Supplementary Information

Evaluation of activated sludge properties' changes in industrial-wastewater pre-treatment: role of residual aluminum hydrolyzed species with different polymerization degree

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Totally 13 pages containing 4 Texts, 6 Tables and 5 Figures.

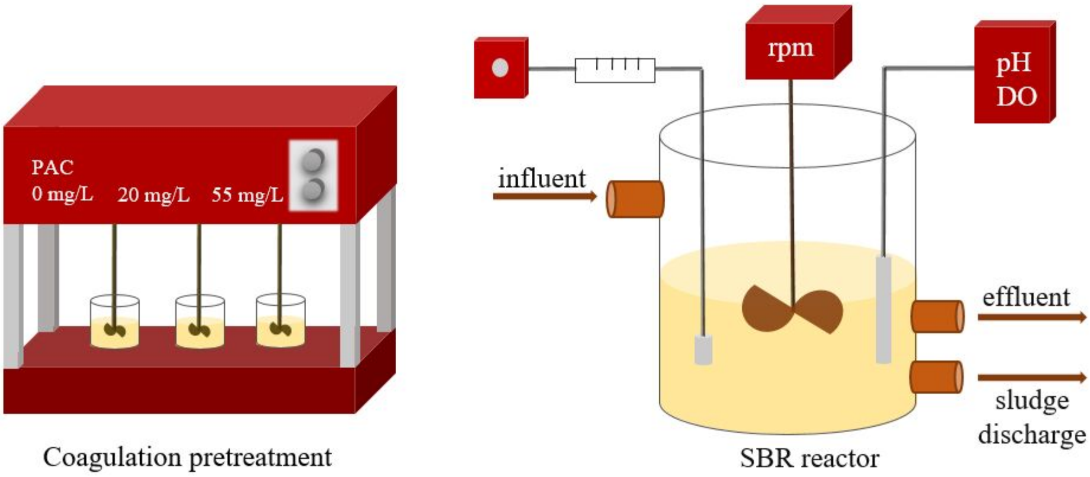

Fig. S1. Reactor device diagram

Text S1. Supplement to the detection method

Usage of ESI-TOF-MS

The sample was injected into the spectrometer at a flow rate of 10 μL/min, and the basic control parameters of the instrument were as follows: the capillary voltage was 3500 V, sample cone voltage was 90 V, extraction cone voltage was 5 V, source temperature was 120 ℃, cone gas (N_2) flow rate was 300 L/h, and the mass range was 50-1000.

Extraction method of EPS

Take a certain volume of sludge mixture, stand for precipitation for 30 min, discard the supernatant, concentrate the sludge at 4 ℃, centrifugation rate of 2100 g for 10 min, then homogenize the sludge and add 10 % NaCl solution with the same volume. The suspension was heat treated at 80 ℃ for 1h to extract EPS. After cooling to room temperature, the sludge was separated by centrifugation at 2100 g. The supernatant was further centrifuged at 12000 g to remove small suspended components, and the resulting supernatant was to be tested.

Text S2. **Aluminum salt form distribution detection**

The general formula of hydroxyl polyaluminum ion, $[AlxOy(OH)z(H_2O)m]^{(3x-2y-z)+}$, was used to infer the aluminum species corresponding to the signals with relative intensity greater than 5 % in the ESI-TOF-MS spectrum; the signals with relative intensity less than 5 % have uncertainty, and in this experiment, they would not be studied. The estimated results of the aluminum salt form are shown in Table S4.

