Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2010, 22(11) 1728–1734
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Characterization of dissolved organic matter in urban sewage using excitation emission matrix fluorescence spectroscopy and parallel factor analysis

Weidong Guo[1,2,*], Jing Xu[1], Jiangping Wang[1], Yingrou Wen[1], Jianfu Zhuo[1], Yuchao Yan[1]

1. State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China. E-mail: wdguo@xmu.edu.cn
2. Joint Key Laboratory of Coastal Study (XMU & FJIO), Xiamen University, Xiamen 361005, China

Received 08 December 2009; revised 14 February 2010; accepted 04 March 2010

Abstract

Wastewater dissolved organic matter (DOM) from different processing stages of a sewage treatment plant in Xiamen was characterized using fluorescence and absorption spectroscopy. Parallel factor analysis modeling of excitation-emission matrix spectra revealed five fluorescent components occurring in sewage DOM: one protein-like (C1), three humic-like (C2, C4 and C5) and one xenobiotic-like (C3) components. During the aerated grit chamber and primary sedimentation tank stage, there was only a slight decrease in fluorescence intensity and the absorption coefficient at 350 nm (a_{350}). During the second aeration stage, high concentration of protein-like and short-wavelength-excited humic-like components were significantly degraded accompanied by significant loss of DOC (80%) and a_{350} (30%), indicating that C1 and C2 were the dominant constituents of sewage DOM. As a result, long-wavelength-excited C4 and C5 became the dominant humic-like components and the DOM molecular size inferred from the variation of spectral slope S (300–650 nm) and specific absorption (a_{280}/DOC) increased. Combination use of F_{max} of C1 and the ratio of C1/C5, or a_{350} may provide a quantitative indication for the relative amount of raw or treated sewage in aquatic environment.

Key words: dissolved organic matter; sewage; fluorescence EEM; parallel factor analysis
DOI: 10.1016/S1001-0742(09)60312-0

Introduction

Organic pollution originating from industrial, agricultural and municipal wastewater discharge has become a common environmental problem in many lakes, rivers, estuaries and coastal waters (Yan et al., 2000; Baker, 2002; Saadi et al., 2006; Wang et al., 2007). Traditional methods to monitor the degree of organic pollution are usually time-consuming wet chemical analysis such as chemical oxygen demand (COD), biological oxygen demand (BOD) and total organic carbon (TOC) (Reynolds and Ahmad, 1997). COD and BOD are indirect indexes which are unable to indicate the true concentration of organic matter in aquatic environment, while, TOC can not provide any compositional and source information for organic substances with complex and heterogenic nature (Teymouri, 2007). On the other hand, the commonly-used methods to study the composition of dissolved organic matter (DOM) (nuclear magnetic resonance, gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, size exclusion chromatography, etc.) need complicated sample pretreatment procedures and are not suitable for on-line or real-time determination.

Excitation-emission matrix (EEM) fluorescence spectroscopy has been suggested as a powerful tool to characterize aquatic DOM which could detect the specific fluorescent fractions in organic matter from different sources with much higher sensitivity (Coble et al., 1996). Traditional "peak-picking" method for EEM has identified humic-like (A, C, M) and protein-like fluorescence peaks (B, T) using simple excitation-emission wavelength pairs of the fluorescence peak locations (Coble et al., 1996). However, the method has some disadvantage and may be unreliable (Stedmon et al., 2003; Teymouri, 2007), as different fluorescence peaks overlap. Recently, it has been suggested that parallel factor analysis (PARAFAC) can decompose EEM of complex mixtures of DOM pool into their independent fluorescent components which may effectively trace the photochemical and microbial reactions of DOM (Stedmon et al., 2003, 2007; Stedmon and Markager, 2005; Stedmon and Bro, 2008). EEM-PARAFAC has also been applied to evaluate the verification of ballast water exchange (Murphy et al., 2006) and the DOM sources in open oceans (Murphy et al., 2008); the algal source of CDOM (Zhang et al., 2009), correlation between water quality parameters (Holbrook et al., 2006; Wang et al., 2007) and the characterization of wastewater and landfill leachate (Teymouri, 2007; Lu et al., 2009).

UV-Visible absorption spectroscopy is another

* Corresponding author. E-mail: wdguo@xmu.edu.cn

spectroscopic approach which can also provide quantitative and qualitative information of DOM (Chin et al., 1994; Stedmon et al., 2000; Guo et al., 2007). Absorption coefficient at a certain wavelength (e.g., 254 nm) can be used as an indicator of DOM concentration (e.g., Weishaar et al., 2003), while absorption ratio at 250 to 365 nm (called E2:E3) or molar absorptivity at 280 nm has been used to trace the changes in the relative size and aromaticity of DOM molecule (De Haan and De Boer, 1987; Chin et al., 1994). Helms et al. (2008) suggested that the spectral slope ratio of 275–295 nm and 350–400 nm (called S_R) appears to be a good proxy for DOM molecular weight.

