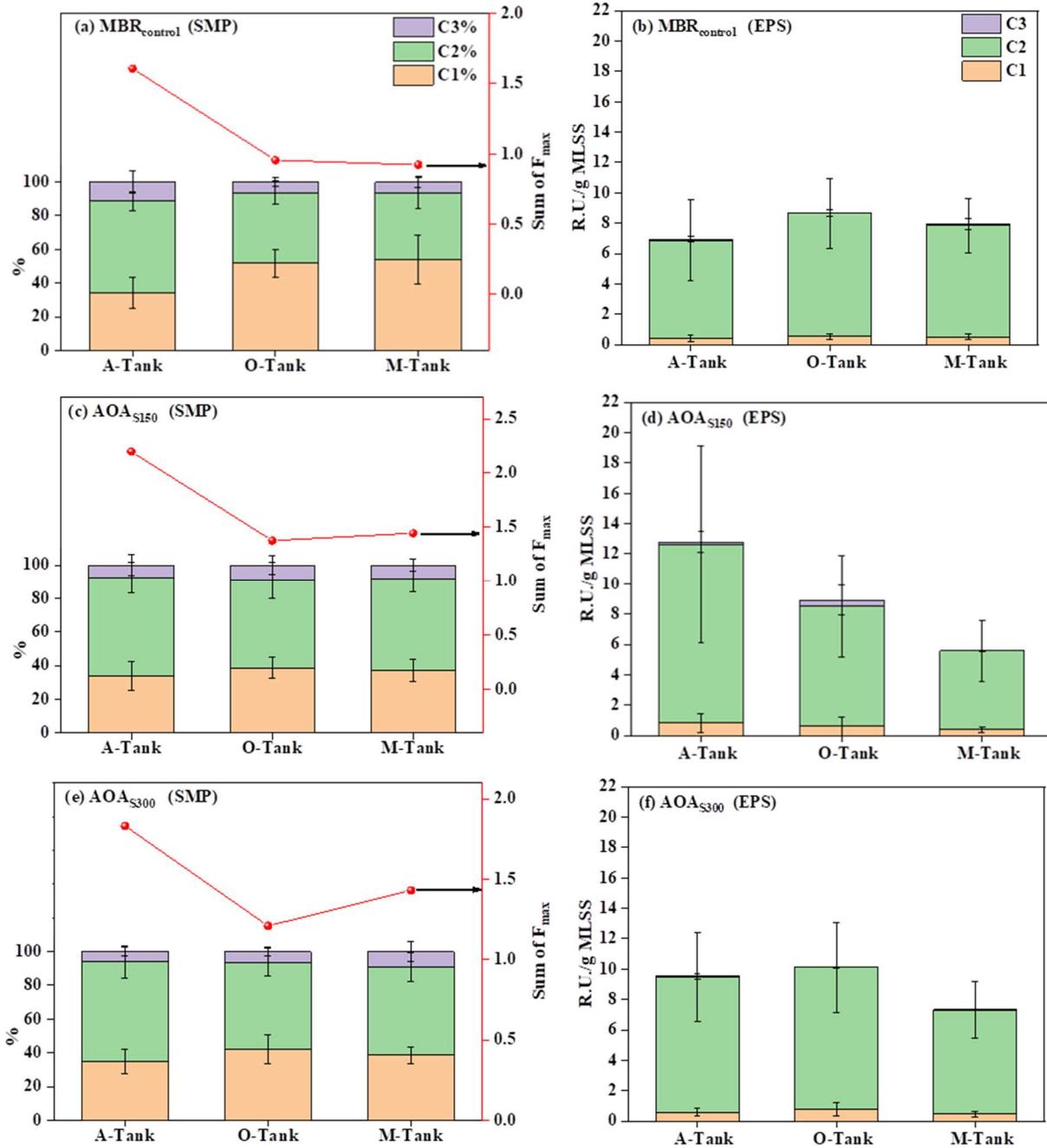
**Figure S3.** The changes of MLSS concentration in different tanks of three bioreactors.



**Figure S4.** The average polysaccharide and protein in SMP in different tanks of MBR<sub>control</sub> (a), AOAs<sub>150</sub> (b), and AOAs<sub>300</sub> (c).



**Figure S5.** EEM-PARAFAC components, humic-like (C1), tryptophan-like (C2), and fulvic-like (C3).



**Figure S6.** The average distribution of EEM-PARAFAC components and sum of  $F_{\max}$  ( $C_1+C_2+C_3$ ) in SMP and EPS of different tanks in MBR<sub>control</sub> (a and b), AOA<sub>S150</sub> (c and d), and AOA<sub>S300</sub> (e and f).

