Supplementary Information for

Characterization of activated sludge in wastewater treatment process using front-face excitation-emission matrix (FF-EEM) fluorescence spectroscopy

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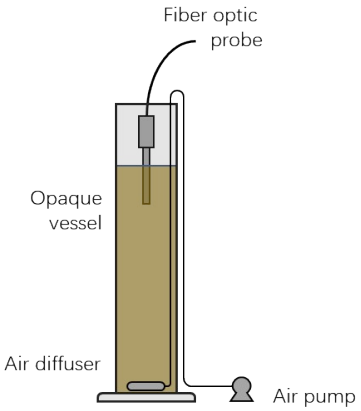

Figure S1 Schematic diagram of fiber optic FF-EEM measurement for activated sludge suspended solid.

The fiber optic probe was vertically inserted into the activated sludge suspended solid. Activated sludge was loaded in an opaque vessel with a working volume of 1 L, to avoid wall effects. To keep the activated sludge suspending, aeration was supplied.

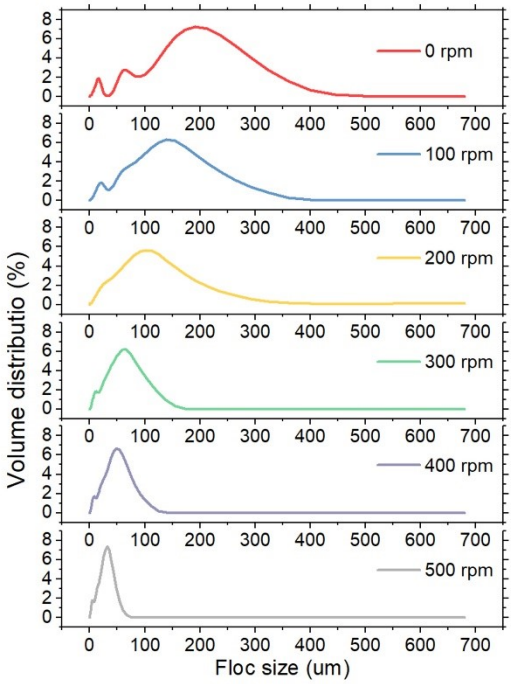

Figure S2 Floc sizes at different stirring speeds.