In this study, wastewater samples were collected from different processing stages of a sewage treatment plant in Xiamen, China. Some seawater samples were also collected from neighboring Yundang Lagoon and Xiamen Bay for comparison. The main objective was to characterize the spectral characteristics of wastewater DOM and to trace its variation during sewage treatment with EEM-PARAFAC and absorption spectroscopy. Such research may provide basic information for sewage plants to improve and optimize their treatment technology. This study will also be helpful to differentiate the organic matter from wastewater source with other natural sources, for example, sea water DOM in coastal waters.

1 Materials and methods

1.1 Sample collection and pretreatment

The sewage treatment process is shown in Fig. 1. The first three stages (S1–S3) referred to primary treatment and S4–S6 secondary treatment. The treated sewage effluents were discharged into Yundang Lagoon, which has a water exchange system with outer Xiamen Bay. Six samples from each processing step (S1–S6), one sample near the outlet in Yundang Lagoon (S7), one sample from the outlet of the Lagoon (S8) and one sample from the outer Xiamen Bay (S9) were collected at the same day. Immediately after sampling, all samples were transported to the laboratory and filtered through precombusted 0.7 μm Whatman GF/F glass fiber filters at 500°C for 5 hr. Approximately 20 mL of the filtrates were stored in amber color bottles and kept frozen for dissolved organic carbon (DOC) measurement. The remaining filtrates were further filtered using 0.2 μm Millipore polycarbonate filters for the EEM and absorption measurements of DOM, which were made within four hours after filtration.

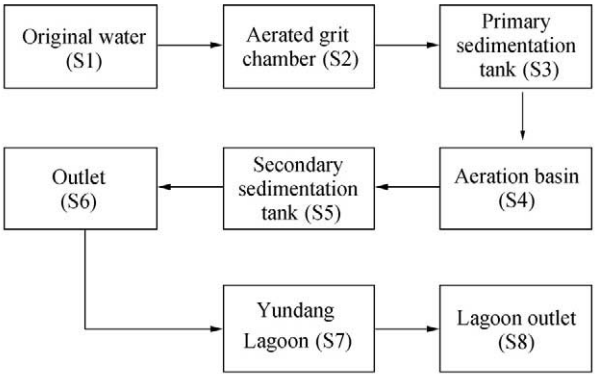

Fig. 1 Treatment process of municipal sewage.

1.2 EEM measurements

EEM fluorescence spectra were obtained using a Cary Eclipse (Varian, USA) equipped with a 150 W Xe arc lamp at PMT voltage of 730 V. EEM were constructed for each sample over excitation wavelengths between 220–450 nm in 5 nm intervals and emission wavelengths between 230–600 nm in 2 nm intervals, with 10 nm and 5 nm bandwidths on excitation and emission modes, respectively. The EEM of Milli-Q water was used as the background level of the instrument, then the fluorescence intensity of samples was normalized to the Raman scattering intensity units (R.U., nm^{-1}) of pure water setting excitation wavelength at 350 nm (Determann et al., 1994).

The basic assumption of fluorescence spectroscopy analysis is that the sample is optically dilute. High absorption properties of sewage samples may cause inner filter effects for fluorescence analysis (Tucker et al., 1992). A dilution experiment was performed for S3 sample with the highest a_{350} (10.06 m^{-1}) to demonstrate such effects. When multiplying with the dilution factor, the relative fluorescent intensities increased with the dilution for traditional tryptophan peak (225 nm/341 nm) and tyrosine peak (225 nm/299 nm) (Fig. 2). However, no inner filter effects was observed for humic-like peak (350 nm/430 nm), due to the lower absorbance at this higher excitation wavelengths. Thus, high absorbance samples were diluted with Milli-Q water to absorbance $a_{350} < 0.08$ to avoid the interference of inner filtering effects for EEM analysis.

1.3 Absorption spectroscopy analysis and DOC measurements

Absorption spectra of original sewage DOM were determined with a 2300 UV-Vis spectrometer (Techcomp, China), with a 10-cm quartz cell. Milli-Q water was used

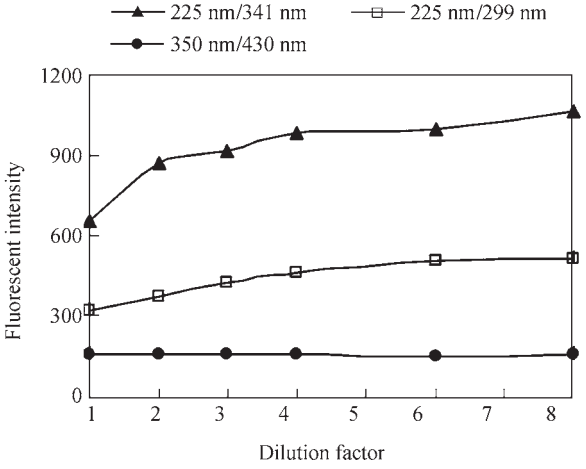

Fig. 2 Dilution experiment showing the inner filter effects for fluorescence analysis of sewage DOM.

as the blank. The scanning wavelength range was 200–800 nm, with a spectral resolution of 1 nm. Absorption coefficient at wavelength of 350 nm (a_{350}) was chosen as an index of DOM concentration. The spectral slope coefficient (S) over a wavelength range of 300–650 nm were calculated using nonlinear regression method.

