Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information: Dynamics of naphthenic acids and microbial community structures in a membrane bioreactor treating oil sands process-affected water: impacts of supplemented inorganic nitrogen and hydraulic retention time

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Supporting information includes 7 tables, 5 figures, and 17 pages in total.

Water chemistry analyses

Samples were collected weekly from the feed by using a disposable syringe, and from the permeate by leaving the effluent tubing in a clean glass bottle untill a volume of 100 mL was achieved. Right after sample collection, Thermo Scientific™ Target2™ Nylon Syringe Filters (0.22 μm) were used to filter water samples prior to analyses. Conventional water chemistry parameters including pH, COD, nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), and nitrite nitrogen (NO_2-N) were measured for the feed and permeate samples in duplicates according to the standard methods (Federation and Association, 2005). To measure the mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) concentration (an indicator of microbial growth), a 30 mL sludge sample was collected weekly from the middle depth of the anoxic and aerobic tanks by using a disposable syringe. The aforementioned analyses were performed right after the samples were collected and pre-filtered. Dissolved oxygen (DO) concentrations in the anoxic and aerobic tanks were monitored through plugging a DO meter (YSI 50B Dissolved Oxygen Meter) probe into the reactor tanks to ensure that anoxic and aerobic conditions were achieved in corresponding tanks. To assess the MBR's performance in reducing the aquatic toxicity of the feed OSPW, the 81.9% screening test Microtox bioassay protocol was performed. The light emitted by the photobacterium *Vibrio fischeri* as a result of its normal metabolic activities was measured using a model 500 Microtox® analyzer (Strategic Diagonostic Inc.). Detailed information on the toxicity analysis is available elsewhere (Chelme-Ayala et al., 2010).

UPLC/HRMS analysis

Chromatographic separations were run on a Waters UPLC Phenyl BEH column (150 × 1 mm, 1.7 µm) using a gradient mobile phase of (A) 10 mM ammonium acetate solution prepared in Optima-grade water and (B) 10 mM ammonium acetate in 50% methanol and 50% acetonitrile, both Optima-grade. Gradient elution was as follows: 1% B for the first 2 min, then ramped to 60% B by 3 min, to 70% B by 7 min, to 95% B by 13 min, followed by a hold until 14 min and finally returned to 1% B, followed by a further 5.8 min equilibration time. The flow was constant at 100 µL/min and column temperature was kept at 50°C, while samples were maintained at 4°C.

Detection was performed with a high resolution Synapt G2 HDMS mass spectrometer (30,000 FWHM) equipped with an electrospray ionization source operating in negative ion mode. The system was controlled using MassLynx® ver. 4.1, tuning and calibration were performed using standard solutions, lucine enkaphenlin and sodium formate, respectively, provided by Waters Corporation (Milford, MA, USA). TargetLynx® ver. 4.1 was used for data analysis of the target compounds, and the relative ratio of each analyte's chromatographic peak area to the internal standard was calculated for subsequent analysis. The Synapt G2 system was permanent calibrated by infusing every 10 seconds the lock mass solution during the analysis of a sample.

Water chemistry analysis results: Chemical oxygen demand (COD) and nitrogen removal, and biomass growth

Table S1 summarizes the permeate COD, NH_4-N and TIN (including NH_4-N, NO_3-N and NO_2-N) concentrations under different supplemented NH_4-N concentrations at an hydraulic retention time (HRT) of 48 hours. As for the permeate water quality at different HRTs, Table S2 is presented. Figure S1 A, B, C and D demonstrate COD, NH_4-N, NO_2-N and NO_3-N

