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Insight into removals of PARAFAC components from dissolved and particulate organic matter in wastewater treatment process by two-dimensional correlation and structure equation modeling

Benxin Yu^{1†}, Dongping Liu^{2†} , Jian Wang^{2,3*} and Yingxue Sun^{1,2*}

Abstract

Background: Most particulate organic matter (POM) cannot be directly degraded in the conventional wastewater treatment, which should be transformed into dissolved organic matter (DOM) through a hydrolysis process. However, non-hydrolyzed POM in the biological treatment can limit treated efficiencies for the wastewater treatment plants (WWTPs) facilities. Hence an operational tool is indispensable for insight into removals of DOM and POM fractions in the WWTP. In this study, excitation-emission matrix fluorescence spectroscopy (EEM) combined parallel factor analysis (PARAFAC), two-dimensional correlation (2D-COS) and structural equation modeling (SEM) was employed to evaluate removals of DOM and POM in a wastewater treatment plant.

Results: Four fluorescence components were identified in DOM and POM substances from the WWTP by EEM combined with PARAFAC, i.e., tyrosine-like (TYLF), tryptophan-like (TRLF), microbial byproduct-like (MBLF), and fulvic acid-like (FALF). In A²/O process, the TYLF and TRLF of DOM were removed to a larger extent than those of MBLF and FALF in anaerobic tank, while TYLF and MBLF of POM were removed to a great extent than those of TRLF and FALF in primary sedimentation and aerobic tanks. By the 2D-COS, a decreasing variation order of DOM fractions in the wastewater treatment process was UV-FALF → MBLF2 → Vis-FALF → TRLF → TYLF, while the decreasing order of POM fractions was Vis-FALF → UV-FALF → MBLF2 → TYLF → MBLF1 → TRLF. SEM revealed that TRLF and TYLF of DOM were degraded by anaerobic microorganism, and TRLF could be transformed partially into FALF. However, TRLF and TYLF of POM were decomposed by aerobic microorganism.

Conclusions: The 2D-COS and SEM can be practicable tools as EEM-PARAFAC for monitoring DOM and POM in the WWTP. The study could present a theoretical support to improving the retrofit of WWTP and formulating emission standards for organic pollutants.

Keywords: Dissolved organic matter, Particulate organic matter, Parallel factor analysis, Two-dimensional correlation spectroscopy, Structural equation modeling, Wastewater

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Background

Wastewater treatment plant (WWTP) has important function on society, removing a volume of contaminants from wastewater before discharging into the aquatic environment. The A²/O process with less

retention time, simple process, and high treatment efficiency was broadly used in WWTP. Organic matter exhibited in wastewater is derived largely from anthropological activities and microbial metabolites, which mainly contains roughly 50% protein and amino acids, 40% carbohydrates, 10% aliphatic compounds, and trace amounts of emerging pollutants, priority contaminants, and surface-active substances [1–3]. Organic matter may be generally divided into dissolved organic matter (DOM) and particulate organic matter (POM) using a simple membrane filtration method [4]. The POM with more than 0.45 μm approximately accounts for more than 50% of the organic loadings of the wastewater in a municipal wastewater treatment plant (WWTP), which is dominated by fresher substances with bacteria, organic debris and algae and protozoa in the wastewater [4, 5]. The POM with relatively low surface area is well known as a restrained factor of biodegradation process, and most of POM cannot be directly degraded in the conventional wastewater treatment, which should be transformed into DOM through a hydrolysis process. The DOM with less than 0.45 μm mainly represents recalcitrant materials, proteins, lipids, polysaccharides, carboxylic acids, and amino acids [1, 6]. The DOM with a relatively higher surface area not only exhibits an important role in improving microbial activities and pollutant degradation, but can induce membrane fouling and disinfection by-products [7, 8].

The conventional wastewater treatment is associated with four continuous processes, i.e., preliminary, primary, biological, and filtration [1, 9, 10]. First, the floating and crude solid-state materials can be removed in the preliminary treatment, in which grid screening and grit chambers are employed [1, 11]. Second, around 70% of organic/inorganic materials with more than 35 μm may be removed in the primary treatment, where sedimentation and clarification tanks are being introduced [1, 12]. Third, the biological treatment is utilized to degrade DOM fractions, in which soluble microbial byproducts and extracellular polymers concomitantly generally occur [13]. Finally, the filtration treatment can be applied to delete partial organic pollutants [1]. Moreover, the most of POM cannot be directly degraded, which should be transformed into DOM through a hydrolysis process [3, 14]. However, non-hydrolyzed POM in the biological treatment can limit treated efficiencies for the WWTPs facilities [3]. Hence the use of an operational tool can be indispensable for insight into removals of DOM and POM fractions in the WWTP, which can be conducive to a calibration for operational parameters, such as temperature, reflux ratio of activated sludge, hydraulic retention time,

influent chemical oxygen demand and dissolved oxygen concentration.