DOC concentrations were determined with Multi N/C 3100 TOC-TN analyzer (Analytik Jena, Germany). Samples were acidified to pH < 2 by HCl, then purged with an oxygen stream for 8 min to remove the inorganic carbon, before high temperature catalytic oxidation. The measurements were performed in triplicates with a fixed instrumental variance < 2%. Potassium hydrogen phthalate combined with Milli-Q water were used as external standard and blank to calculate DOC concentrations. The accuracy of measurements was daily verified with low carbon water (LCW) and deep sea water (DSW) produced by University of Miami, USA.

1.4 PARAFAC modeling

PARAFAC decomposes the fluorescence signals X into F tri-linear components according to the number of fluorophores present in the samples:

$$X_{ijk} = \sum_{f=1}^{F} c_{if} b_{jf} a_{kf} + \sigma_{ijk} \quad (1)$$

where, F defines the number of components in the model, X_{ijk} is the intensity of fluorescence for the sample i at emission wavelength j and excitation wavelength k, c_{if} is the score for the f_{th} component and related to the concentration of fluorophore f in sample i, b_{jf} and a_{kf} are linearly related to the fluorescence quantum efficiency (fraction of absorbed energy emitted as fluorescence) of the jth and kth analyte at emission wavelength j respectively. Finally, σ_{ijk} is the error term representing the variability not accounted for by the model. The principle behind the model is to minimize the sum of squared residual σ_{ijk}.

Split-half analysis was further performed to validate the reliability of the model results (Stedmon et al., 2003). The fluorescence intensity of each component was represented by $F_{\max}$ (R.U., i.e. Raman units) (Stedmon and Markager, 2005)

In this study, PARAFAC modeling was accomplished through the use of "N-way toolbox for MATLAB version 3.1" in MATLAB 7.5 technical computing program (Mathworks Inc., Cambridge, MA).

2 Results and discussion

2.1 Fluorescent components of sewage DOM

Five separate fluorescent components were identified for sewage DOM by the PARAFAC model (Fig. 3). All components had multiple peaks, except for component C3. In general, these components have single emission maxima with two excitation maxima, however, C1 had a shoulder at 300 nm. These fluorescent components showed many similarities with the previously identified peaks (Table 1). Component C1 is composed of two excitation maxima (at 220 and 275 nm), with one emission peak centered at 339 nm and a shoulder at 300 nm (Fig. 3, Table 1), corresponding well to the fluorescent peaks of pure tryptophan and pure tyrosine (Mayer et al., 1999). Thus, the component is a typical protein-like substance. The results also indicated that both tryptophan-like and tyrosine-like components in sewage DOM had the same origin.

Component C2 (240, 280/377 nm) is similar to humic-like components corresponding to traditional peak M and A (Murphy et al., 2008). An intense excitation peak for component C3 occurred at 230 nm with the emission maxima at 345 nm (Fig. 3, Table 1). It is not similar to any of the traditionally identified peaks in river and marine environments. However, it was dominant in the landfill leachate and sewage samples (220–230/340–370 nm) and was called as xenobiotic organic matter (e.g., naphthalene) (Baker and Curry, 2004, Teymouri, 2007). Hence, C3 can be taken as a typical indicator component of sewage DOM. Furthermore, the overlap of C3 (230/345 nm) with short-wavelength-excited peak (220/339 nm) of tryptophan-like component C1 suggested that the fluorescent peak located at 220–230/330–350 nm can not be taken for granted as reliable indication of tryptophan component.