concentrations over time, respectively. The permeate COD level throughout the study was close to that of the original OSPW COD. To better understand COD changes during the MBR process, t-test was conducted between original OSPW COD and permeate COD concentrations within each of the six stages. Compared with original OSPW COD, t-test P values for MBR permeate COD are 0.162, 0.851, 0.519, and 0.289 at A75H48, A50H48, A25H48, and A25H72, respectively. Thus, the MBR process did not significantly change the OSPW indigenous COD ($P >> 0.05$) over the four stages. In contrast, MBR permeate at A25H24 and A25H12 showed significant COD reduction ($P < 0.05$) with a P of 0.017 and 0.002, respectively, implying that the operating conditions used at A25H24 and A25H12 were more favourable for OSPW indigenous COD degradation. Average COD degradation rates of 10.9% and 10.7% were achieved in the MBR at A25H24 and A25H12, respectively. The better COD removal in those two stages might result from enhancement of rejection by biofouling layer (Khor et al., 2007) given that more frequent severe membrane fouling were witnessed within the two periods (data not shown).

Table S3 describes MLSS and MLVSS concentrations and MLVSS/MLSS ratios under different operating conditions. As recirculation was performed from the aerobic tank to the anoxic tank at a rate of 2-fold the influent flowrate, an average value of the measured solids concentrations in the anoxic and aerobic tank was calculated for each sampling day. The MLVSS/MLSS ratio was relatively stable within a range of 0.66 – 0.74 over the whole study in spite of different operating conditions. The typical MLVSS/MLSS ratio is 0.8 – 0.9 in an activated sludge system (Metcalf et al., 2003). The lower MLVSS/MLSS ratio in this study could be attributed to the long SRT. In Table S3, the MLSS and MLVSS concentrations behave in great congruity. Thus, MLVSS is picked to describe how biomass concentration changed over time under each operating condition. When the HRT was shortened to 24 hours at A25H24 and 12 hours at A25H12, the MLVSS

concentration fell to an average level of ~2390 mg/L and ~3020 mg/L, respectively. The bacterial growth in the system was likely to be impacted by the intensified OSPW organic loading under shorter HRTs.

Table S1 Permeate COD and inorganic nitrogen under different NH_4-N concentrations (HRT = 48 hours)

Supplemented NH_4-N	**COD**	**COD removal**	**NH_4-N**	**NH_4-N removal**	**TIN**	**TIN removal**
mg/L	mg/L	%	mg/L	%	mg/L	%
75	211.4±28.1	-5.4±9.4	0.16±0.21	99.8±0.3	11.1±10.6	86.2±13.1
50	206.4±22.5	-3.3±12.5	0.06±0.12	99.9±0.2	26.9±11.1	67.0±13.3
25	220.8±20.7	1.3±12.5	0.39±0.96	98.4±4.0	5.6±3.5	93.0±4.2

Table S2 Permeate COD and inorganic nitrogen at different HRTs (25 mg NH_4-N/L)

HRT	**COD**	**COD removal**	**NH_4-N**	**NH_4-N removal**	**TIN**	**TIN removal**
hours	mg/L	%	mg/L	%	mg/L	%
72	238.9±18.6	5.8±13.6	0.10±0.17	99.6±0.7	15.5±6.6	80.7±8.0
48	220.8±20.7	1.3±12.5	0.39±0.96	98.4±4.0	5.6±3.5	93.0±4.2
24	226.1±29.9	10.9±2.0	0.18±0.21	99.4±0.8	12.2±1.4	84.6±1.8
12	209.0±14.3	10.7±3.5	0.06±0.09	99.8±0.4	20.4±6.9	74.7±8.3

Table S3 Biomass concentrations and MLVSS/MLSS ratios at different operating conditions

Operating condition	**Biomass concentration**		**MLVSS/MLSS**
	MLVSS (mg/L)	MLSS (mg/L)	
A75H48	3636±689	4982±949	0.73±0.01
A50H48	2965±534	4360±879	0.68±0.02
A25H48	3801±252	5340±395	0.71±0.01
A25H72	3087±347	4633±548	0.67±0.01
A25H24	2386±109	3309±164	0.72±0.01
A25H12	3020±239	4288±336	0.70±0.01

Table S4 Pearson Correlation Coefficient between the major orders and removal rates of classical NAs with different n