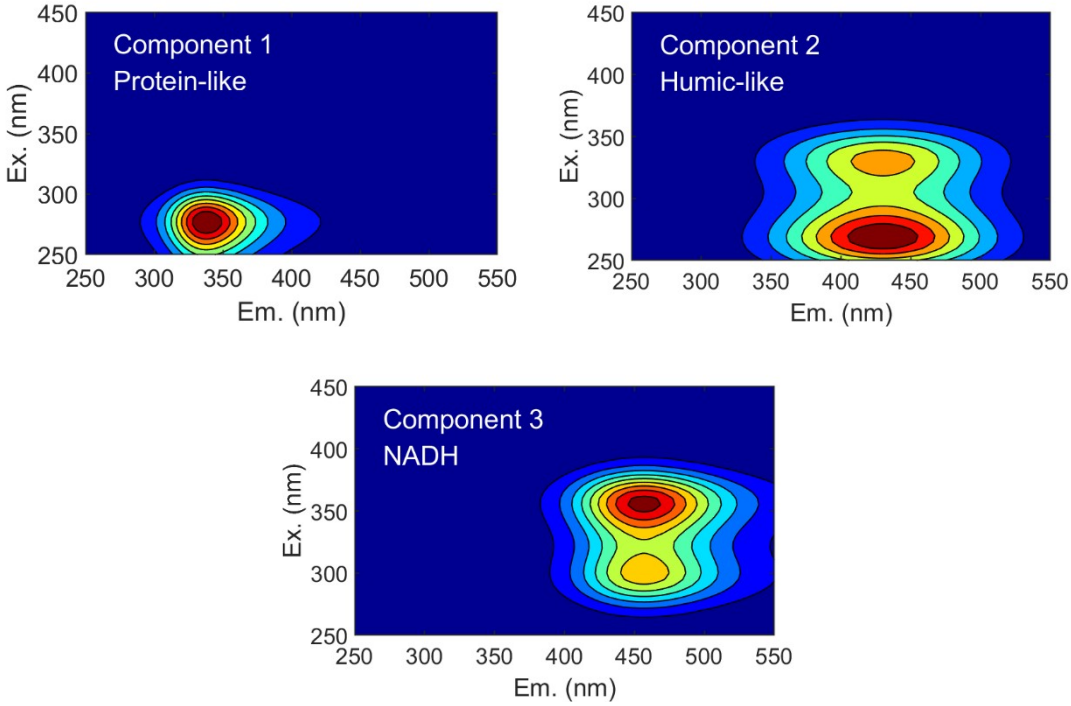

Figure S3 PARAFAC components decomposed from EEMs acquired with cuvette FF-EEM (the suspended activated sludge samples)

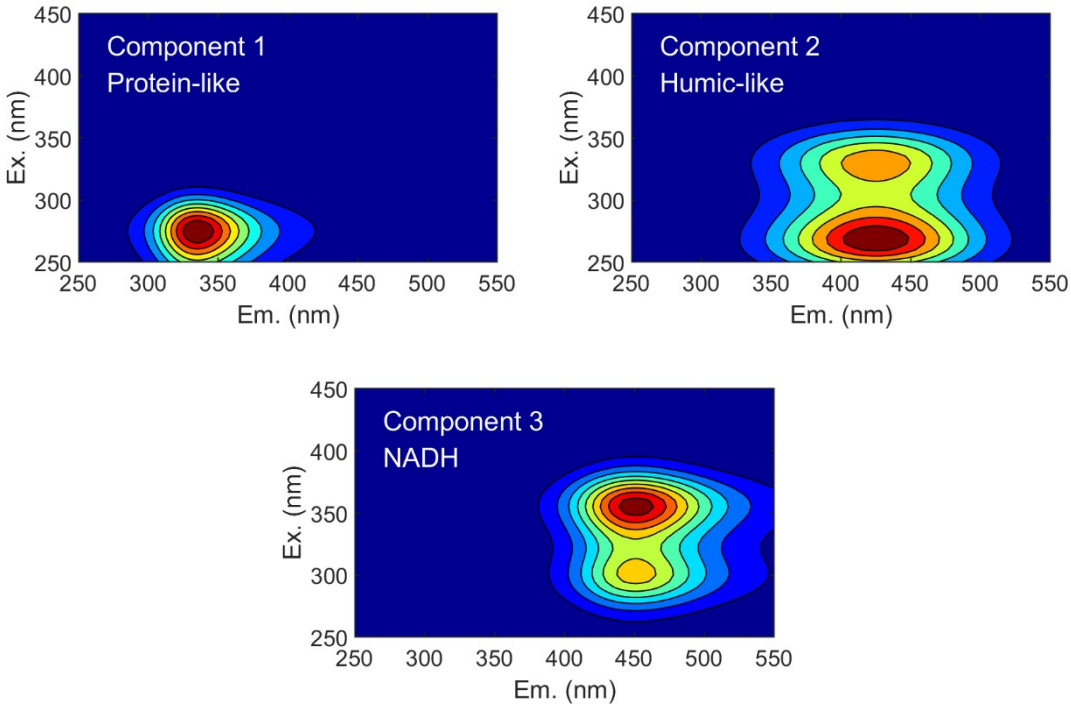

Figure S4 PARAFAC components decomposed from EEMs acquired with Optic fiber FF-EEM (the suspended activated sludge samples)

