Supplementary Information:

Membrane fouling characteristics of membrane bioreactor (MBR) under salinity shock: Extracellular Polymeric Substances (EPS) and optimization of operating parameters

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☆ EPS extraction method

EPS can be divided into soluble EPS (S-EPS, or SMP) and adhesive EPS (B-EPS), and the latter can be divided into loosely attached EPS (LB-EPS) and tightly attached EPS (TB-EPS). Using heating extraction method, the steps are as follows:

At the end of each experimental stage, 10 mL of sludge adhered to the membrane was enriched, added with 0.01 M NaCl buffer solution and centrifuged at 4 ℃ 550 r/min for 15 min, and the supernatant was filtered through 0.45 μm organic microfiltration Membrane to obtain the SMP solution; add 0.01 M NaCl buffer to the remaining sludge to resuspend to the original volume, sonicate for 2 min, centrifuge at 4 ℃ 9000 r/min for 20 min, the supernatant is over 0.45 μm organic LB-EPS is obtained by the microporous filter membrane; then add 0.01 M NaCl to the remaining sludge to resuspend the weight to the original volume, heat in a water bath at 70 ℃ for 40 min, and cool to room temperature at 4 ℃ at 20000 r/min Centrifuge for 20 min, and pass the supernatant through a 0.45 μm organic microporous membrane to obtain TB-EPS. At the same time, the concentration of sludge VSS was measured.

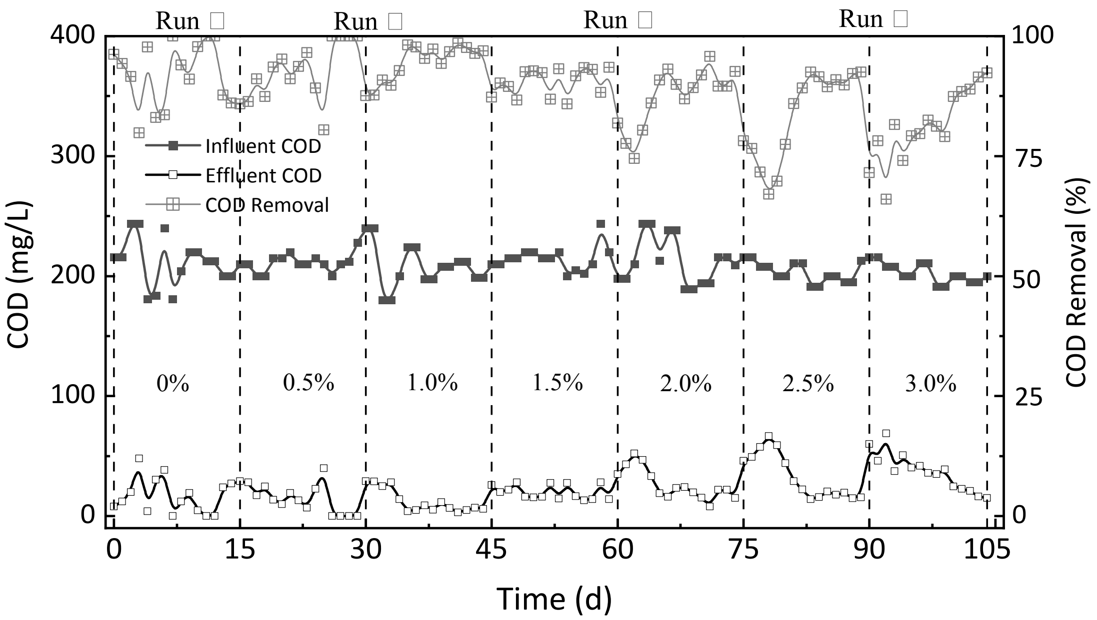
☆ Optimize the design of experimental operating parameters

The intermittent filtration cycle of the fixed reactor was continuous water output for 4 min, stop pumping for 1 min. When HRT was used as a variable, the fixed C/N was 3: 1, DO was 3.5 - 4.5 mg/L; When C/N was variable, fixed HRT was 24.0 h, DO was 2.0 - 3.0 mg/L; when DO was variable, fixed HRT was 24.0 h, C/N was 5:1. When the system runs stably under the conditions of various operating parameters, start membrane fouling rate determination experiment, and finally determine the content of extracellular secretions.