Major orders over the study duration	Molecular carbon number n														
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Proteobacteria;c_β-Proteobacteria;o_Rhodocyclales	0.122	0.215	0.248	0.726	0.739	0.706	0.714	0.755	0.743	0.428	0.165	-0.138	-0.090	-0.411	-0.570
Proteobacteria;c_β-Proteobacteria;o_ASSO-13	-0.364	0.036	0.290	0.562	0.229	0.135	0.036	0.152	0.143	-0.099	-0.339	-0.724	-0.609	-0.691	-0.946
Proteobacteria;c_β-Proteobacteria;o_Nitrosomonadales	0.544	-0.059	0.207	0.277	-0.128	-0.226	-0.289	-0.183	-0.159	-0.353	-0.561	-0.833	-0.664	-0.680	-0.821
Bacteroidetes;c_Cytophagia;o_Cytophagales	0.670	0.393	-0.057	-0.212	0.197	0.273	0.311	0.174	0.093	0.479	0.755	0.981	0.827	0.857	0.766
Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales	-0.123	-0.072	0.363	-0.090	-0.435	-0.446	-0.445	-0.478	-0.405	-0.280	-0.289	-0.407	-0.629	0.056	0.088
Bacteroidetes;c_[Saprospirae];o_[Saprospirales]	-0.042	-0.192	-0.207	-0.693	-0.697	-0.658	-0.633	-0.743	-0.749	-0.377	-0.075	0.200	0.088	0.497	0.596
Nitrospirae;c_Nitrospira;o_Nitrospirales	-0.340	-0.628	-0.550	-0.880	-0.860	-0.807	-0.738	-0.789	-0.730	-0.634	-0.420	-0.008	0.010	0.171	0.590
Planctomycetes;c_Phycisphaerae;o_Phycisphaerales	-0.795	-0.698	-0.320	-0.353	-0.661	-0.695	-0.668	-0.569	-0.455	-0.776	-0.916	-0.831	-0.634	-0.673	-0.301
Proteobacteria;c_β-Proteobacteria;o_Burkholderiales	0.075	-0.041	-0.262	-0.710	-0.575	-0.526	-0.456	-0.569	-0.574	-0.220	0.064	0.426	0.392	0.582	0.730
Proteobacteria;c_γ-Proteobacteria;o_Xanthomonadales	-0.634	-0.438	-0.021	-0.179	-0.554	-0.597	-0.568	-0.488	-0.368	-0.612	-0.787	-0.814	-0.709	-0.558	-0.291
Acidobacteria;c_[Chloracidobacteria];o_RB41	-0.008	-0.141	-0.133	-0.649	-0.666	-0.627	-0.587	-0.695	-0.684	-0.332	-0.067	0.190	0.063	0.500	0.621
Proteobacteria;c_α-Proteobacteria;o_Rhizobiales	-0.662	-0.436	-0.477	-0.443	-0.509	-0.532	-0.420	-0.319	-0.205	-0.562	-0.730	-0.457	-0.056	-0.563	-0.097
Cyanobacteria;c_4C0d-2;o_MLE1-12	-0.145	-0.295	0.008	-0.429	-0.582	-0.549	-0.467	-0.505	-0.390	-0.354	-0.335	-0.188	-0.323	0.131	0.470
[Thermi];c_Deinococci;o_Deinococcales	0.205	0.038	-0.096	-0.573	-0.482	-0.434	-0.410	-0.548	-0.583	-0.126	0.203	0.451	0.285	0.693	0.689

Table S5 Pearson Correlation Coefficient between the major orders and removal rates of classical NAs with different Z