Excitation-emission matrix (EEM) fluorescence, well known as the rapidity, cheapness and simplicity of the tool have been applied to trace variations of DOM fractions in the WWTP [15, 16]. Recently EEM combined with parallel factor analysis (PARAFAC) can not only discriminate different fluorescent components of DOM in the WWTP, but determine their relative abundances [17, 18]. Moreover, two-dimensional correlation spectroscopy (2D-COS) can separate overlapped peaks by amplification of the narrowband signals in the second dimension, and distinguish sequential orders of any subtitle changes in dependence on external perturbations [19, 20]. The fluorescence 2D-COS has been applied to monitor DOM fractions in the WWTP [21], but less characterize inter/inner-variations of PARAFAC components with the intensities of excitation loadings from EEM-PARAFAC, especially PARAFAC components of POM. Structural equation modeling (SEM) is a statistical method to provide hypotheses of relationships among observed and latent variables, which could be used to reveal the latent transformation of PARAFAC components. Furthermore, SEM has been widely applied in market, ecology, management, construction, medicine, and environment studies [22–24].

The objectives of this study are to (i) extract the fluorescent components of DOM and POM from EEM combined with PARAFAC, and evaluate their removals in the WWTP; (ii) trace variation order of the PARAFAC components using 2D-COS, and (iii) reveal the latent transformation of PARAFAC components by SEM.

Materials and methods

Sample collection and DOM-POM isolation

An urban WWTP situated in northern China was selected to evaluate removals of DOM and POM fractions in the treatment process. A typical treatment craft of anaerobic/anoxic/oxic (A^2/O) process is performed for simultaneous removals of nitrogen, phosphorous and organic matter, whose capacity of wastewater treatment is approximately 1.0 million $\text{m}^3 \text{d}^{-1}$. The sampling sites were situated in effluents of the different treatment units, which were concerned with the preliminary grit chamber (PGC), primary sedimentation (PRS), anaerobic (ANA), anoxic (ANO), facultative (FAC), the aerobic (AER), and secondary sedimentation (SES). Duplicate wastewater samples with a 5-L Kemmerer Water Sampler were collected in each treatment unit, poured into glass BOD bottles and transferred to the laboratory in a freezer at 4 $^\circ\text{C}$ [25]. After 4 h, samples were detected.

Before DOM and POM were separated from the wastewater samples, the samples should keep standing for an

hour to precipitate the suspended solids [3]. This could reduce the influence of the activated sludge on the separation. POM was isolated from the wastewater sample using a glass fiber filter of 0.45 μm pore diameter, and the filtrate was defined as DOM [4]. The organic matter on the 0.45 μm filter was considered as POM, which was isolated into 10 mL of 0.1 N NaOH for 24 h in the dark at room temperature [26]. The mixed solution passed through the 0.45 μm filter, whose pH was adjusted closely to that of the original wastewater sample. Finally, the filter as the POM solution was amended to the corresponding solution volume of DOM.

EEM determination and parallel factor analysis

EEM spectroscopies were determined in a 1.0 cm quartz cell using a Hitachi Fluorescence Spectrophotometer (F-7000) equipped with fluorescence solutions 1.00.000 for data processing. The slit widths of excitation and emission were fixed at 5 nm bandpass, and scan speed was at 2400 nm min^{-1} . The range of the excitation wavelengths was 240–450 nm at 5 nm intervals, while the emission wavelengths ranged from 260 to 550 nm at 5 nm intervals. After all spectroscopies were corrected for inner-filtering effects, Raman signals were deleted using Quinine sulfate calibrations [27]. The corrected EEM spectroscopies were subtracted from their respective procedural blanks.

PARAFAC based on MATLAB software package with DOM Fluor Toolbox 1.7, was applied for EEM data to extract fluorescent components of DOM and POM [28]. The optimal number of the identified components were validated by split-half analysis, residual analysis, and visual inspection. The maximum fluorescence intensity (F_{max}) of the independent component has the same units (Raman units), which could be used to explicit its partial abundance. The relative proportions of the different PARAFAC components in a given treatment unit could be measured as % of F_{max} sum of all components for the individual component (% F_{max}).

Two-dimensional correlation and structural equation modeling

Due to the wastewater samples collected from the consecutive treatment units in the WWTP, the order of units could be defined as the specific external perturbations. Hence, 2D-COS was applied for the fluorescence data of the excitation spectroscopies originated from PARAFAC components, to competently discriminate the order of the fluorescence peaks in either components or in a component. 2D-COS excitation spectroscopies were conducted with a program of “2D Shige software”, which was released from Kwansei-Gakuin University, Japan [29].

SEM is associated with path, factor and regression analyses, which can be generally utilized as a multivariate tool of distinguishing multiple correlations among many potential variables [20]. In this study, SEM was performed to reveal the latent transformation of PARAFAC components of DOM and POM in the wastewater treatment process. SEM was operated by the software package AMOS (IBM Corporation Software Group, Somers, NY) with the maximum-likelihood estimation method [23].