Component C4 (230, 340/422 nm) reflects UVA humic-like substances whose main constituent is fulvic acid (FA), while component C5 (260, 380/467 nm) is thought to represent humic materials exported from terrestrial sources and its constituent is primarily humic acid (HA) (Kowalczuk et al., 2003). The broader excitation and emission band and the longest excitation and emission wavelength of C5 suggested that it is associated with humic matter composed of high molecular weight and high aromatic organic compounds (Stedmon et al., 2003; Kowalczuk et al., 2009). Both C4 and C5 are widespread fluorescent components in river, estuary and coastal environments (Coble, 1996; Stedmon et al., 2003; Murphy et al., 2008;

Table 1 Positions of the fluorescence maxima of the five components

Fluorescent components	Peak position λEx/Em (nm)	Description	Reference
C1	220, 275/339 220, 275/300	Protein-like (tryptophan-like + tyrosine-like)	220–230, 270–280/340–350 nm (Mayer et al., 1999)
C2	240, 280/377	Peak M and A	< 260, 325/385 nm (Murphy et al., 2008)
C3	230/345	Xenobiotic-like substance	220–230/340–370 nm (Baker and Curry, 2004)
C4	230, 340/422	UV humic-like	230, 325/416 nm (Stedmon et al., 2003) 250, 320/420 nm (Lu et al., 2009)
C5	260, 380/467	Visible humic-like	250, 360/460 nm (Lu et al., 2009) 260, 370/490 nm (Murphy et al., 2008)

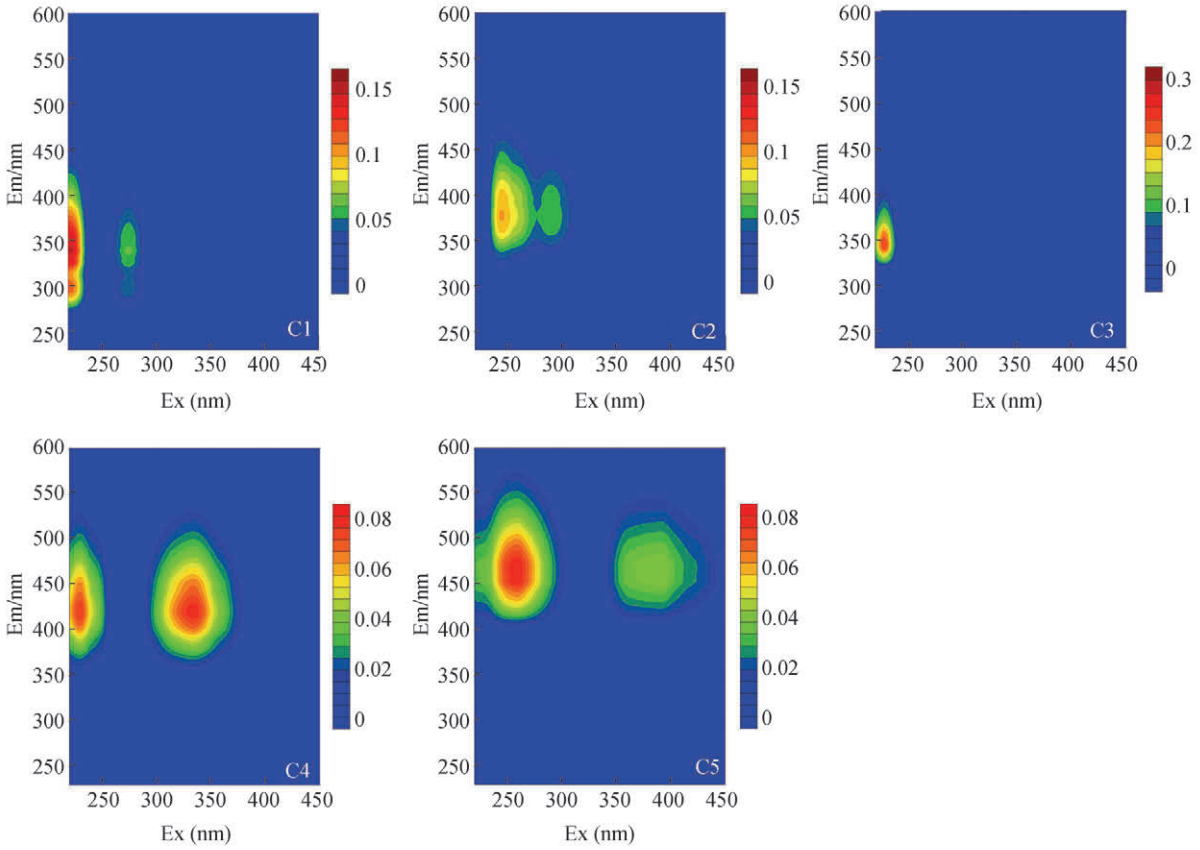

Fig. 3 Fluorescent components of sewage DOM identified by the PARAFAC model.

Lu et al., 2009). It should be noted that, the traditional short wavelength UV-excited peak A designated by Coble (1996) was not a single component, but actually a combination of C2, C4 and C5 fluorescent components for sewage DOM. Furthermore, peaks in traditional "peak A" area have close link with long wavelength excited peaks like M and C.