Major orders over the study duration	Absolute value of hydrogen deficiency Z									
	0	2	4	6	8	10	12	14	16	18
Proteobacteria;c_β-Proteobacteria;o_Rhodocyclales		0.445	0.585	0.794	0.895	0.591	0.567	0.208	0.338	0.367
Proteobacteria;c_β-Proteobacteria;o_ASSO-13		-0.291	-0.076	0.286	0.464	0.188	0.292	-0.384	-0.328	0.057
Proteobacteria;c_β-Proteobacteria;o_Nitrosomonadales		-0.560	-0.440	-0.061	0.150	-0.053	0.152	-0.614	-0.578	-0.166
Bacteroidetes;c_Cytophagia;o_Cytophagales		0.613	0.530	0.060	-0.291	0.215	-0.105	0.762	0.680	0.355
Bacteroidetes;c_Flavobacteriia;o_Flavobacteriales		-0.543	-0.576	-0.398	-0.376	-0.347	-0.114	-0.350	-0.426	-0.070
Bacteroidetes;c_[Saprospirae];o_[Saprospirales]		-0.419	-0.519	-0.771	-0.920	-0.585	-0.611	-0.132	-0.288	-0.296
Nitrospirae;c_Nitrospira;o_Nitrospirales		-0.524	-0.703	-0.849	-0.786	-0.803	-0.686	-0.411	-0.488	-0.663
Planctomycetes;c_Phycisphaerae;o_Phycisphaerales		-0.784	-0.855	-0.526	-0.162	-0.620	-0.257	-0.897	-0.846	-0.723
Proteobacteria;c_β-Proteobacteria;o_Burkholderiales		-0.161	-0.346	-0.655	-0.826	-0.408	-0.462	0.044	-0.070	-0.233
Proteobacteria;c_γ-Proteobacteria;o_Xanthomonadales		-0.722	-0.800	-0.426	-0.124	-0.458	-0.072	-0.787	-0.745	-0.500
Acidobacteria;c_[Chloracidobacteria];o_RB41		-0.383	-0.512	-0.727	-0.865	-0.535	-0.521	-0.116	-0.256	-0.254
Proteobacteria;c_α-Proteobacteria;o_Rhizobiales		-0.376	-0.613	-0.385	-0.048	-0.332	-0.013	-0.646	-0.517	-0.679
Cyanobacteria;c_4C0d-2;o_MLE1-12		-0.410	-0.605	-0.518	-0.437	-0.485	-0.214	-0.325	-0.357	-0.307
[Thermi];c_Deinococci;o_Deinococcales		-0.162	-0.253	-0.592	-0.845	-0.374	-0.497	0.144	-0.018	-0.068

Table S6 α diversity parameters of microbial communities at different operating conditions

Stage	**Parameters** (N = 14640 sequences/sample)		
	Chao1[a]	**Observed species**[b]	**Shannon**[c]
A75H48	1239.3	551	5.29
A50H48	882.5	480	5.16
A25H48	1055.2	485	4.39
A25H72	948.9	496	4.88
A25H24	890.1	439	4.80
A25H12	732.0	427	4.31

a. Chao1 index is usually used to estimate the total number of species within a community;
b. Observed species refers to the total count of unique OTUs in the sample;
c. Shannon index is a value of evenness that combines species richness and abundance to describe how different species are numerically distributed within a community.

Table S7 Total abundances of top 5 identified genera under each operating condition

Stages	Count of total genera	Count of the 5% most abundant genera	Total abundance of the 5% most abundant genera
A75H48	177	9	79%
A50H48	151	8	75%
A25H48	181	9	90%
A25H72	132	7	70%
A25H24	98	5	73%
A25H12	122	6	83%