Results and discussion

Extraction of PARAFAC components

A strong peak and four weak shoulders were represented in the EEM spectroscopy of DOM from the wastewater sample at the preliminary grit chamber (Fig. 1a). Based on published literatures for EEM spectra of organic matter [30], peak T at $\lambda_{\text{Ex}}/\lambda_{\text{Em}}=270\text{--}290/330\text{--}370$ nm might be associated with tryptophan-like fluorescence substance (TRLF), while shoulder B at $\lambda_{\text{Ex}}/\lambda_{\text{Em}}=260\text{--}280/300\text{--}330$ nm might be relative to tyrosine-like fluorescence substance (TYLF). Shoulders A and C at $\lambda_{\text{Ex}}=250\text{--}280$ nm and $340\text{--}370$ nm with the same λ_{Em} (430–470 nm) might be referred to UV fulvic-like (UV-FALF) and visible fulvic-like fluorescence substance (Vis-FALF), respectively. Shoulder M located between shoulders A and peak C might be microbial byproduct-like fluorescence substance (MBLF). The intensities of TRLF and TYLF were much higher than those of FALF and MBLF. This indicated that the former was dominant in DOM, which could be assigned with fresh and less degraded fresher and more recalcitrant materials derived from anthropogenic activities [31]. After the consecutive treatment, the intensities of shoulder B and peak T fell to a much larger extent than those of shoulders M, A and C (Fig. 1b).

The intensities of EEM spectra of POM from the wastewater at the PGC were much less than those of DOM (Fig. 1a and c), which attributed that about 60% of the total suspended solids should be removed after the preliminary grit chamber. Moreover, the still existing particles could be mostly disintegrated into smaller products by a hydrolysis process, which could be further degraded by bacteria and microbials [3]. Interestingly a distinct peak at $\lambda_{\text{Ex}}/\lambda_{\text{Em}}=240\text{--}260/360\text{--}390$ nm in EEM spectra of POM could be concerned with TRLF (Fig. 1c and d), which had been partially degraded. In the wastewater treatment process, the trends of shoulder B and peak T of POM were similar to those of DOM.

Four different fluorescent components were extracted from EEM spectroscopies of DOM and POM using PARAFAC modeling (Fig. 2). The component I (C1) with a single peak at $\lambda_{\text{Ex}}/\lambda_{\text{Em}}=275/330$ nm similar to shoulder B, could be TYLF, and the component II (C2) with

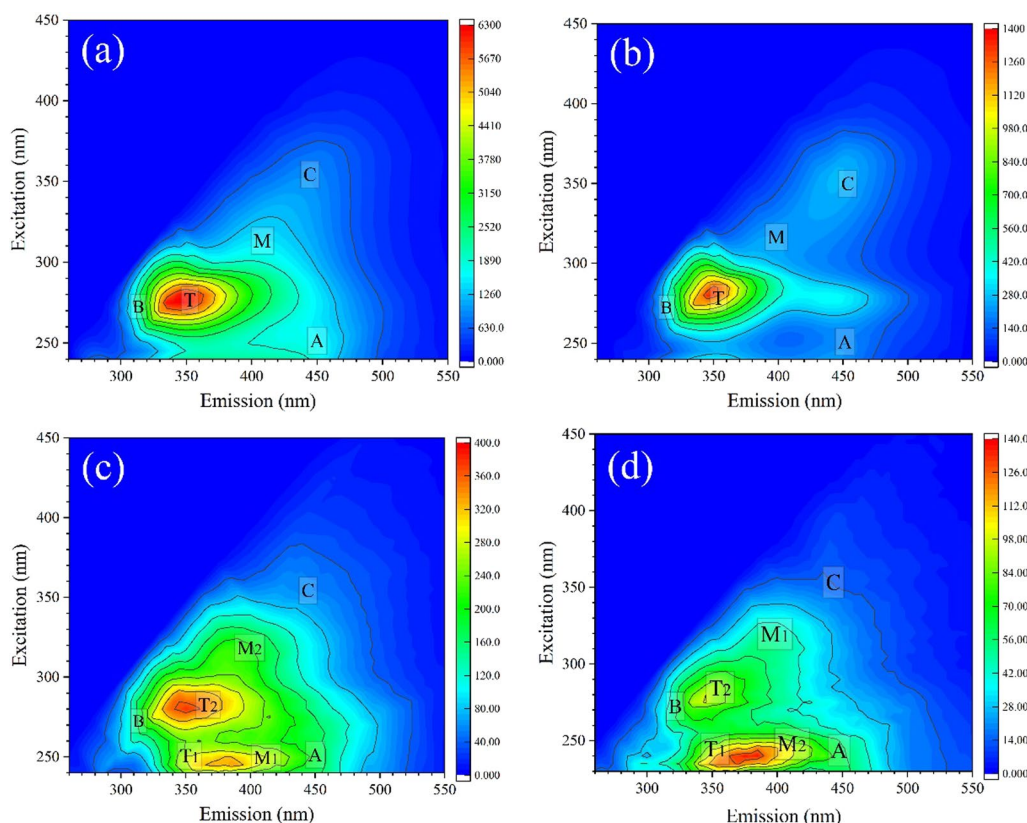


Fig. 1 EEM spectroscopies of DOM from the wastewater at the PGC **a** and SES **(b)**, and of POM from the wastewater at the PGC **c** and the SES **d** in the WWTP