According to Fig. S2, the main form of aluminum in PAC solution is Al_{13}, whose peaks were m/z 337 (100 %), m/z 255 (60.66 %), m/z 213 (27.76 %), m/z 328 (24.44 %), m/z 237 (21.60 %), m/z 409 (11.76 %), m/z 286 (8.89 %), m/z 80 (8.85 %), m/z 364 (6.35 %), m/z 418 (5.96 %). There are also other forms of aluminum such as Al_1 (m/z 79, 97), Al_2 (m/z 157, 193), Al_3 (m/z 181, 199, 217), Al_4 (m/z 105, 123, 223). As for R2 and R3, because after coagulation occurs, part of aluminum reacted with pollutants and produced $Al(OH)_3$ precipitates and negatively charged substances, but ESI-TOF-MS could only detect positively charged aluminum substances, so in (b), (c), The types and signal intensity of the detectable aluminum form have decreased significantly. Analyzing the mass spectrum, it could be obtained that, in the supernatant of R2, the signal peaks with higher intensity are as follows (from large to small): Al_{13} (m/z 213, 100 %), Al_5 (m/z 247, 22.45 %), Al_4 (m/z 223, 22.15 %), Al_2 (m/z 193, 21.60 %), Al_1 (m/z 79, 19.75 %), and in the supernatant of R3, the signal peaks with higher intensity are as follows (from large to small): Al_{13} (m/z 355, 100%, m/z 213, 79%), $Al(OH)_2{}^+$ (m/z 61, 76.10%), $[Al(OH)_2(H_2O)]^+$ (m/z 79, 43.95%).

According to the results of ESI-TOF-MS characterization, aluminum forms were divided into five categories: monomer and dimer aluminum (Al_1-Al_2), low-polymer aluminum (Al_3-Al_5), medium-polymer aluminum (Al_6-Al_{10}), High polymer aluminum (Al_{11}-Al_{20}) and Alu (the unmeasured part, $Al(OH)_3$ or $Al(OH)_4^-$). Take the sum of the peak intensities of all aluminum forms in the original PAC solution (Fig. S2.a.) as the total number (Alt) of aluminum in the experimental groups, the value of Alu was equal to Alt minus [(Al_1-Al_2) + (Al_3-Al_5) + (Al_6-Al_{10}) + (Al_{11}-Al_{20})]. Thus the morphology distribution map of aluminum in the three solutions could be obtained.