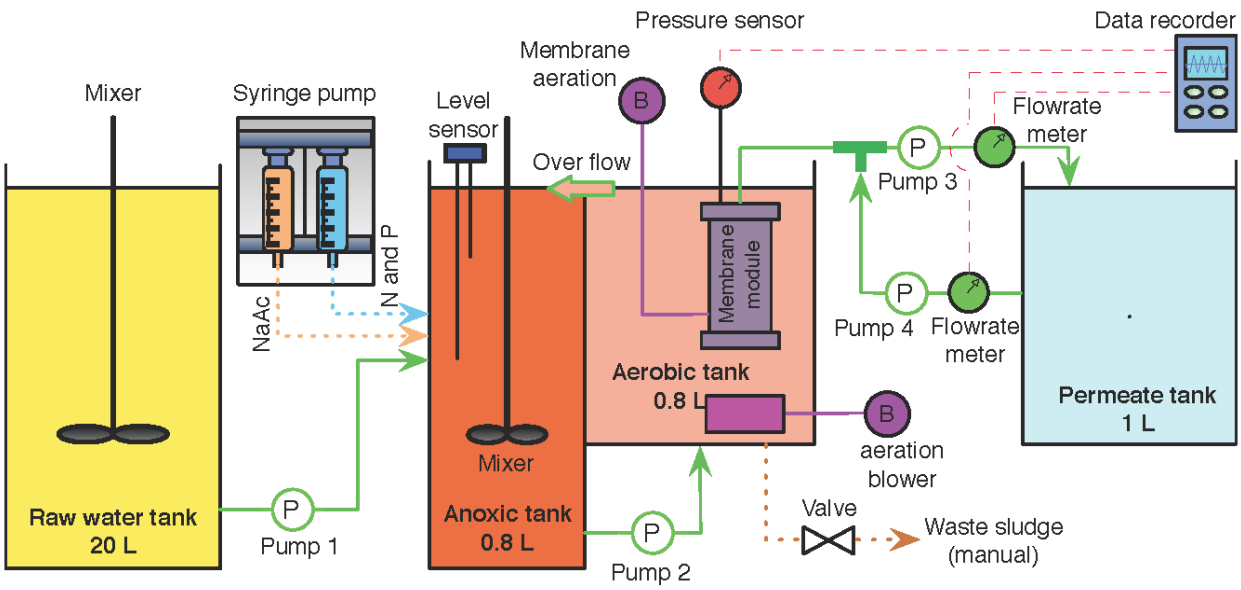

Figure S1 Schematic diagram of the MBR configuration (Adapted from Xue et al. (2016)).