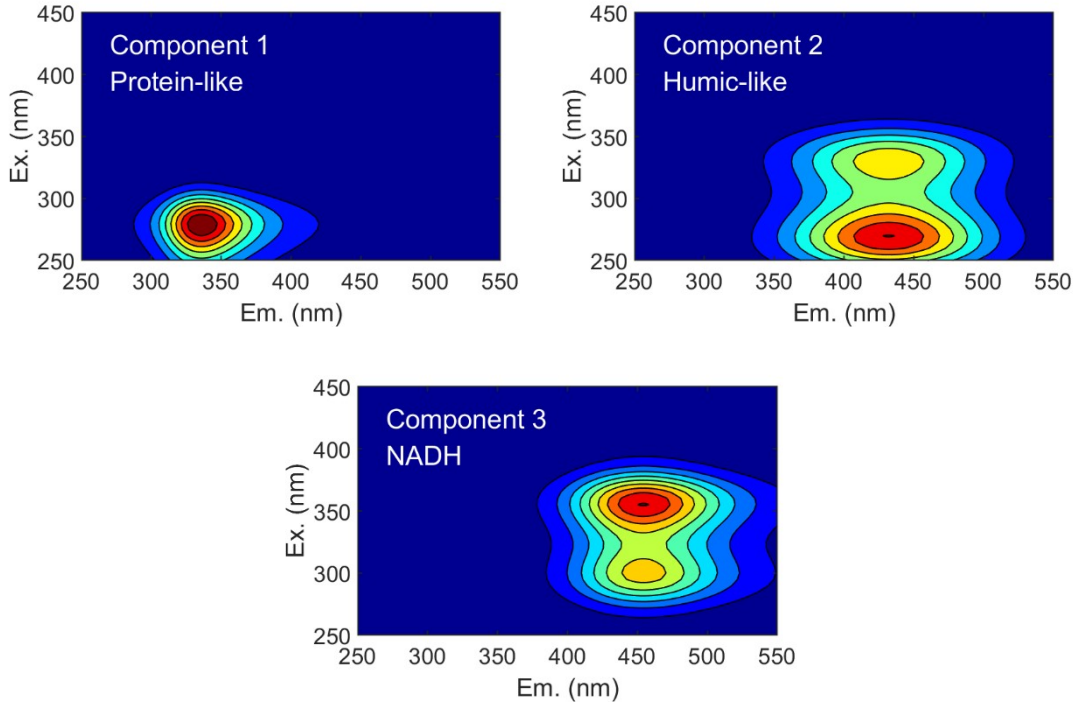

Figure S5 PARAFAC components decomposed from EEMs acquired with conventional right-angle EEM (the SMP, EPS, and IPS samples)

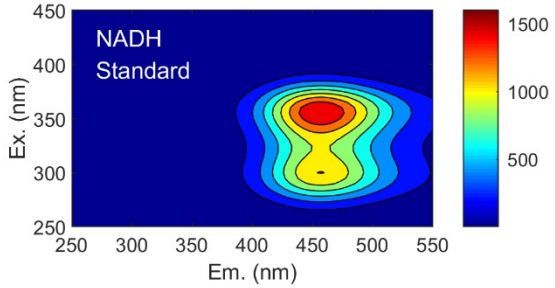

Figure S6 The EEM of NADH standard solution (5 mg/L) obtained with cuvette right-angle EEM

A commercially available disodium slat of NADH was purchased (N106933, Aladin, China), and dissolved in pure water at a concentration of 5 mg/L. It was subjected to the conventional cuvette right-angle EEM measurement.