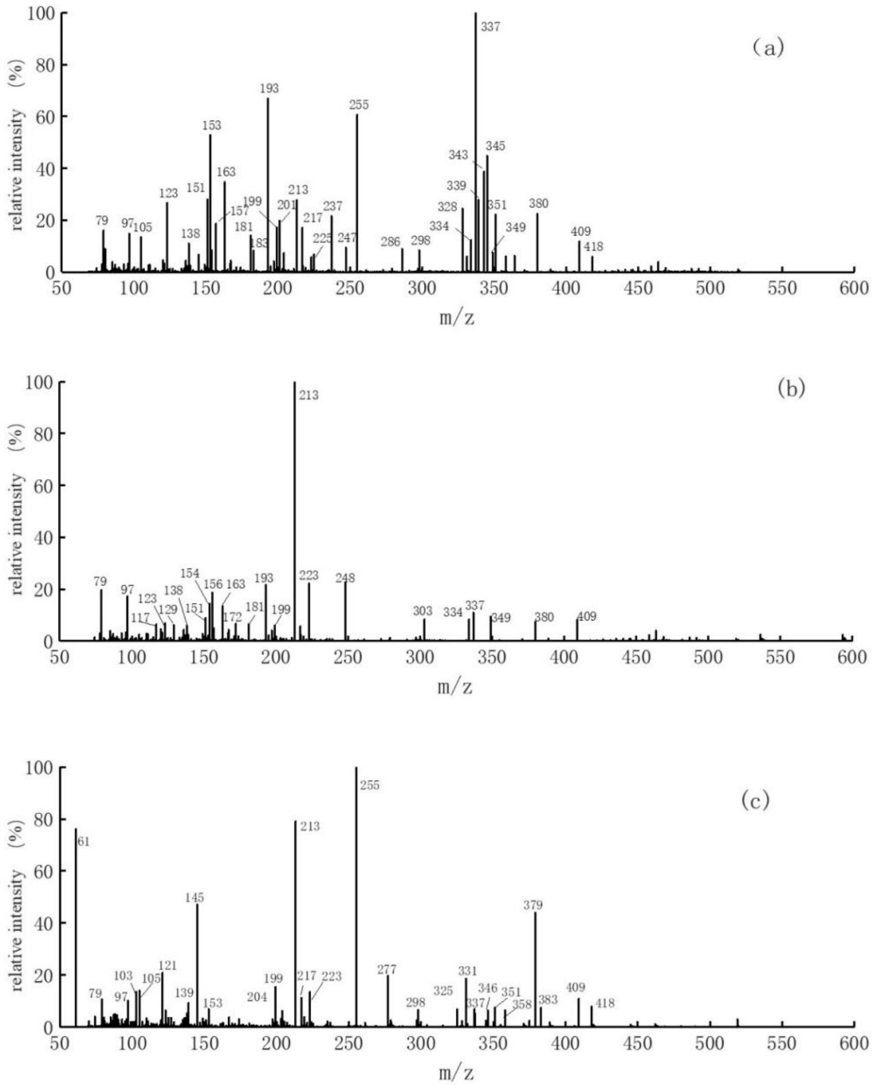

Fig. S2. Mass spectra of three groups of solutions (a) reference solution; (b) supernatant of R1; (c) supernatant of R2

Text S3. **Sludge concentration**

The sludge concentration changes of R1, R2 and R3 were shown in Fig. S3. The changes of MLSS, MLVSS and MLVSS/MLSS of the blank control group R1 were not significant during the entire operation phase. The three indicators of R2 had a slow upward tendency, and gradually stabilized due to regular sludge discharge in the later period. The sludge concentration of R3 was also generally on the rise, with the most striking change. Because of the excessive residual aluminum salt concentration, a large amount of positively charged polymeric aluminum after hydrolysis would be electrically neutralized or attached through adsorption bridging with negatively charged sludge. Then the colloids would get together and fulfill the aggregation after destabilization. As a result, the mass of activated sludge increased rapidly. When the addition of aluminum salt stopped, the sludge volume gradually returned to normal thanks to the residual sludge discharge of the system at regular intervals.

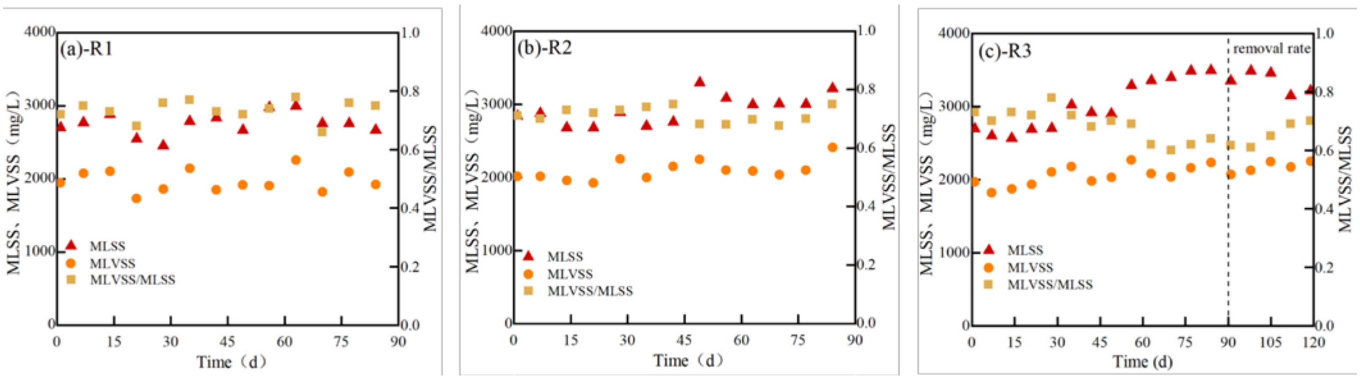

Fig. S3. Change of sludge concentration in the reactor

Text S4.