2.2 Influence of treatment process on the fluorescent properties of sewage DOM

Original sewage water (S1) has high concentration of protein-like and xenobiotic components and relatively low concentration of humic-like components (Fig. 4). During the aerated grit chamber and primary sedimentation tank stage, there were only small decreases in protein-like (C1), xenobiotic (C3) and short wavelength excited humic-like

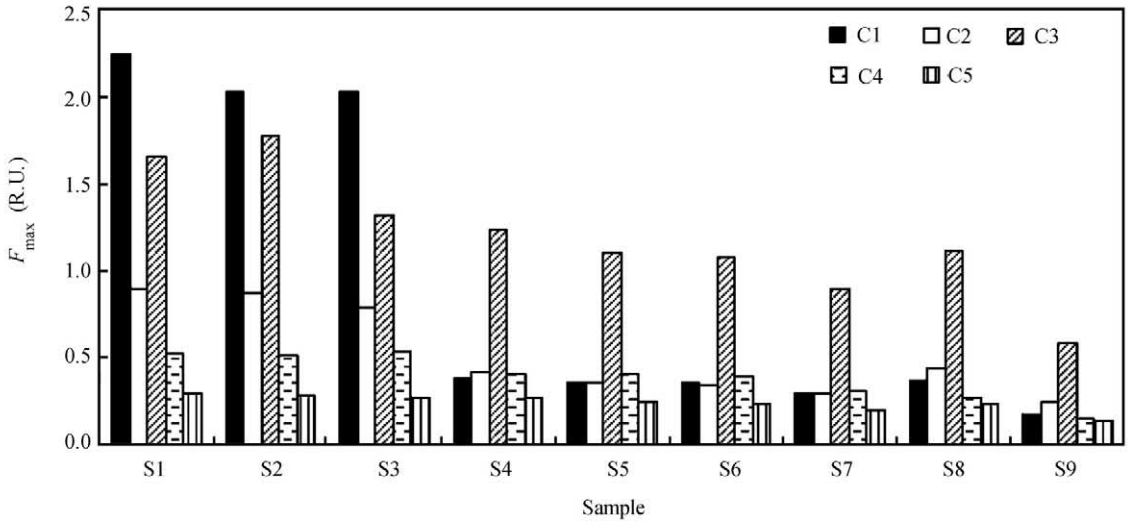

Fig. 4 Distribution and variation of five fluorescent components along the sampling gradient (S1–S9).

(C2) components. The longer wavelength excited humic-like (C4, C5) components showed no obvious variation. However, during the second aeration process, a large decrease was observed for protein-like component (81%), followed by two humic-like component (48% for C2 and 24% for C4) (Fig. 4). Xenobiotic and humic-like component C5 showed small decrease (< 6%). From this tank to the outlet, only C2 and C3 components showed relatively large decrease (13%–15%). Through the whole treatment process, protein-like (C1), short wavelength excited humic-like (C2) and xenobiotic (C3) components were highly degraded, while the longer wavelength excited humic-like (C4, C5) components just showed low degree of degradation. The ratio of C1/C4 and C1/C5 decreased significantly from 4.2 to 0.9 and 7.5 to 1.5, respectively. These results support the previous suggestions to use protein-like fluorescence for the determination of wastewater BOD (Reynolds and Ahmad, 1997) and detection of contamination events in recycled water systems (Henderson et al., 2009).

2.3 Variations of DOC and adsorption parameters during sewage process

The high DOC and a_{350} concentrations of the original sewage showed small variation during the primary treatment stage. This might be expected as the major role of this primary stage sedimentation. However, during the secondary treatment stage, DOC concentration showed an obvious decrease (ca. 80%) while the decrease of absorption coefficient a_{350} was only about 30% (Fig. 5a, b). When compared with the significant loss of protein-like (C1) and short wavelength-excited humic-like (C2) fluorescent component, it can be inferred that C1 and C2 fluorescent components would be the dominant constituents of sewage DOM which were labile and can be easily degraded during aeration process. This can be further supported by the very good relationship between C1 and DOC (r = 0.99, n = 9) and C2 and DOC (r = 0.97, n = 9).

On the other hand, the relatively small decrease of both a_{350} and two longer wavelength excited humic-like (C4, C5) fluorescent components from the primary to the secondary treatment stage suggested that the two components may show much close relationship with a_{350}, which was supported by good correlation between C4 and a_{350} (r = 0.96, n = 9) and C5 and a_{350} (r = 0.83, n = 9). This is to be expected when considering the excitation characteristics of the fluorescent components (Fig. 2).