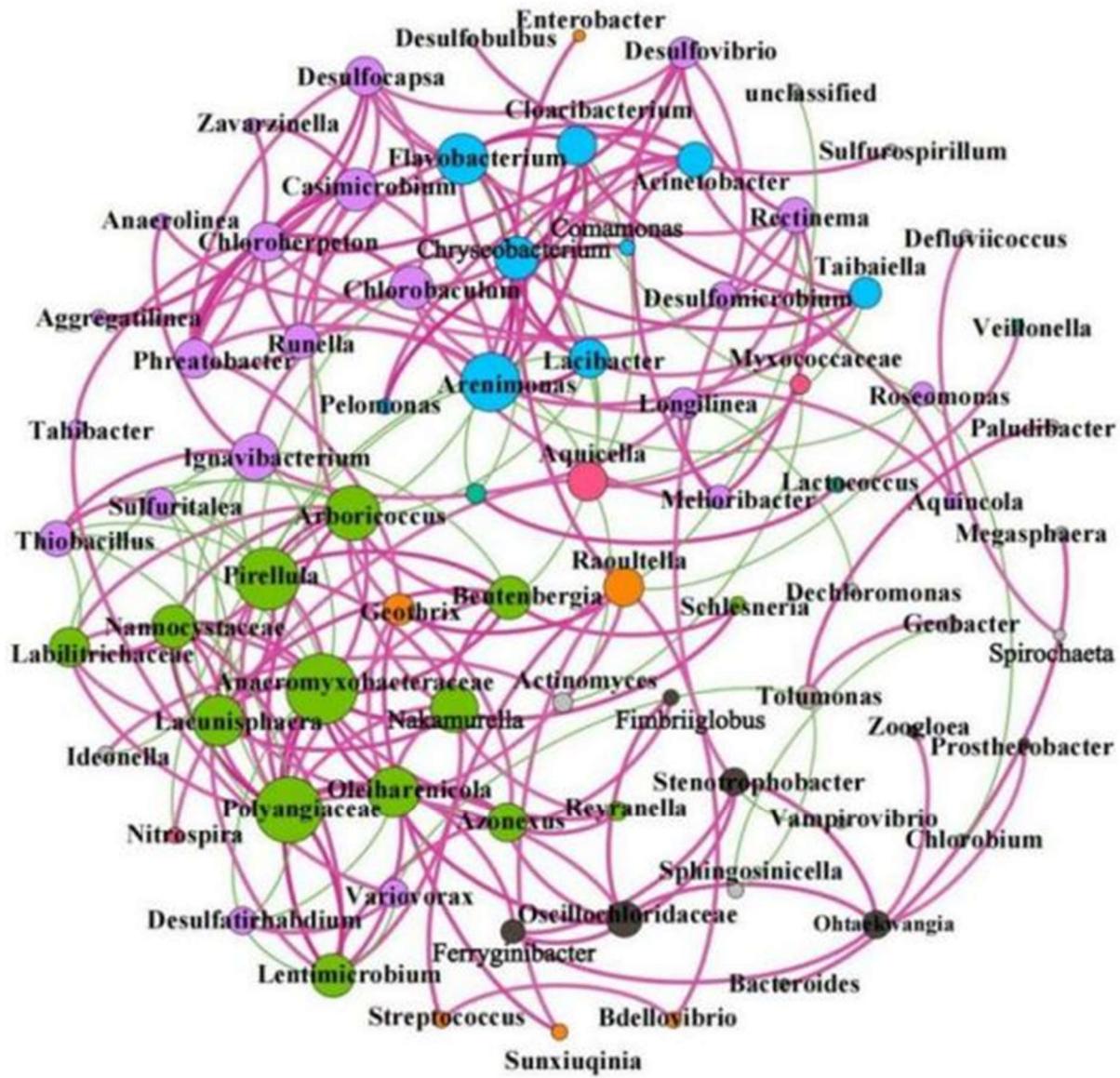


Figure S7. The network analysis among functional microorganisms in three bioreactors.

## References

1. Maqbool, T., Li, C., Qin, Y., Zhang, J., Asif, M. B., Zhang, Z. . A year-long cyclic pattern of dissolved organic matter in the tap water of a metropolitan city revealed by fluorescence spectroscopy. *Sci. Total Environ.* **2021**, 771, 144850. <https://doi.org/10.1016/j.scitotenv.2020.144850>
2. Huang, M., Li, Z., Huang, B., Luo, N., Zhang, Q., Zhai, X., Zeng, G. Investigating binding characteristics of cadmium and copper to DOM derived from compost and rice straw using EEM-PARAFAC combined with two-dimensional FTIR correlation analyses. *J. Hazard. Mater.* **2018**, 344, 539-548. <https://doi.org/10.1016/j.jhazmat.2017.10.022>
3. Stedmon, C. A., Bro, R. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnol. Oceanogr.-Meth.* **2008**, 6(11), 572-579. <https://doi.org/10.4319/lom.2008.6.572>
4. Rodríguez-Vidal, F. J., García-Valverde, M., Ortega-Azabache, B., González-Martínez, Á., Bellido-Fernández, A. Characterization of urban and industrial wastewaters using excitation-emission matrix (EEM) fluorescence: Searching for specific fingerprints. *J. Environ. Manage.* **2020**, 263, 110396. <https://doi.org/10.1016/j.jenvman.2020.110396>