According to the study by Chen et al.[1], the fluorescence spectrum of 3D-EEM could be divided into five regions, corresponding to protein tyrosine and protein Tryptophan-like, soluble microbial by-products, fulvic acids and humic acids. Fig. S4 showed the three-dimensional fluorescence spectrum of the sample sludge EPS. Table S6 summarized the 3D-EEM fluorescence peak positions and fluorescence intensity of eight EPS samples. There were three main peaks in the 3D-EEM diagram. Peak A and Peak C were located in the region Ex / Em = 250~380 nm / 380~540 nm, which belonged to humic acids. Peak B was located in the region Ex/Em = 250~335 nm / Within 280~380 nm, it belonged to the category of soluble microbial by-products. The fluorescence spectra of b, c, d of the samples from the three reactors (run for 30 days) were similar. Compared with inoculated sludge (a), the peak value of peak A in R1 (b) decreased significantly, and there was no significant change in peak A of R2 (c) and R3 (d), while the intensity of peak B of the three samples increased remarkably. On the 90th day, peak A in the three systems all redshifted, peak B disappeared, and a new peak C appeared. The fluorescence intensity of humic acid substances was the strongest in all samples, indicating the highest protein content in EPS[2]. Compared with R1 (e) and R2 (f), the peak value of peak A in R3 (g) increased sharply, indicating that the protein content of EPS secreted by R3 sludge increased significantly on the 90th day. This test result was consistent with the above. In the h chart (R3, 120th day), the peak value of peak A dropped significantly, and peak B appeared again with a higher intensity, indicating that after the aluminum salt addition was stopped, the structure of sludge EPS could be restored to a certain extent to the state of the early stage of operation.