Table S1 Optimized test operation parameter design

Operating parameters	Ⅰ	Ⅱ	Ⅲ
HRT /(h) (C/N = 3:1, DO = 3.5 ~ 4.5 mg/L)	12.0	16.0	24.0
C/N (HRT = 24.0 h, DO = 2.0 ~ 3.0 mg/L)	3:1	5:1	8:1
DO /(mg/L) (HRT = 24.0 h, C/N = 5:1)	0.5 ~ 1.5	2.0 ~ 3.0	3.5 ~ 4.5

☆ **MBR system operating efficiency during salinity acclimation**



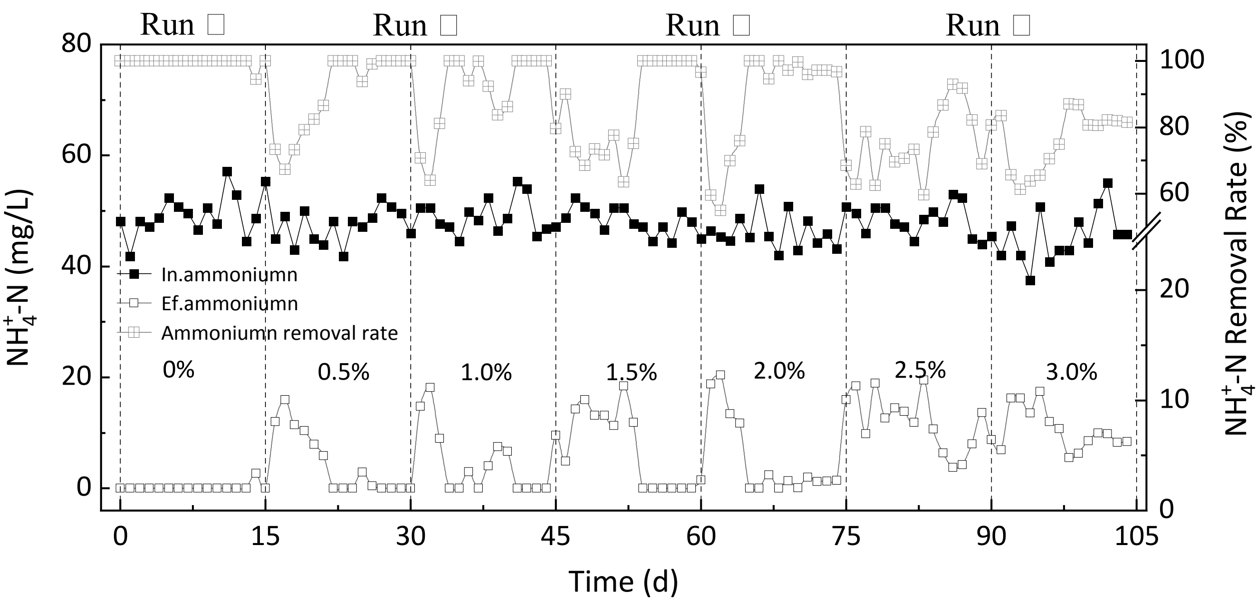


Fig.S1 Evolution of COD and NH_4^+-N in the reactor

☆ Correlation analysis between EPS and TMP

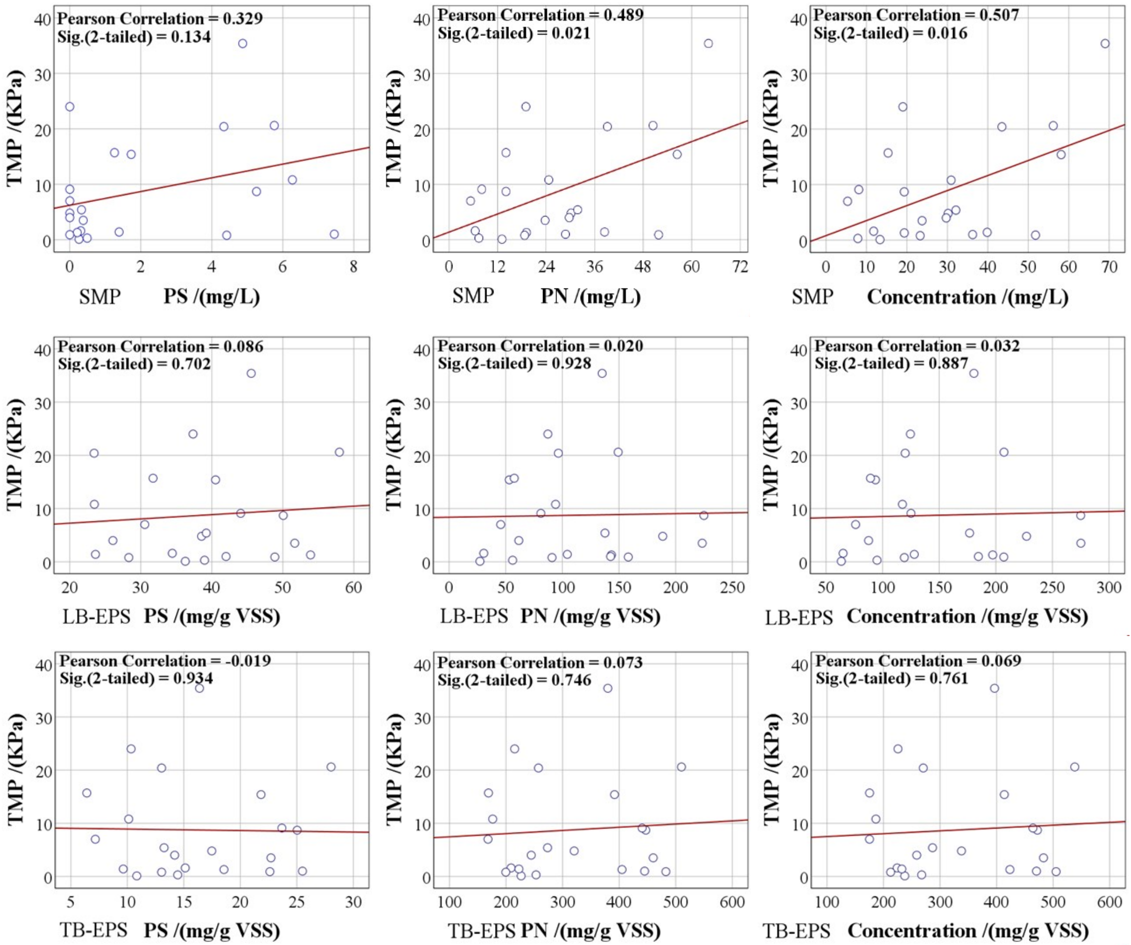


Fig.S2 EPS and TMP correlation analysis

Table S2 Surface Energy Spectrum Analysis of MBR Membrane under Different Salinity

Weight (%) / Salinity (%)	Elements												
	C	O	F	P	Na	K	Ca	Fe	Al	Si	S	Zn	Mg
New Membrane	66.76	16.09	16.27	0.88	-	-	-	-	-	-	-		
0	44.91	37.27	-	3.96	0	0.67	1.15	3.26	3.53	3.75	0.37	0.69	0.43
1.0	55.17	28.2	-	2.9	1.7	0.97	0.54	3.37	2.5	3.26	1.39	-	-
2.0	55.48	19.92	10.25	0.76	1.97	0.59	0.61	3.41	2.17	3.15	0.31	0.86	0.52
3.0	57.43	24.17	7.87	2.61	1.04	0.39	0.64	2.85	0.96	1.73	0.31	-	-