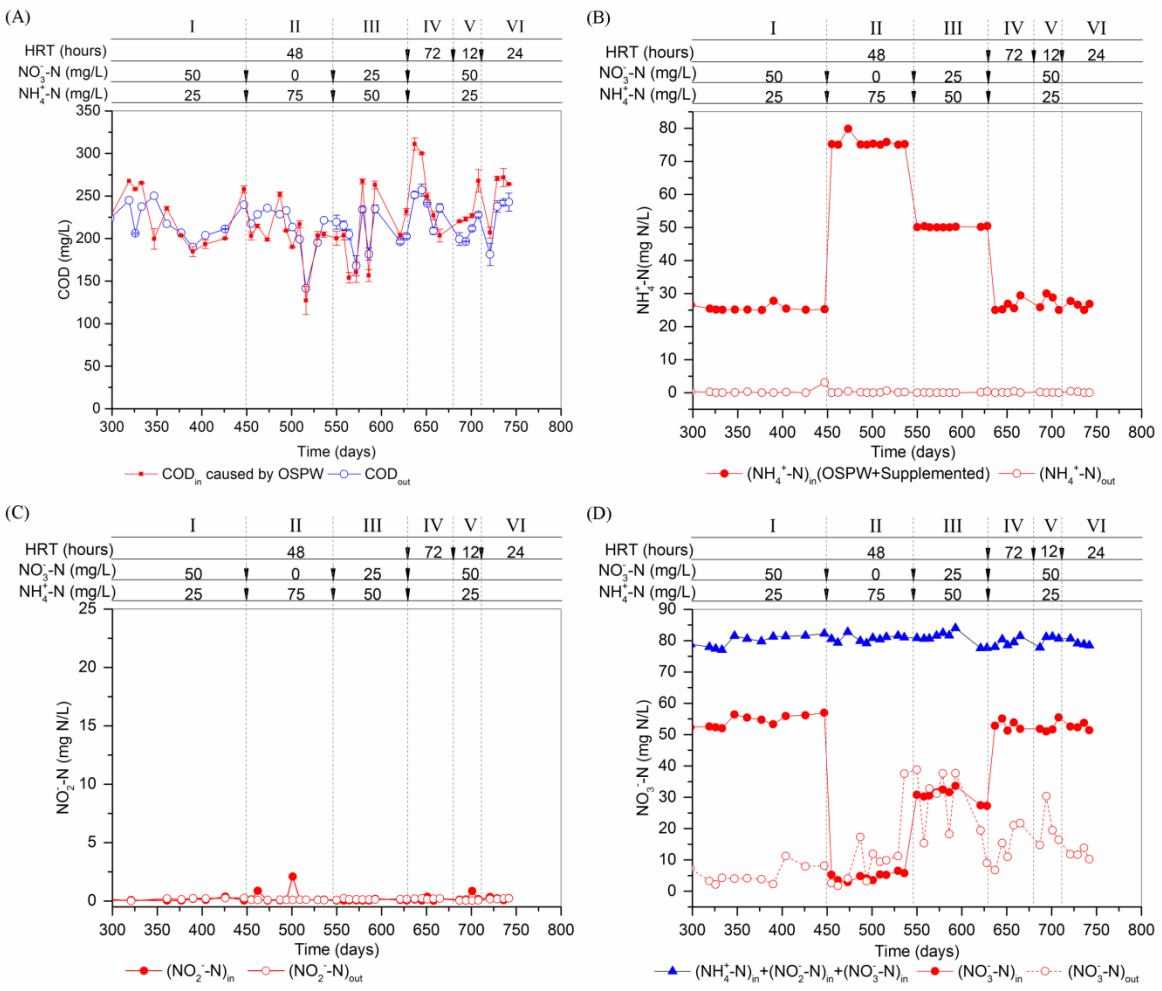

Figure S2 Water chemistry parameters over time: (A) COD; (B) NH_4-N; (C) NO_2-N; and (D) NO_3-N. (Note: Since ammonium and nitrate were supplemented directly into the MBR anoxic tank with pumped syringes, influent NH_4-N in chart B is a sum of OSPW indigenous NH_4-N and supplemented NH_4-N. Similarly, influent NO_3-N concentration in chart D is a sum of OSPW indigenous NO_3-N and supplemented NO_3-N.)