only a peak at $\lambda_{Ex}/\lambda_{Em} = 285/350$ nm similar to peak T, could be TRLF. The component III (C3) exhibited two peaks at $\lambda_{Ex}/\lambda_{Em} = 240/400$ nm (M1) and $300/400$ nm (M2) resembled as shoulder M, and should be MBLF. The component IV (C4) also displayed primary and secondary peaks at $\lambda_{Ex}/\lambda_{Em} = 360$ and $275/450$ nm comparable to shoulders C and A, and should be Vis-FALF and UV-FALF, respectively.

Evaluating removals of PARAFAC components

Figure 3a–c showed the abundances, relative proportions, and removal efficiencies of PARAFAC components of DOM in the WWTP. In the wastewater process, the decreasing order of total Fmax of C1 to C4 was PGC (13,321.03) > PRS (10,107.43) > ANA (3890.67) > FAC (3596.55) > ANO (3631.09) > AER (2935.13) > SES (2669.48), whose decreasing order of the removal efficiencies was ANA > (61.51%) > PRS (24.12%) > AER (19.17%) > SES (9.05%) > ANO (7.56%) > FAC (-0.96%). This indicated that DOM fractions could be mostly removed in the anaerobic tank. The Fmax of the C1 and C2 reduced to much greater extents than those of the C3 and C4, which elaborated

that TYLF and TRLF were mostly removed, especially in the anaerobic tank. The C1%Fmax values were relatively constant (38.93%–49.88%) in the treatment process, while the C2%Fmax showed firstly increasing from 28.95% to 51.79% then decreasing to 41.42%. The C3%Fmax kept less than 10% (except for 14.66% in the preliminary grid chamber), so was the C4. Interestingly, the sum of the C1 and C2 were much more than 78.84% in a given unit, verified that TYLF and TRLF were dominant components of DOM in the wastewater. It was reported that the protein-like fluorophores are typically labile to biodegradation, and humic-like components require further treatments such as adsorption, coagulation, and advanced oxidation technologies [32, 33]. The removal efficiencies of C1 at the PGC to PRS were much higher than those at the rest sites, so were C3 and C4. This indicated that TRLF, MBLF and FALF were mostly degraded in anaerobic tank by anaerobic microorganisms, besides they were partially removed through absorption and sedimentation in the primary sedimentation tank. Furthermore, the removal efficiencies of C2 at the ANA and AER were much higher than those at the other sites, indicating that TRLF was