The large variation in DOC and a_{350} concentrations and contents of fluorescence components during the treatment process implied that the relative molecular size of DOM may be also changed, which was convinced by indicators as spectral slope S (300–650 nm) value, the slope ratio S_R, E2:E3 and specific absorption at 280 nm (calculated by a_{280} divided by DOC). The reliability of these optical parameters to reflect DOM molecular size was verified by size exclusion chromatography (SEC) (De Haan and De Boer, 1987; Chin et al., 1994; Helms et al., 2008). The higher S value (Fig. 5c), low specific absorption (Fig. 5d) and higher S_R and E2:E3 ratios for S1–S3 samples suggested that the relative molecular size should be lower for these DOM samples which had high proportion of degradable protein-like components. The following decrease of S and S_R and increase of specific absorption indicated the molecular size of DOM for S4–S6 samples increased along the treatment process. This is consistent with the recalcitrant nature of these DOM which were not effectively degraded even during the aeration process.

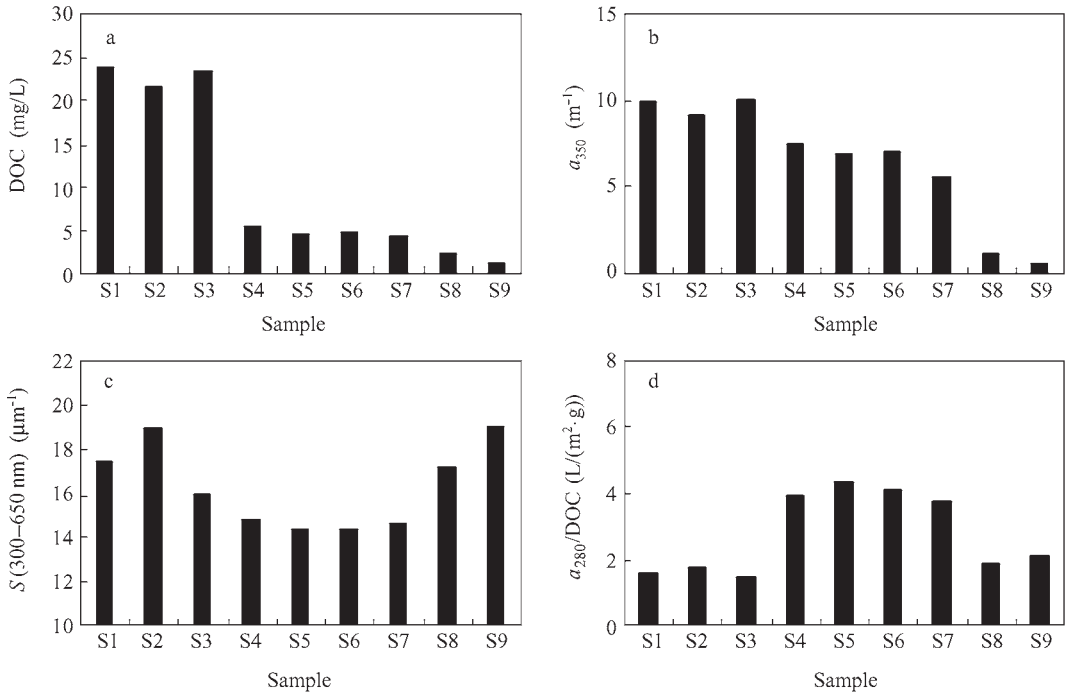

Fig. 5 Distribution and variation of DOC concentration (a), absorption coefficient a_{350} (b), spectral slope S (300–650 nm) (c) and specific absorption at 280 nm (d) along the sampling gradient (S1–S9).

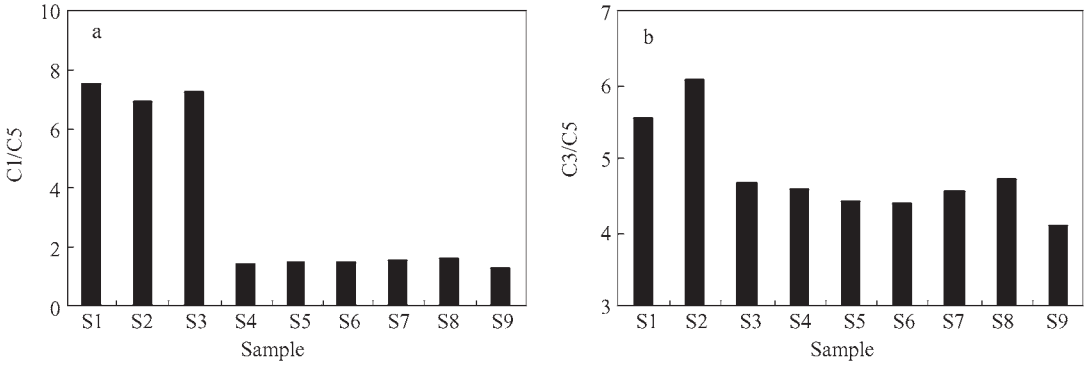

Fig. 6 Variation of the ratio of the protein (a), and xenobiotic (b) to humic fluorescence along the sampling gradient (S1–S9).