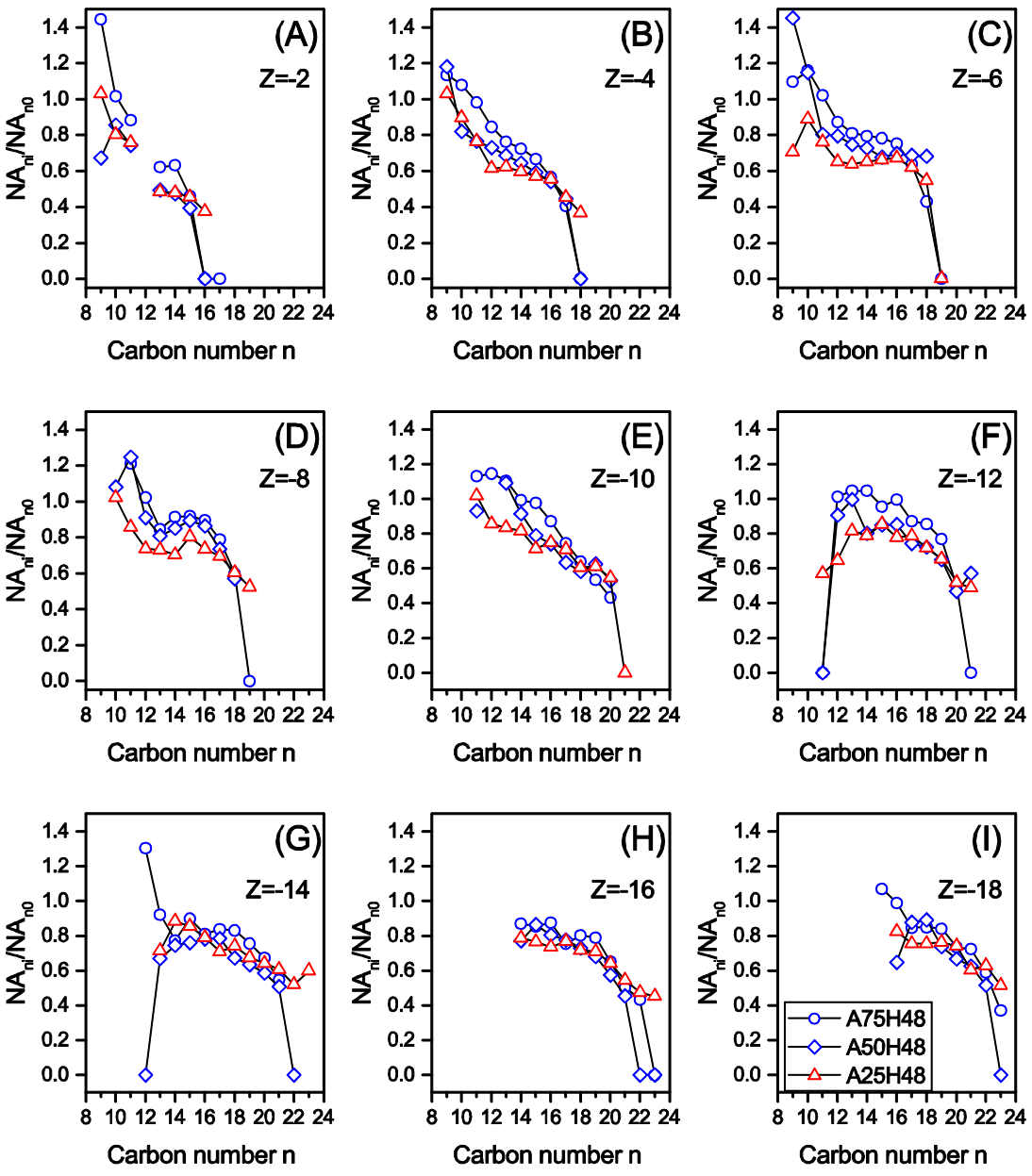

Figure S3 The relative remaining abundance of NAs with different carbon numbers (NA_{ni}/NA_{n0}) within each Z series of the permeate samples under different supplemented NH_4-N concentrations