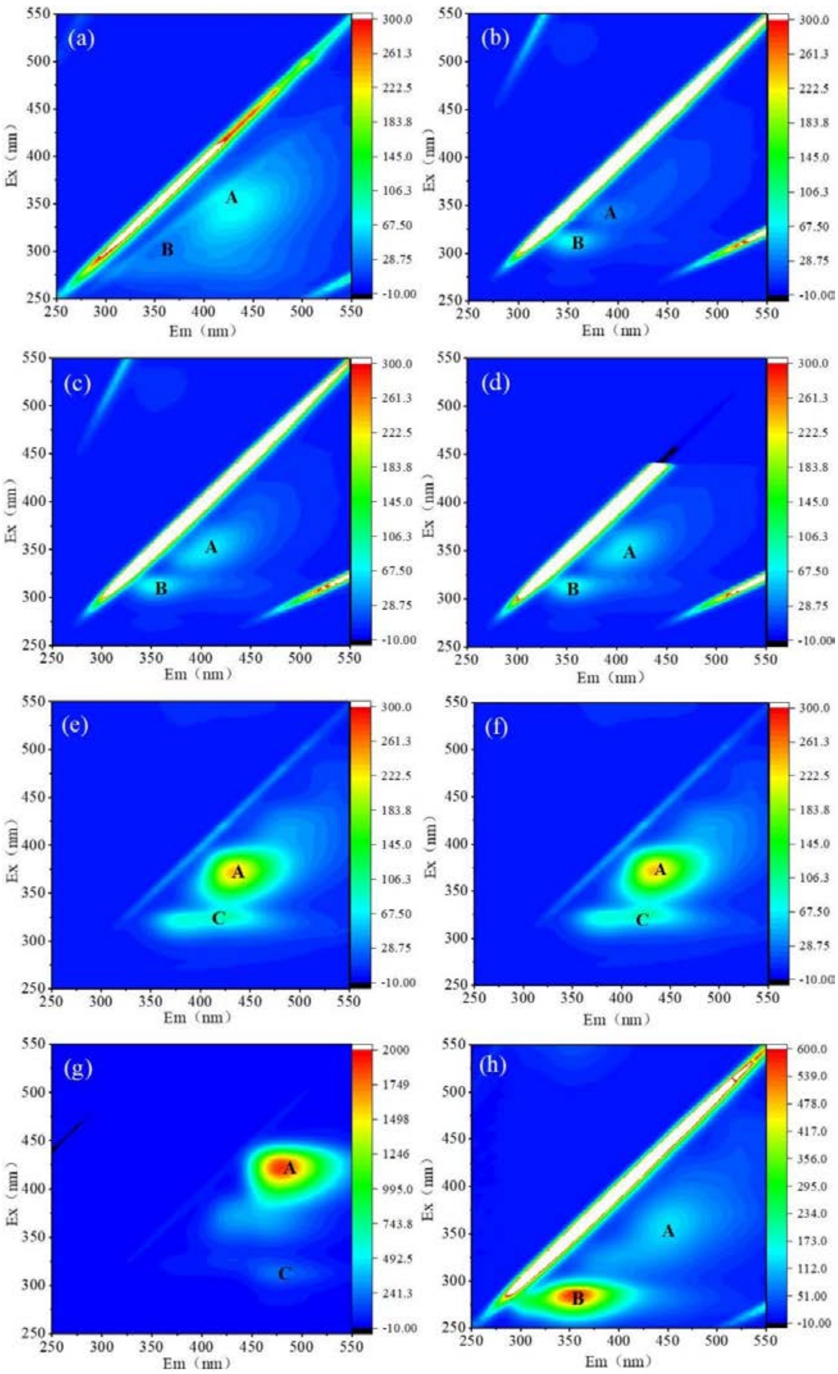

Fig. S4. The three-dimensional fluorescence spectrum of sludge EPS, a: inoculated sludge EPS; b-d: R1, R2, R3 sludge samples on the 30th day; e-g: R1, R2, R3 sludge samples on the 90th day; h: R3 120th day sludge sample

Text S5.

The stretching vibrations at 3422 cm^{-1}, 3447 cm^{-1}, and 3418 cm^{-1} represented hydrogen bond O-H; near 2965 cm^{-1} was produced by the C-H stretching vibration of polysaccharide molecules and alkane organic matter; near 1420 cm^{-1} was produced by C=O symmetrical stretching of

-COO- group; 1651 cm^{-1}, 1647 cm^{-1}, and 1638 cm^{-1} represented C=O symmetrical protein stretching vibration, belonging to protein amide I band (1700-1600 cm^{-1}); Protein amide II band (1600-1500 cm^{-1}) is produced by N-H bending and C-N stretching vibration; Amide III band (1300-1200 cm^{-1}) was attributed to C-N extension; 1105 cm^{-1}, 1088 cm^{-1} may be related to the stretching vibration of polysaccharide COC, C-OH and C-O; 1047 cm^{-1} represented the production of phosphorylated protein and alcohols C-OH vibration; 874 cm^{-1} was related to O-P-O asymmetric stretching vibration in nucleic acid; 500-800 cm^{-1} belonged to the fingerprint area and was related to the vibration of aromatic amino acids and nucleotide rings.