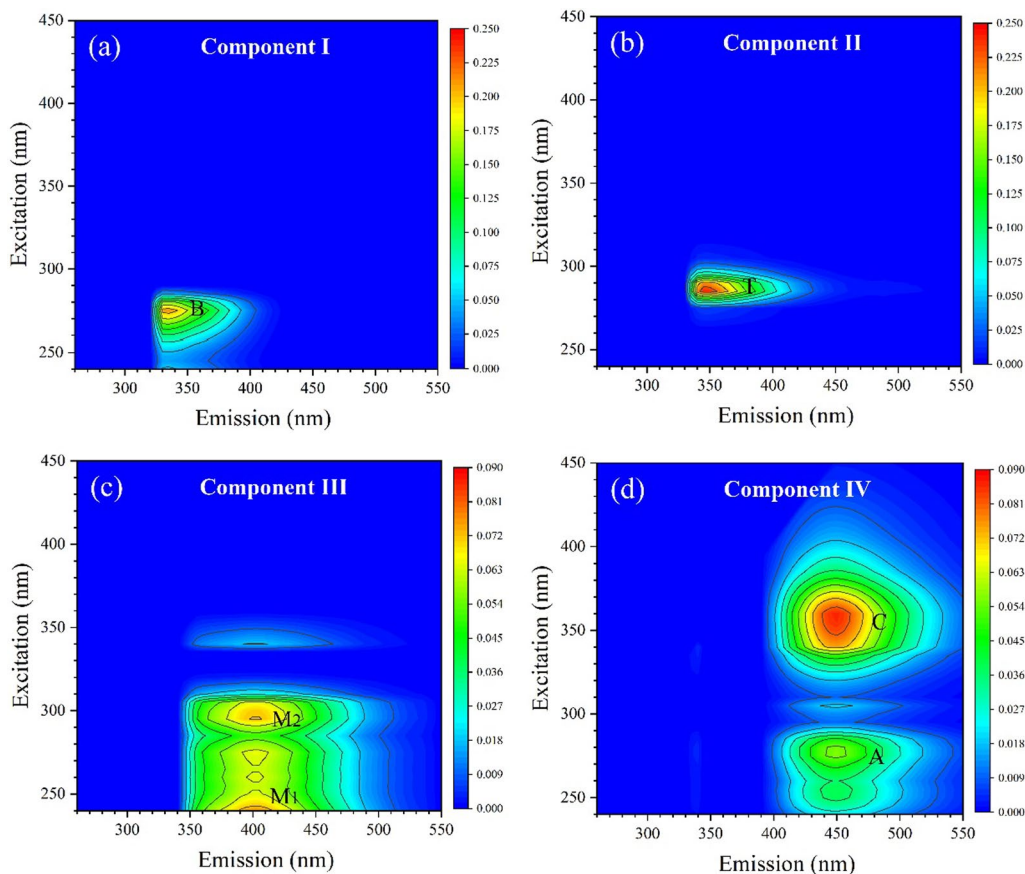


Fig. 2 PARAFAC components extracted from EEM spectroscopies of DOM and POM in the WWTP

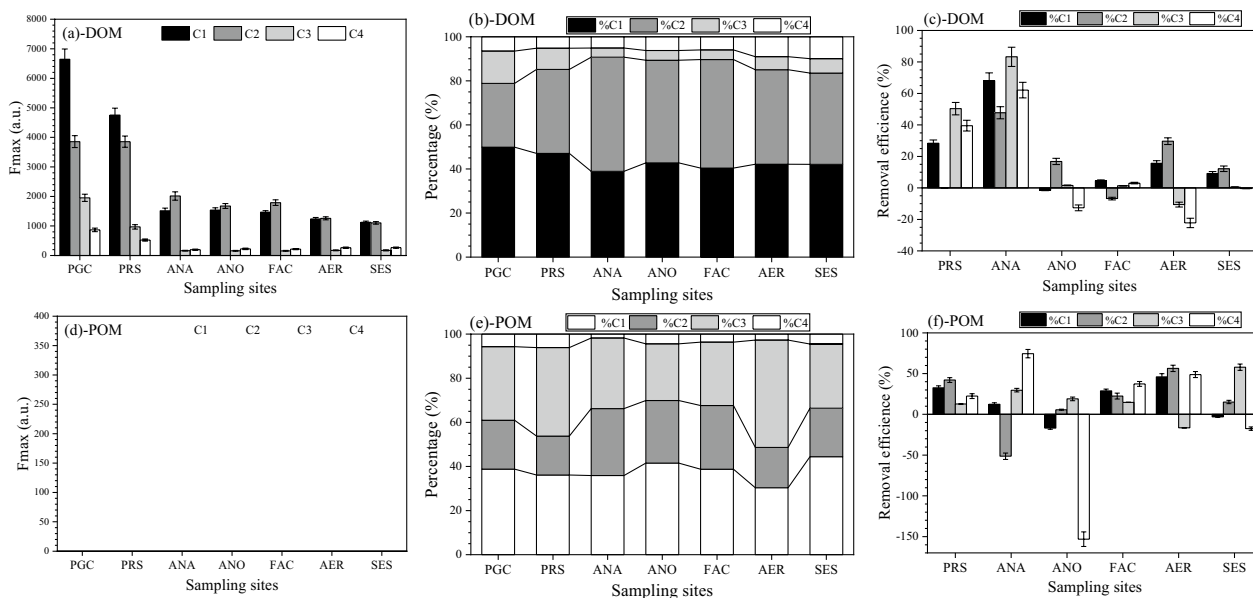


Fig. 3 Distributions of abundances, relative proportions, and removal efficiencies of PARAFAC components extracted from DOM **a-c** and POM **d-f** in the WWTP

mostly degraded in anaerobic and aerobic tanks by microorganisms.