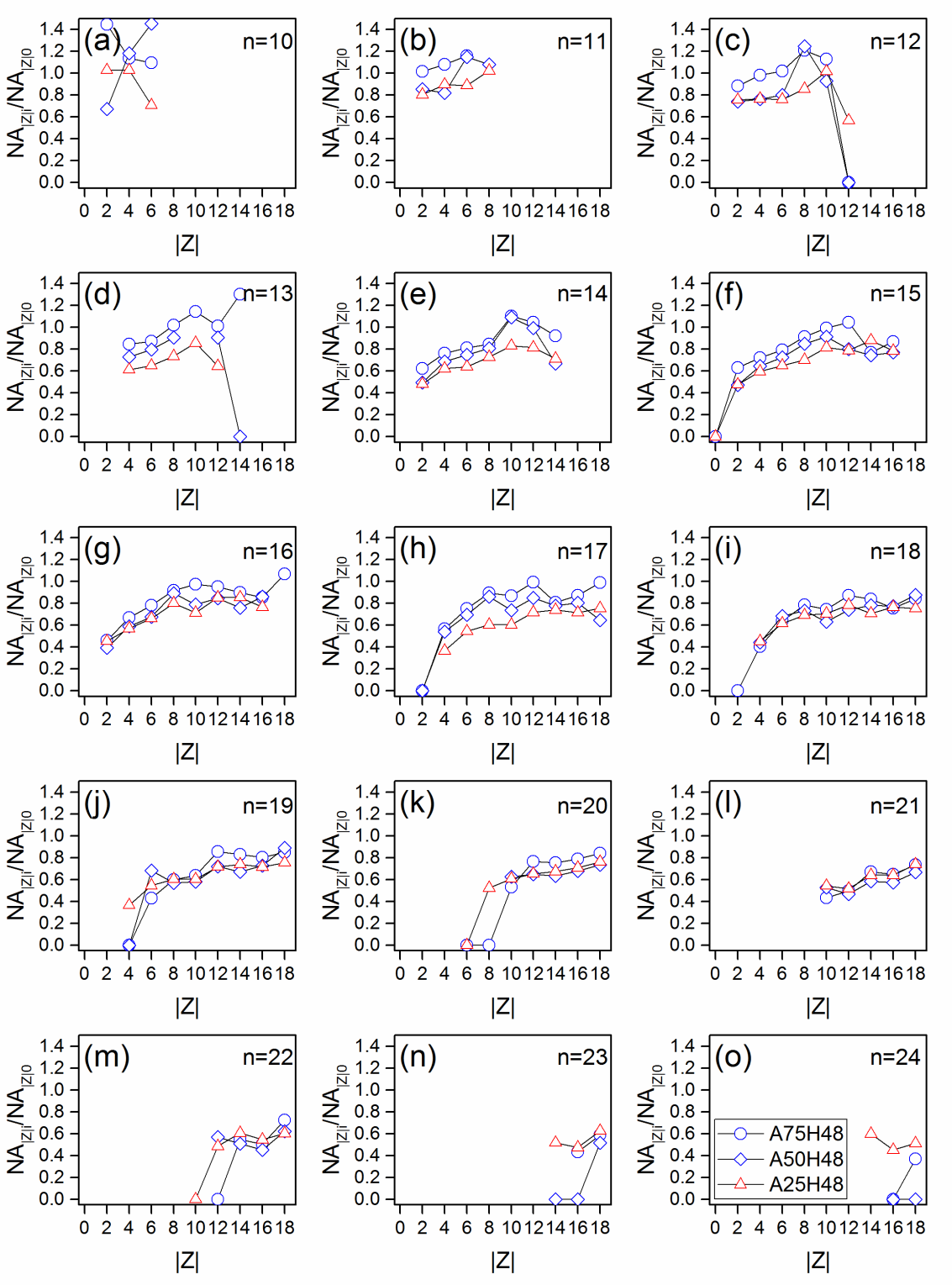

Figure S4 The relative remaining abundance of NAs with different hydrogen deficiencies (NA_{Zi}/NA_{Z0}) within each *n* series of the permeate samples under different supplemented NH_4-N concentrations

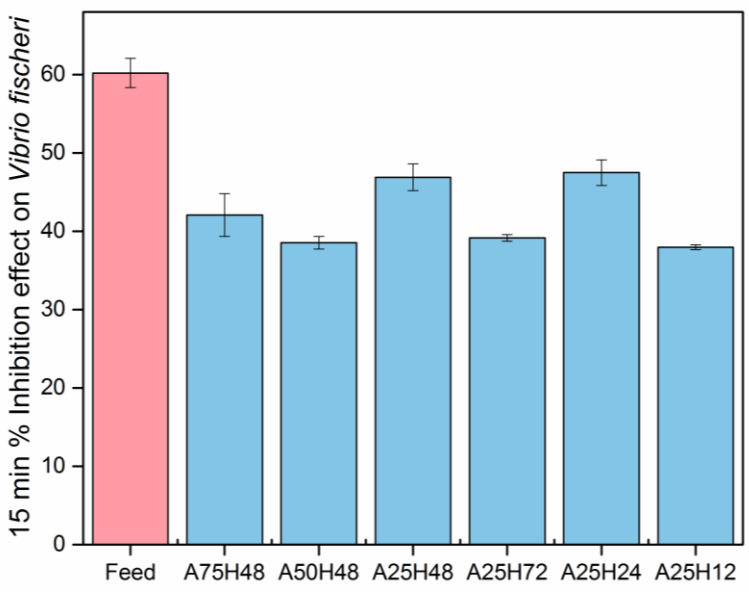

Figure S5 Microtox toxicity of the MBR feed and permeates based on the 81.9% screening test

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