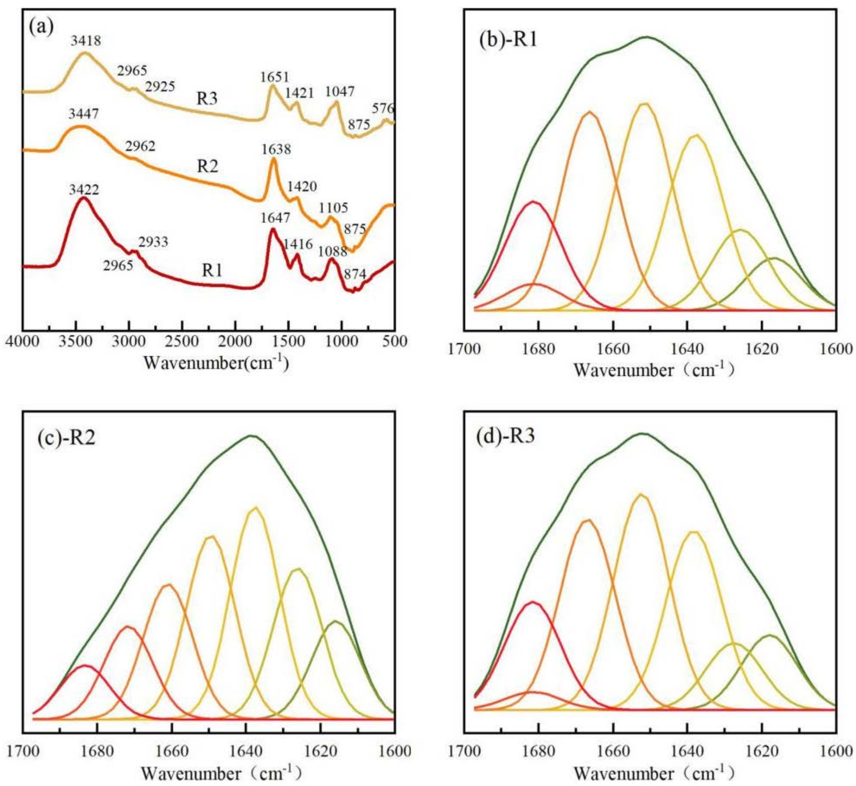

Fig. S5 (a) FTIR spectrum of sludge EPS; (b) R1 (c) R2 (d) R3 sludge EPS amide I area curve fitting diagram

Table S1. influent water quality of experimental group

unite：mg/L

	SS	COD	NH_4^+-N	TN	TP	Residual aluminum
R2 Supernatant after coagulation pretreatment	≤6	380±10	32±3.5	35±3.5	≤0.5	0.0144
R3 Supernatant after coagulation pretreatment	≤10	430±20	34.5±4	37.5±4	≤0.5	0.1132

Table S2. composition of artificial water distribution

unite：mg/L

compound medicine	NaAc	NH_4Cl	KH_2PO_4	$NaHCO_3$	$MgSO_4$	$CaCl_2$	microelement
Dosage of control group	580	150	1.5	10	10	5	0.5 mL/L
Dosage of experimental group	1200	168	16	10	10	5	0.5 mL/L

Table S3. trace element dosage

medicament	dosage(g/L)	medicament	dosage(g/L)
$FeCl_3 \cdot 6H_2O$	1.50	$ZnSO_4 \cdot 7H_2O$	0.12
KI	0.18	$Na_2MoO_4 \cdot 2H_2O$	0.06
H_3BO_3	0.15	$CuSO_4 \cdot 5H_2O$	0.03
$MnCl_2 \cdot 4H_2O$	0.12	EDTA	10
$CoCl_2 \cdot 6H_2O$	0.15		

Table S4. Speculation results of aluminum salt speciation

Kinds	Signal	Substance
Al_1	61、79、97	$Al(OH)_2^+ \cdot n(H_2O)$; n=0-2
Al_2	103、121、139、157、193	$Al_2O_2(OH)^+ \cdot n(H_2O)$; n=0-3,5
Al_3	181、199、217	$Al_3O_4^+ \cdot n(H_2O)$; n=2-4
Al_4	105、123	$Al_4(OH)_9^{3+} \cdot n(H_2O)$; n=3,6
	223、277	$Al_4O_5(OH)^+ \cdot n(H_2O)$; n=1,4
Al_5	247	$Al_5O_7^+$
Al_6	154、163、172	$Al_6O_7(OH)_2^{2+} \cdot n(H_2O)$; n=1-3
Al_7	117、129	$Al_7O_9(OH)_3^{3+} \cdot n(H_2O)$; n=1,3
Al_8	204	$Al_8O_{12}^{2+}$
Al_9	145、151	$Al_9O_{12}^{3+} \cdot n(H_2O)$; n=0,1
	157、163	$Al_9O_{10}(OH)_4^{3+} \cdot n(H_2O)$; n=0,1
Al_{10}	183、201、225	$Al_{10}O_{10}(OH)_7^{3+} \cdot n(H_2O)$; n=0,3,7
Al_{12}	334	$Al_{12}O_{14}(OH)_6^{2+} \cdot (H_2O)$
Al_{13}	213、237	$Al_{13}O_{18}^{3+} \cdot n(H_2O)$; n=0,4
	328、337、346、355、364	$Al_{13}O_{18}(OH)^{2+} \cdot n(H_2O)$; n=0,1,2,3,4
	409、418	$Al_{13}O_8(OH)_{20}^{2+} \cdot n(H_2O)$; n=0,1
Al_{14}	358、349	$Al_{14}O_{20}^{2+} \cdot n(H_2O)$; n=0,1
Al_{15}	379	$Al_{15}O_{21}(OH)^{2+}$
Al_{16}	303	$Al_{16}O_{20}(OH)_5^{3+} \cdot 4(H_2O)$
Al_{18}	331、343	$Al_{18}O_{20}(OH)_{11}^{3+} \cdot n(H_2O)$; n=0,2
Al_{19}	345、351	$Al_{19}O_{22}(OH)_{10}^{3+} \cdot n(H_2O)$; n=0,1
Al_{20}	383	$Al_{20}O_{26}(OH)_5^{3+} \cdot 6(H_2O)$