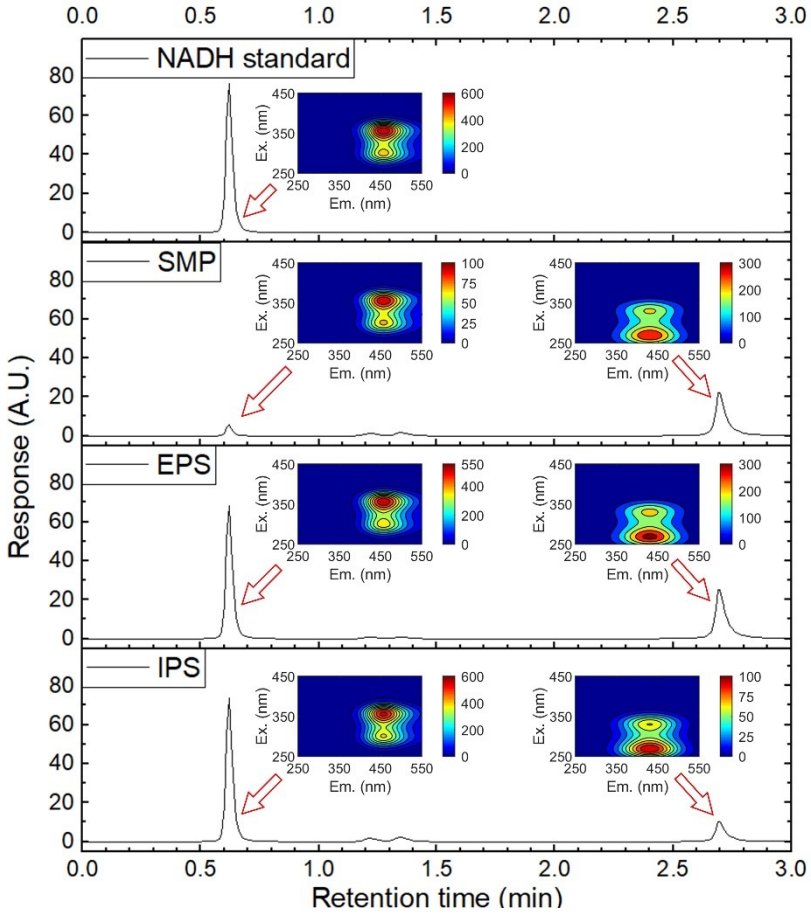

Figure S7 The liquid chromatography of NADH standard (5 mg/L), SMP, EPS, and IPS. 30% methanol and 70% H_2O were used as eluent. An Eclipse XDB-C18 column (4.6 mm in diameter, 150 mm in length, and particle size was 5 mm) was used. The injection volume was 10 μL, and the flow rate was 0.3 mL/min. A fluorescence detector was used, and the Ex and the Em wavelengths were set to be 350 nm and 450 nm respectively. The 1st-minute eluent and the 3rd-munite eluent of each sample were collected and subjected to EEM measurement, and the EEM contour plots were showed on the chromatography.

To further confirm the component was NADH, a liquid chromatography (LC) experiment was conducted. Due to the activated sludge sample could not be subjected

to LC measurement, only liquid solution samples (i.e NADH standard solution, SMP, EPS, and IPS) were examined. LC with a fluorescence detector was used, and the Ex and Em wavelengths of the detector were set to be the same as the fluorescence peak of NADH standard (Ex/Em = 350/450 nm). As shown in Fig. R2, the SMP, EPS, and IPS samples showed the same peak at around 0.62 min, which possessed the same retention time as the NADH standard (at 0.62 min). The LC eluent at the 1st minute was collected for each sample and subjected to the fluorescence EEM measurement with a micro cuvette. The EEMs of the 1st-minute eluent from different samples showed the same contour with the peak at Ex/Em = 350/450 nm, and these EEMs were also identical to the PARAFAC Component 3 (as shown in Fig. S3, S4, and S5). Therefore, the identical EEM contour and the same retention time in LC may evidence that the components were NADH.

Moreover, SMP, EPS, and IPS also showed a clear peak at around 2.70 min, this wouldn't be the NADH due to the different retention times. The LC eluents at the 3rd minute of these samples were collected and their EEMs were measured. The EEMs showed the same contour with the peak at Ex/Em = 280,340/430 nm, which was similar to the humic-like substances component (Component 2 in Fig. S3, S4, and S5).

With this result, it is considered that Component 3 in the PARAFAC model may be NADH.