Figure 3d–f exhibited the abundances, relative proportions, and removal efficiencies of PARAFAC components extracted from POM in the WWTP. In the wastewater units, the Fmax sum of C1 to C4 in the PGC (765.73) was the highest, followed by the PRS (555.42), ANO (494.62), ANA (489.88), FAC (376.89), AER (259.32) and SES (182.87). However, the removal efficiencies of POM fractions in the site AER was the highest (31.19%), followed by the SES (29.48%), PRS (27.47%), FAC (23.80%), ANA (11.80) and ANO (-0.97). This indicated that POM fractions could be deeply removed through adsorption and sedimentation in the primary and secondary sedimentation tanks, and degraded by aerobic microorganisms in the aerobic tank [3, 32]. Much higher proportions of the Fmax of the C1 and C3 were removed than those of the C2 and C4 in the successive treatment units (Fig. 3d), which indicated that TYLF and MBLF were highly much degraded. The mean of the C1%Fmax ($37.98 \pm 4.49\%$) was the highest in the wastewater treatment process, followed by the C3 ($33.95 \pm 7.94\%$), C2 ($23.97 \pm 5.22\%$) and C4 ($4.11 \pm 1.55\%$). Noticeable, the sum of the C1 and C3 were much more than 67.22% in each unit (Fig. 3e), expounding that TYLF and MBLF were representative components of POM in the wastewater. The removal efficiency of the C1 was highest at the site AER, followed by the PRS, FAC, ANA, SES and ANO, and the removal efficiency of the C2 was the highest at the site AER too, followed by the PRS, FAC, SES, ANO and ANA (Fig. 3f). These protein-like substances were mainly removed in the aerobic tank. This attributed that the protein-like should be broken into smaller products through the hydrolysis process [3], which could be further metabolized by bacteria and microbial. The descending order of the C3 removal efficiencies was SES > ANA > ANO > FAC > PRS > AER, indicating that MBLF were mostly removed in the second sedimentation tank. The descending order of the C4 removal efficiencies was ANA > AER > FAC > PRS > SES > ANO, explaining that FALF substances were mostly degraded by anaerobic microbial in the anaerobic tank.

Inter/inner dynamic-variations of PARAFAC components

There were six peaks in PARAFAC components (Fig. 2), whose changing order could be identified by hetero 2D-COS and 2D-COS in the seven successive treatment units. This could reveal inter/inner dynamic-variations of PARAFAC components of DOM or POM from the wastewater in the WWTP.

Figure 4 exhibited synchronous and asynchronous maps of the hetero 2D-COS based on excitation loadings of PARAFAC components of DOM. There was a positive

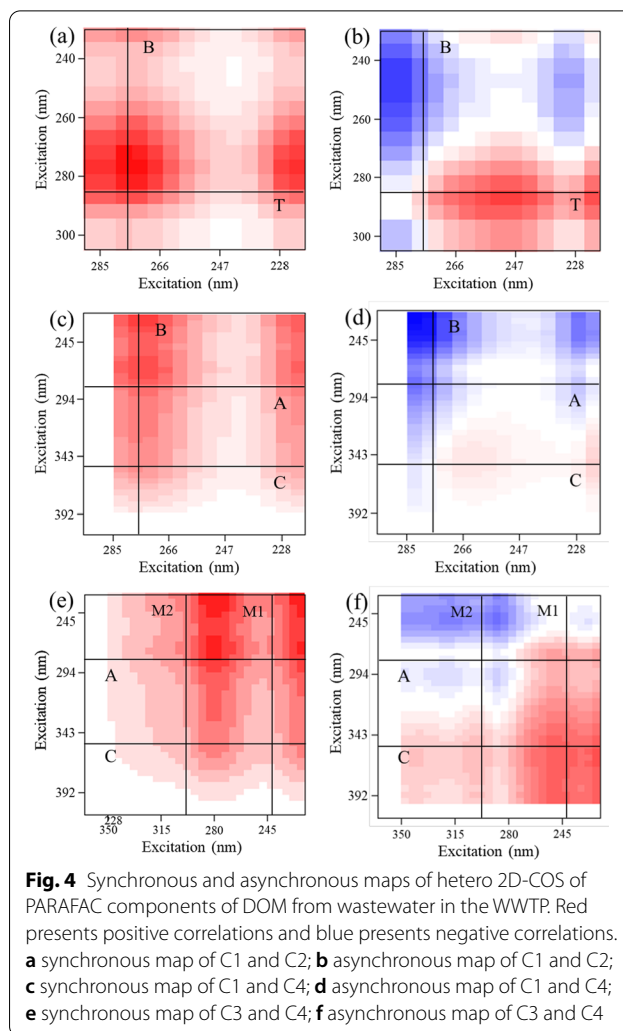


Fig. 4 Synchronous and asynchronous maps of hetero 2D-COS of PARAFAC components of DOM from wastewater in the WWTP. Red presents positive correlations and blue presents negative correlations. **a** synchronous map of C1 and C2; **b** asynchronous map of C1 and C2; **c** synchronous map of C1 and C4; **d** asynchronous map of C1 and C4; **e** synchronous map of C3 and C4; **f** asynchronous map of C3 and C4

relationship between peaks B and T in both the synchronous map and asynchronous map (Fig. 4a, b), indicating that the changing order was B → T according to Noda’s rule [30]. Peak B had positive correlations with peaks A and C in the synchronous map (Fig. 4c), while negative correlations with the peaks A and C in the asynchronous map (Fig. 4d), elaborating that the changing order was A and C → B. Peaks A and C presented positive correlations with peak M1 in either synchronous or asynchronous maps (Fig. 4e, f), proving that the changing order M1 → A and C. There was a positive relationship between peaks M2 and A in the synchronous map and a negative relationship in the asynchronous map (Fig. 4e, f), explaining that the change order was A → M2. Peak M2 had a positive correlation with peak C in the synchronous and asynchronous map (Fig. 4e, f), indicating that the changing order was M2 → C. In summary, the changing order of the six peaks was M1 → A → M2 → C → B → T, indicating that the continuous dynamic variation of MBLF

occurred in the successive treatment units, while disconnected dynamic variations of TRLF and TYLF. This indirectly proved that TRLF and TYLF could be removed in the anaerobic/anoxic units.