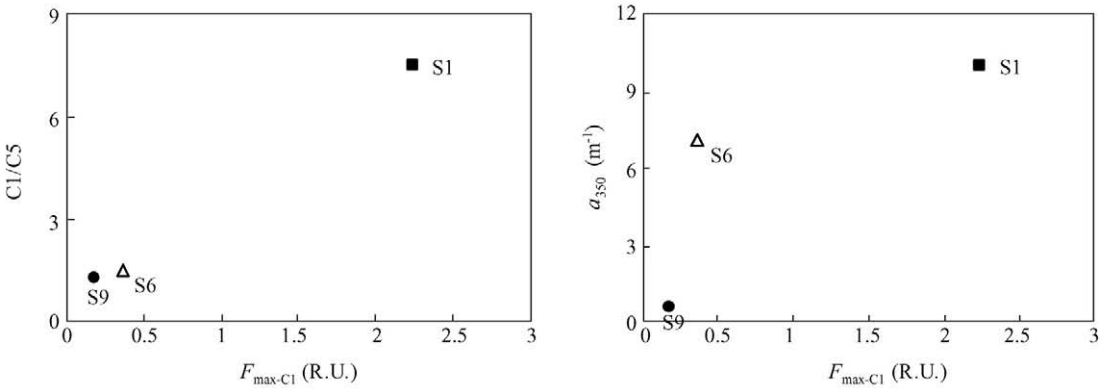

Fig. 7 Comparison of raw sewage (S1), treated sewage (S6) and background seawater (S9).

2.4 Spectroscopic indication of the sewage sources in aquatic environment

The obvious differences in DOM optical properties between treated and untreated sewage with seawater provide a useful spectroscopic indicator to trace the sewage sources in aquatic environment. The untreated sewage has high concentration of DOC, a_{350} and protein-like component (C1) compared with those of seawater (Table 2). It also has very high ratio of protein-like component to visible humic-like component (C1/C5) (Fig. 6a). As a result, the content of protein-like fluorescence (C1) and C1/C5 can be used as an indicator for the input of sewage organic pollution in various aquatic environments (Fig. 7). Since CDOM contents in high salinity sea area of Xiamen Bay showed no obvious seasonal variation (Guo et al., 2007), if we take S9 as the background seawater sample, it is easy to quantify the relative amount of raw sewage in a marine sample using a simple calculation. This can be easily satisfied with monitoring the fluorescence intensity at excitation/emission wavelength of both 275 nm/339 nm and 380 nm/467 nm in normal or in-situ investigation.

Table 2 Ratio of optical parameters and DOC between raw or treated sewage and seawater

Parameter	Raw sewage (S1)/ Seawater (S9)	Treated sewage (S6)/ Seawater (S9)
C1	12.1	2.0
C2	3.50	1.4
C3	2.84	1.9
C4	3.40	2.6
C5	2.10	1.7
a_{350}	16.7	11.9
DOC	19.7	4.0

This indicator system is not suitable for tracing the treated sewage in aquatic environment as the difference of its C1/C5 ratio with background seawater is very small. However, the difference in absorption coefficient at 350 nm (a_{350}) between them is significant. Hence, the combination use of the content of protein-like fluorescence (C1) and a_{350} may provide a quantification indication for the percent amount of treated sewage for a marine sample in sewage-influenced environment (Fig. 7).

3 Conclusions

Excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC) of wastewater DOM from a sewage treatment plant revealed high concentration of degradable protein-like (C1) fluorescence and the occurrence of xenobiotic-like (C3) component. This technique provides a fast and sensitive way to monitor the qualitative and quantitative variation of DOM during the whole sewage treatment process. The combination use of the protein-like (C1), the ratio of protein-like to visible humic-like component (C1/C5) and absorption coefficient a_{350} may provide quantification tracer for the relative amounts of raw or treated sewage for any marine sample. This indicator system may further be used in the on-line or in situ monitoring of organic pollution in aquatic environment.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 40776041, 40676046), the National High Technology Research and Development Program of China (No. 2007AA091704) and the Program for New Century Excellent Talents in Fujian Province University. The authors would like to thank two reviewers for their constructive comments on the manuscript.

References

Baker A, 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. *Water Research*, 36(1): 189–195.

Baker A, Curry M, 2004. Fluorescence of leachates from three contrasting landfills. *Water Research*, 38(10): 2605–2613.

Chin Y P, Alken G, O'Loughlin E, 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environmental Science and Technology*, 28(11): 1853–1858.

Coble P G, 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, 51(4): 325–346.

De Haan H, De Boer T, 1987. Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic Laken Tjeukemeer. *Water Reserach*, 21(6): 731–734.

Determann S, Reuter R, Wagner P, Willkomm R, 1994. Fluorescent matter in the eastern Atlantic Ocean. I: Methods of measurement and near-surface distribution. *Deep-Sea Research Part I*, 41(4): 659–675.