Table S3 Fluorescence spectral parameters of SMP and EPS under different salinity

Samples	Salinity (%)	Peak A (A_1) (nm)	Intensity	Peak B (nm)	Intensity	Peak C (nm)	Intensity	Peak D (A_2) (nm)	Intensity
SMP	0	Ex:270-280 Em:300-310	25.74	Ex:310-360 Em:400-450	7.18	Ex:220-230 Em:300-310	2.35	Ex:270-280 Em:410-450	5.56
	1.0		42.16		8.8		5.00		/
	2.0		25.11		9.87		1.66		4.70
	3.0		21.73		9.34		/		6.57
LB-EPS	0		13.10		2.45		1.08		2.06
	1.0		20.38		/		/		/
	2.0		22.86		3.56		1.57		2.35
	3.0		22.65		9.74		/		7.82
TB-EPS	0		129.01		40.20		16.60	(285/355)	207.50
	1.0		134.72		42.67		17.53		173.15
	2.0		22.78		3.54		1.57		/
	3.0		/		42.46		11.43		145.35
Composition		High excitation light tyrosine protein [1]		Humic acid-like organics [2]		Low excitation light tyrosine protein [1,2]		Fulvic acid-like organics (Tryptophan-like protein [3,4])	

☆ Optimization test response surface analysis

Table S4 Response surface test factors and level values

Factors	Code	Factor level		
		-1	0	1
HRT /(h)	A	12	16	24
C/N	B	3	5	8
DO /(mg/L)	C	1	2.5	4