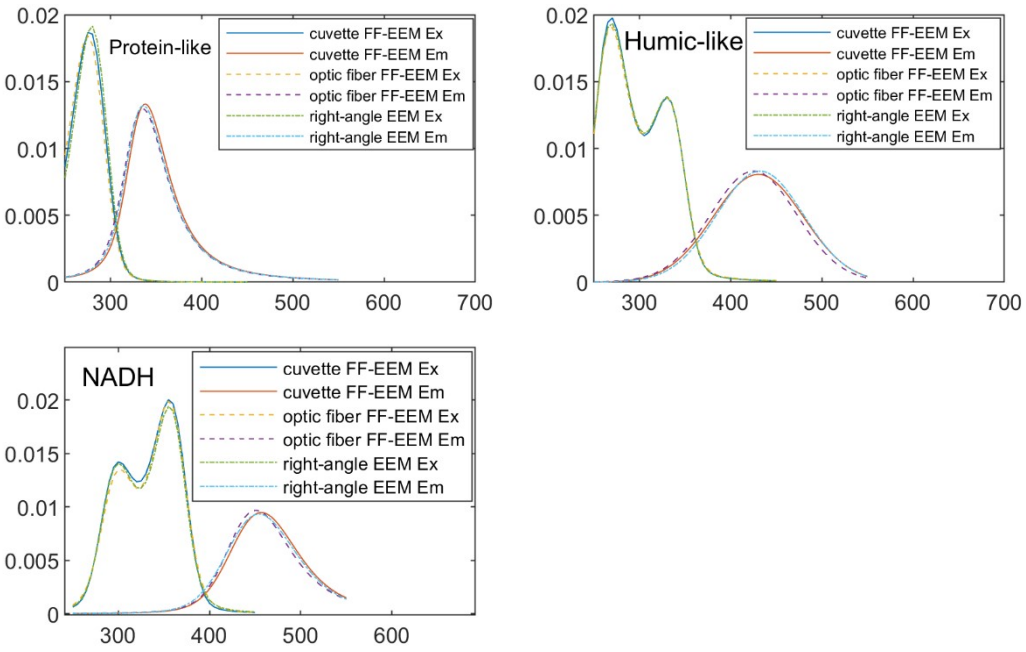

Figure S8 Comparison of the Ex loading and Em loading of each component from different PARAFAC models.

Table S1 Tucker's Congruence coefficient of Ex loadings or Em loadings of the protein-like component in different PARAFAC models

	Protein-like Ex loading			Protein-like Em loading		
	Cuvette FF-EEM	Optic fiber FF-EEM	Right-angle EEM	Cuvette FF-EEM	Optic fiber FF-EEM	Right-angle EEM
Cuvette FF-EEM	1	0.9745	0.9895	1	0.9916	0.9894
Optic fiber FF-EEM	0.9745	1	0.9623	0.9916	1	0.9923
Right-angle EEM	0.9895	0.9623	1	0.9894	0.9923	1

Table S2 Tucker's Congruence coefficient of Ex loadings and Em loadings of the humic-like component in different PARAFAC models

	Humic-like Ex loading			Humic-like Em loading		
	Cuvette FF-EEM	Optic fiber FF-EEM	Right-angle EEM	Cuvette FF-EEM	Optic fiber FF-EEM	Right-angle EEM
Cuvette FF-EEM	1	0.9879	0.9983	1	0.9849	0.9918
Optic fiber FF-EEM	0.9879	1	0.9893	0.9849	1	0.9942
Right-angle EEM	0.9983	0.9893	1	0.9918	0.9942	1

Table S3 Tucker's Congruence coefficient of Ex loadings and Em loadings of the NADH component in different PARAFAC models

	NADH Ex loading			NADH Em loading		
	Cuvette FF-EEM	Optic fiber FF-EEM	Right-angle EEM	Cuvette FF-EEM	Optic fiber FF-EEM	Right-angle EEM
Cuvette FF-EEM	1	0.9616	0.9902	1	0.9708	0.9924
Optic fiber FF-EEM	0.9616	1	0.9892	0.9708	1	0.9941
Right-angle EEM	0.9902	0.9892	1	0.9924	0.9941	1

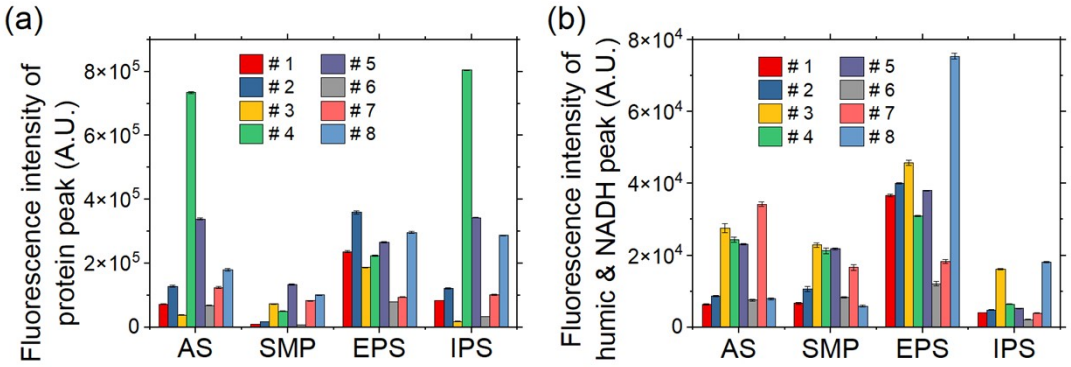

Figure S9 Fluorescence intensity at protein peak (Ex/Em 280/340 nm) in activated sludge, SMP, EPS, and IPS (a), fluorescence intensity at humic & NADH peak (Ex/Em 340/440 nm) in activated sludge, SMP, EPS, and IPS.