Figure 5 showed synchronous and asynchronous maps of hetero 2D-COS and 2D-COS based on excitation loadings of PARAFAC components of POM. Peak B was positively related to peaks M1 and M2 in the synchronous map (Fig. 5a), while negative related to peaks M1

and M2 (Fig. 5b). This suggested that the varying order should be M1 and M2 → B. Peak T was positively relative with peak M1 in both synchronous and asynchronous maps (Fig. 5c, d), indicating that the varying order was T → M1. Peak T was positively relative with peak M2 in the synchronous map, while negatively relative with peak M2 (Fig. 5c, d). This suggested that the varying order was M2 → T. Peaks M1 and M2 had positive correlations with peaks A and C, while negative correlations with peaks A and C (Fig. 5e, f). This proposed that the varying order was A and B → M1 and M2. Peak A represented a positive correlation with peak C (Fig. 5g), while a negative correlation with peak C in the asynchronous map (Fig. 3h). This indicated that the varying order was C → A. Based on the above results, the varying order of the peaks was C → A → M2 → T → M1 → B, indicating that FALF showed a successive variation in the wastewater treatment process, while TRLF showed an undulated variation. This indirectly validated that TRLF of POM could be degraded in the anaerobic and anoxic units.

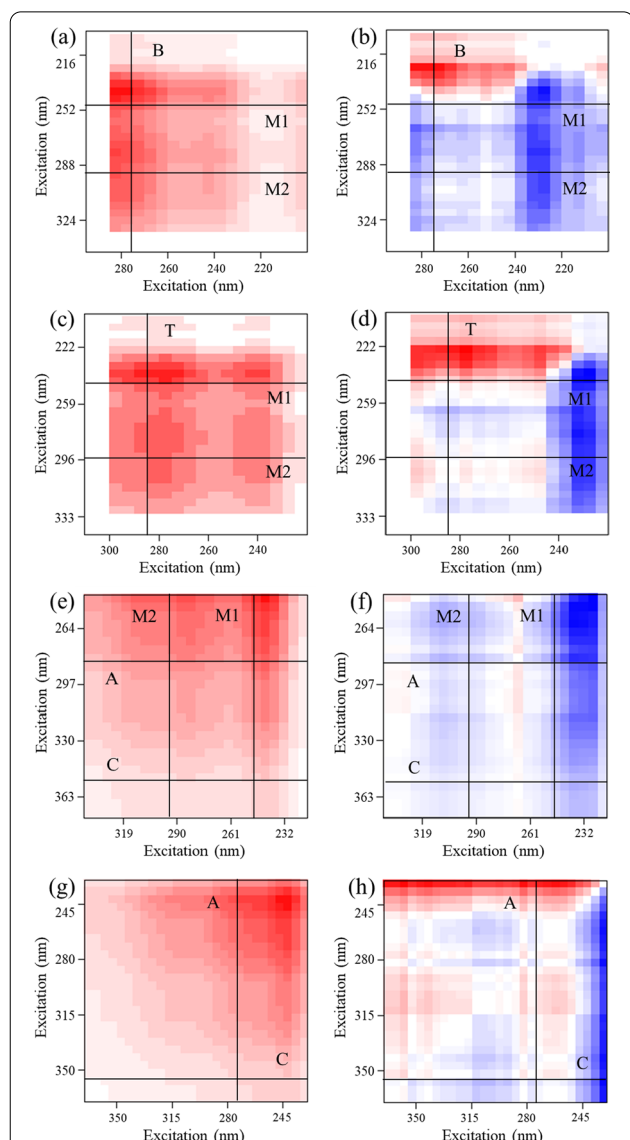


Fig. 5 Synchronous and asynchronous maps of hetero 2D-COS and 2D-COS of PARAFAC components of POM from wastewater in the WWTP. Red presents positive correlations and blue presents negative correlations. **a** synchronous map of C1 and C3; **b** asynchronous map of C1 and C3; **c** synchronous map of C2 and C3; **d** asynchronous map of C2 and C3; **e** synchronous map of C3 and C4; **f** asynchronous map of C3 and C4; **g** synchronous map of C3; **h** asynchronous map of C3

Latent transformation of PARAFAC components

An SEM based on the hypothetical model could be developed as a modeling with an endogenous latent variable and four observed variables. Meanwhile, the former was associated with the removal efficiencies of Fmax sum of PARAFAC components of DOM or POM, and the latter was concerned with the Fmax of C1 to C4.