Guo W D, Stedmon C A, Han Y C, Wu F, Cheng Y Y, Hu M H, 2007. The conservative and non-conservative behavior of chromophoric dissolved organic matter in Chinese estuarine waters. *Marine Chemistry*, 107(3): 357–366.

Helms J R, Stubbins A, Ritchie J D, Minor E C, Kieber D J, Mopper K, 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. *Limnology and Oceanography*, 53(3): 955–969.

Henderson R K, Baker A, Murphy K R, Hambly A, Stuetz R M, Khan S J, 2009. Fluorescence as a potential monitoring tool for recycled water systems: A review. *Water Research*, 43(4): 863–881.

Holbrook R D, Yen J H, Grizzard T J, 2006. Characterizing natural organic material from the Occoquan Watershed (Northern Virginia, U.S.) using fluorescence spectroscopy and PARAFAC. *Science of the Total Environment*, 361(1-3): 249–266.

Kowalczuk P, Cooper W J, Whitehead R F, Durako M J, Sheldon W, 2003. Characterization of CDOM in an organic-rich river and surrounding coastal ocean in the South Atlantic Bight. *Aquatic Sciences*, 65(4): 384–401.

Kowalczuk P, Cooper W J, Durako M J, Kahn A E, Gonsior M, 2009. Characterization of dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: Interannual variability. *Marine Chemistry*, 113(3-4): 182–196.

Lu F, Chang C H, Lee D J, He P J, Shao L M, Su A, 2009. Dissolved organic matter with multi-peak fluorophores in landfill leachate. *Chemosphere*, 74(4): 575–582.

Mayer L M, Schick L L, Loder T C, 1999. Dissolved protein fluorescence in two maine estuaries. *Marine Chemistry*, 64(3): 171–179.

Murphy K R, Ruiz G M, Dunsmuir W T M, Waite T D, 2006. Optimized parameters for fluorescence-based verification of ballast water exchange by ships. *Environmental Science and Technology*, 40 (7): 2357–2362.

Murphy K R, Stedmon C A, Waite T D, Ruiz G, 2008. Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. *Marine Chemistry*, 108(1-2): 40–58.

Reynolds D M, Ahmad S R, 1997. Rapid and direct determination of wastewater BOD values using a fluorescence technique. *Water Research*, 31(8): 2012–2018.

Saadi I, Borisover M, Armon R, Laor Y, 2006. Monitoring of effluent DOM biodegradation using fluorescence, UV and DOC measurements. *Chemosphere*, 63(3): 530–539.

Stedmon C A, Markager S, Kaas H, 2000. Optical properties and signatures of chromophoric dissolved organic matter (CDOM) in Danish coastal waters. *Estuarine Coastal Shelf Science*, 51(2): 267–278.

Stedmon C A, Markager S, Bro R, 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry*, 82(3-4): 239–254.

Stedmon C A, Markager S, 2005. Resolving the variability in dissolved organic matter fluorescence in a temperate estuary and its catchment using PARAFAC analysis. *Limnology and Oceanography*, 50(2): 686–697.

Stedmon C A, Markager S, Tranvik L, Kronberg L, Slatis T, Martinsen W, 2007. Photochemical production of ammonium and transformation of dissolved organic matter in the Baltic Sea. *Marine Chemistry*, 104 (3-4): 227–240.

Stedmon C A, Bro R, 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnology and Oceanography: Methods*, 6: 572–579.

Teymouri B, 2007. Fluorescence spectroscopy and parallel factor analysis of waters from municipal waste sources. University of Missouri, Columbia, USA.

Tucker S A, Amszi V L, Acree W E, 1992. Primary and secondary inner filtering – effect of $K_2Cr_2O_7$ on fluorescence emission intensities of quinine sulfate. *Journal of Chemical Education*, 69(1): A8.

Wang Z G, Liu W Q, Zhao N J, Li H B, Zhang Y J, Si-Ma W C et al., 2007. Composition analysis of colored dissolved organic matter in Taihu Lake based on three dimension excitation-emission fluorescence matrix and PARAFAC model, and the potential application in water quality monitoring. *Journal of Environmental Sciences*, 19(7): 787–791.

Weishaar J L, Aiken G R, Bergamaschi B A, Fram M S, Fugii R, Mopper K, 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environmental Science and Technology*, 37(20): 4702–4708.

Yan Y, Li H, Myrick M L, 2000. Fluorescence fingerprint of waters: Excitation emission matrix spectroscopy as a tracking tool. *Applied Spectroscopy*, 54(10): 1539–1542.

Zhang Y L, van Dijk M A, Liu M L, Zhu G W, Qin B Q, 2009. The contribution of phytoplankton degradation to chromophoric dissolved organic matter (CDOM) in eutrophic shallow lakes: Field and experimental evidence. *Water Research*, 43(18): 4685–4697.