Table S5 Response surface test results

Number	Actual value of independent variable level			Membrane fouling rate $\lvert K_J \rvert$	Ammonia removal efficiencyR.E / (%)
	A	B	C		
1	12	5	4	1.011E+13	39.4
2	16	5	2.5	4.175E+12	83.4
3	16	5	2.5	3.892E+12	83.2
4	24	3	2.5	7.091E+12	84.8
5	16	5	2.5	4.089E+12	83.2
6	12	5	1	8.839E+12	38.6
7	24	5	4	1.032E+13	85.7
8	12	8	2.5	7.765E+12	40.6
9	16	3	4	4.21E+12	84.2
10	12	3	2.5	4.343E+12	40.3
11	16	5	2.5	4.005E+12	84.5
12	16	8	1	4.672E+12	73.5
13	24	5	1	1.001E+13	72.6
14	16	5	2.5	4.31E+11	83.6
15	16	8	4	4.341E+12	85.6
16	24	8	2.5	1.41E+13	84.8
17	16	3	1	3.65E+12	72.3

Table S6 Analysis of variance of the cubic model of membrane fouling rate $\lvert K_J \rvert$

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-Value**	**P-Value**	
Model	1.601E+26	12	1.334E+25	523.26	< 0.0001	significant
A-HRT	7.226E+23	1	7.226E+23	28.35	0.0060	
B-C/N	3.149E+23	1	3.149E+23	12.35	0.0246	

C-DO	1.359E+22	1	1.359E+22	0.5332	0.5057

Continued

AB	3.217E+24	1	3.217E+24	126.18	0.0004
AC	2.309E+23	1	2.309E+23	9.06	0.0396
BC	1.985E+23	1	1.985E+23	7.79	0.0493
A^2	8.541E+25	1	8.541E+25	3350.50	< 0.0001
B^2	1.823E+24	1	1.823E+24	71.52	0.0011
C^2	6.918E+24	1	6.918E+24	271.37	< 0.0001
A^2B	7.736E+24	1	7.736E+24	303.46	<0.0001
A^2C	2.595E+23	1	2.595E+23	10.18	0.0335
AB^2	5.979E+24	1	5.979E+24	234.24	0.0001
Pure Error	1.020E+23	4	2.549E+22		
Cor Total	1.602E+26	16			

R^2 = 0.9994， $R_{Adjusted}^2$ = 0.9975；Since the regression analysis uses a confidence level of 95%, P <0.01 indicates that the interaction is extremely significant, P <0.05 indicates that the interaction is significant, and P> 0.05 indicates that the interaction is not significant.

Table S7 Variance analysis of the third model of ammonia nitrogen removal efficiency (R.E)

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-Value**	**P-Value**	
Model	5690.72	12	474.23	1624.06	< 0.0001	significant
A-HRT	1411.69	1	1411.69	4834.55	< 0.0001	
B-C/N	1.35	1	1.35	4.64	0.0976	
C-DO	156.80	1	156.80	537.00	< 0.0001	
AB	0.0225	1	0.0225	0.0771	0.7951	
AC	37.82	1	37.82	129.53	0.0003	
BC	0.0100	1	0.0100	0.0342	0.8622	
A^2	2943.57	1	2943.57	10080.73	< 0.0001	
B^2	0.3165	1	0.3165	1.08	0.3566	
C^2	56.85	1	56.85	194.70	0.0002	
A^2B	0.5732	1	0.5732	1.96	0.2338	
A^2C	23.30	1	23.30	79.80	0.0009	
AB^2	8.77	1	8.77	30.04	0.0054	
Pure Error	1.17	4	0.2920			
Cor Total	5691.88	16				

$R^2 = 0.9998$，$R_{Adjusted}^2 = 0.9992$；Since the regression analysis uses a confidence level of 95%, $P < 0.01$ indicates that the interaction is extremely significant, $P < 0.05$ indicates that the interaction is significant, and $P > 0.05$ indicates that the interaction is not significant.

Reference

[1] Peng Zhu Z H, Huimin Li. PARAFAC Method Used for Analysis of Three-Dimensional Fluorescence Spectra of DOM in Taihu Lake[J]. Spectroscopy and Spectral Analysis, 2013, 33(06): 1619-1625.

[2] Gao P, Guo L, Sun J, et al. Enhancing the hydrolysis of saline waste sludge with thermophilic bacteria pretreatment: New insights through the evolution of extracellular polymeric substances and dissolved organic matters transformation[J]. Science of the Total Environment, 2019, 670.

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[4] Shao Y, Zhang H, Buchanan I, et al. Comparison of extracellular polymeric substance (EPS) in nitrification and nitritation bioreactors[J]. International Biodeterioration & Biodegradation, 2019, 143: 104713.