The modeling with Chi-square=52.887, Degree of freedom=3 and Probability level=0.000 showed a marginal acceptance for the latent transformation of DOM fractions (Fig. 6a), for the Chi-square with less than 5.0 was available [34, 35]. This could attribute that C3 or C4 had a weak effect on the total PARAFAC component efficiencies, as proved by relatively small path coefficients (0.22 or 0.22). This indirectly that the percentages of the C3 and C4 were smaller than those of the C1 and C2. The C1 with a path coefficient of -1.00 had a strongly negative direct effect on the removal efficiencies, indicating that C1 should be removed in the anaerobic tank [32].

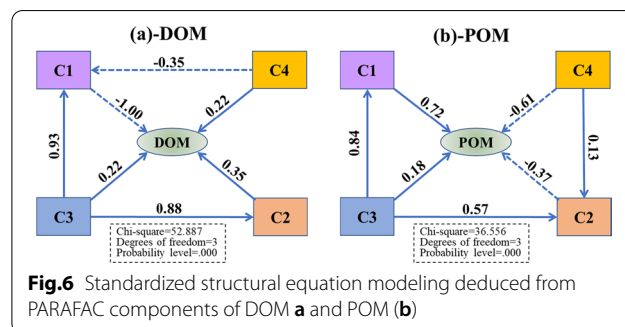


Fig. 6 Standardized structural equation modeling deduced from PARAFAC components of DOM **a** and POM **(b)**

C2 with the path coefficient of 0.35 showed a positive effect on the removal efficiencies, elaborating that C2 were continuously removed in the treatment process. C3 with the path coefficients of 0.93 or 0.88 showed a strongly positive direct effect on the C1 or C2, indicating C3 showed an indirect effect on the removal efficiencies. This indirectly verified that TYLF and TRLF could be degraded by microorganism, especially in the anaerobic tank [32]. Furthermore, C4 showed an indirect effect on the removal efficiencies through C1, as insight into the path coefficient of -0.35. This indirectly evidenced that C1 could be degraded partially into C4 in the wastewater treatment.

The model deduced from PARAFAC components of POM was referred as the Chi-square = 36.556, Degree of freedom = 3 and Probability level = 0.000 represented a rough acceptance too (Fig. 6b). This could contribute to a poor direct influence of the C3 with the path coefficient of 0.18 on the removal efficiencies, and a weak direct influence of C4 with the path coefficient of 0.13 on the C2. This indirectly validated that C1 and C3 was the representative component of POM, instead of the C2 and C4. C1 with the path coefficient of 0.72 displayed a direct positive influence on the removal efficiencies, demonstrating that TYLF could be unceasingly removed in the successive treatment units. However, C2 and C4 with the path coefficients of -0.37 and -0.61 respectively, displayed direct negative influences on the removal efficiencies, demonstrating that the variations of C2 and C4 were apparently unstable (Fig. 3c). C3 displayed a directly positive influence on the C1 or C2, as confirmed by the relatively large path coefficients (0.84 and 0.57). This indicated that C3 displayed an indirectly positive influence on the removal efficiencies through the C1 and C2. Undeniably, this indirectly proved that TYLF and TRLF should be decomposed by microorganism too. Interestingly, C4 displayed an indirectly positive influence on the removal efficiencies by the C2 with the path coefficient of 0.13. This could attribute that C2 and C4 should be partially degraded into dissolved organic matter.

Conclusions

2D-COS and SEM can be practicable tools as EEM-PARAFAC for monitoring DOM and POM in the WWTP. TRLF and TYLF were the representative components of DOM in wastewater, which were removed to a greater extent than those of MBLF and FALF. TRLF and MBLF were the dominated components of POM, which were removed to the larger extent than those of TYLF and FALF. The decreasing variation order of DOM fractions was MBLF1 → UV-FALF → MBLF2 → Visible-FALF → TRLF → TYLF, while the order of POM fractions was Visible-FALF → UV-

FALF → MBLF2 → TYLF → MBLF1 → TRLF. TRLF and TYLF of DOM were degraded by anaerobic microorganism, and TRLF could be transformed partially into FALF. However, TRLF and TYLF of POM were decomposed by aerobic microorganism.

Abbreviations

DOM: Dissolved organic matter; POM: Particulate organic matter; TYLF: Tyrosine-like fluorescence substances; TRLF: Tryptophan-like fluorescence substances; MBLF: Microbial byproduct-like fluorescence substances; FALF: Fulvic acid-like fluorescence substances; PARAFAC: Parallel factor analysis; 2D-COS: Two-dimensional correlation spectroscopy; SEM: Structural equation modeling; WWTP: Wastewater treatment plant; PGC: Preliminary grit chamber; PRS: Primary sedimentation; ANA: Anaerobic; ANO: Anoxic; FAC: Facultative; AER: Aerobic; SES: Secondary sedimentation.

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Authors' contributions

BY carried out the experiments, analyzed the data and wrote the manuscript; DL analyzed the data and modified the manuscript; JW and YS wrote the manuscript and improved the language of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets obtained and analyzed in the research can be available from the first author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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