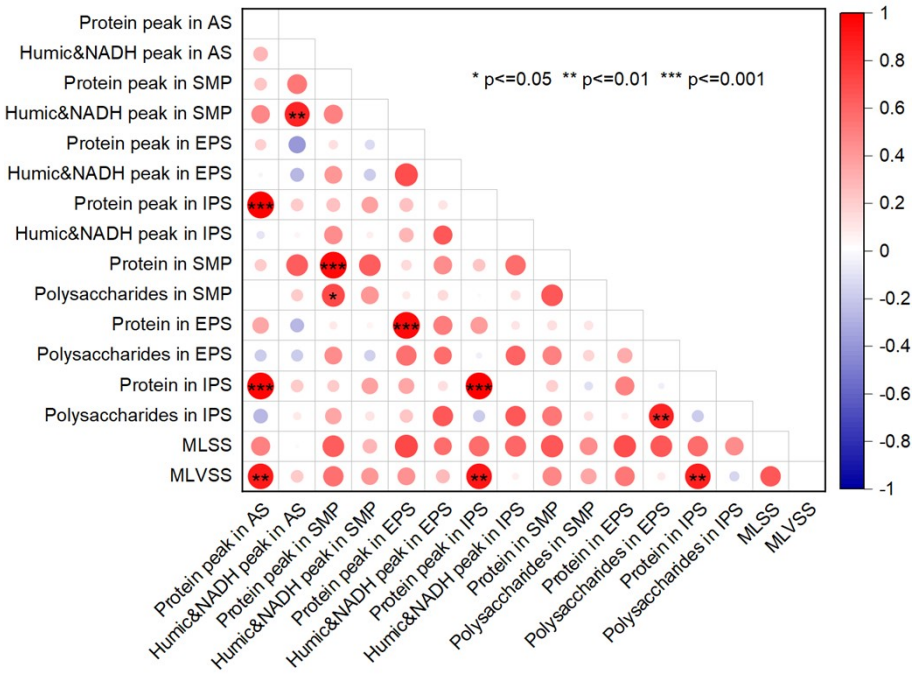

Figure S10 Correlation between fluorescence peak intensity (protein peak: Ex/Em 280/340 nm, and humic & NADH peak: Ex/Em 340/440 nm) and other properties of activated sludge. The color and the diameter of circles indicated the R2 values, and the asterisks indicated the p-value from ANOVA.

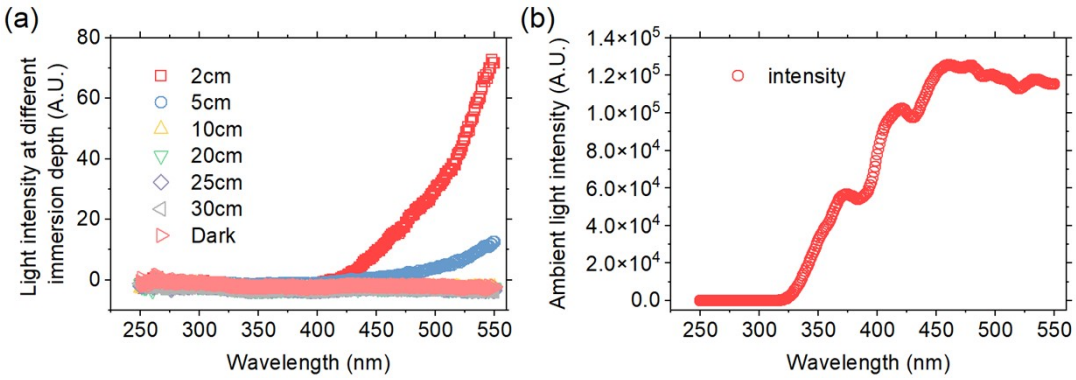

Figure S11 Light intensity at different immersion depths in activated sludge suspension (a), and ambient light intensity (b)