Table S5. Elemental analysis in sludge under different aluminum salt concentrations

element	R1		R2		R3		R3-recovery period	
	quality （%）	atom （%）	quality （%）	atom （%）	quality （%）	atom （%）	quality （%）	atom （%）
C	62.85	71.67	49.28	58.69	28.87	39.02	46.68	56.15
O	28.09	24.4	42.53	37.44	47.24	47.94	42.79	38.63
Na	0.85	0.51	1.23	0.76	1.8	1.27	1	0.63
Al	2.01	1.03	2.96	1.44	10.08	6.07	4.73	2.53
Si	1.24	0.61	0.46	0.23	2.19	1.27	0.64	0.33
P	1.57	0.7	1.4	0.64	4.72	2.47	2.16	1.01
S	0.84	0.36	0.62	0.27	0.8	0.4	0.5	0.23
Cl	0.11	0.04	0.25	0.1	0.15	0.07	—	—
K	0.11	0.04	0.1	0.04	0.14	0.06	—	—
Ca	0.76	0.26	0.75	0.27	2.24	0.91	0.99	0.36
Ti	0.04	0.01	0.03	0.01	0.12	0.04	0.07	0.02
Fe	1.4	0.35	0.48	0.12	1.65	0.48	0.44	0.11
Ni	0.14	0.03	—	—	—	—	—	—
total	100	100	100	100	100	100	100	100

The EDS analysis data were shown in Table S5. The main components in the sludge were C, O, P and the essential trace elements Al, Fe, Na, K, Ca, Ti, etc. The mass ratio of Al in R3 sludge was 10.08 %, which was significantly higher than R2 (2.96 %) as the result of long-term accumulation of residual aluminum salts in the activated sludge.

Table S6. The position and substance of EPS's three-dimensional fluorescence spectrum peaks

Sample number	Peak A			Peak B			Peak C		
	Ex/Em	Peak value	substance	Ex/Em	Peak value	substance	Ex/Em	Peak value	substance
a	350/430	74		285/315	39				
b	340/388	35		313/351	73				
c	351/403	66		313/353	76	Dissolved			
d	351/407	67	Humic	313/351	79	microbial			Humic
e	374/434	239	Acids			by-produc	327/425	97	Acids
f	374/434	263				ts	327/425	116	
g	421/477	1889					314/477	232	
h	365/450	127		285/355	574				

References

1. Sheng, G.-P. and H.-Q. Yu, *Characterization of extracellular polymeric substances of aerobic and anaerobic sludge using three-dimensional excitation and emission matrix fluorescence spectroscopy.* Water Research, 2006. **40**(6): p. 1233-1239.
2. Iorhemen, O.T., et al., *Aerobic granular sludge membrane bioreactor (AGMBR): Extracellular polymeric substances (EPS) analysis.* Water Research, 2019. **156**: p. 305-314.