

Spectroscopic characterization of dissolved organic matter in coking wastewater during bio-treatment: full-scale plant study

Ronghua Xu, Huase Ou, Xubiao Yu, Runsheng He, Chong Lin and Chaohai Wei

ABSTRACT

This paper taking a full-scale coking wastewater (CWW) treatment plant as a case study aimed to characterize removal behaviors of dissolved organic matter (DOM) by UV spectra and fluorescence excitation-emission matrix-parallel factor analysis (PARAFAC), and investigate the correlations between spectroscopic indices and water quality parameters. Efficient removal rates of chemical oxygen demand (COD), dissolved organic carbon (DOC) and total nitrogen (TN) after the bio-treatment were 91.3%, 87.3% and 69.1%, respectively. UV₂₇₀ was proven to be a stable UV absorption peak of CWW that could reflect the mixture of phenols, heterocyclics, polynuclear aromatic hydrocarbons and their derivatives. Molecular weight and aromaticity were increased, and also the content of polar functional groups was greatly reduced after bio-treatment. Three fluorescent components were identified by PARAFAC: C1 (tyrosine-like), C2 (tryptophan-like) and C3 (humic-like). The removal rate of protein-like was higher than that of humic-like and C1 was identified as biodegradable substance. Correlation analysis showed UV₂₇₀ had an excellent correlation with COD ($r = 0.921$, $n = 60$, $P < 0.01$) and DOC ($r = 0.959$, $n = 60$, $P < 0.01$) and significant correlation ($r = 0.875$, $n = 60$, $P < 0.01$) was also found between C2 and TN. Therefore, spectroscopic characterization could provide novel insights into removal behaviors of DOM and potential to monitor water quality real-time during CWW bio-treatment.

Key words | coking wastewater, fluorescence excitation-emission matrix, parallel factor analysis, UV spectra, water quality monitoring

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LIST OF ABBREVIATIONS AND SYMBOLS

CWW	coking wastewater	CDOM	chromophoric dissolved organic matter
CWWTP(s)	coking wastewater treatment plant(s)	UV ₂₅₄	ultraviolet absorbance at 254 nm
DOM	dissolved organic matter	SUVA	specific UV absorbance at 254 nm
EEM	excitation-emission matrix	A _{240–400}	integral area of UV spectra calculated from 240 to 400 nm
PARAFAC	parallel factor analysis	S _R	ratio of the slope of the 275–295 nm region and that of the 350–400 nm region
COD	chemical oxygen demand	QSU	quinine sulfate unit
DOC	dissolved organic carbon		
TN	total nitrogen		
HRT	hydraulic retention time		
DO	dissolved oxygen		
PAH(s)	polynuclear aromatic hydrocarbon(s)		
HC(s)	heterocyclic(s)		
A/O ¹ /O ²	anoxic/aerobic ¹ /aerobic ²		
A/O ¹ /H/O ²	anoxic/aerobic ¹ /hydrolysis/aerobic ²		

INTRODUCTION

Coal is the main energy resource in China. The wide exploitation and chemical processes of it produce a hazardous

waste called coking wastewater (CWW), which brings about a series of severe environmental problems. CWW is composed of complex inorganic and organic contaminants such as ammonia, cyanide, thiocyanide, phenolic compounds, polynuclear aromatic hydrocarbons (PAHs), polycyclic nitrogen-, oxygen- and sulfur-containing heterocyclics (HCs) and acyclic compounds, most of which are carcinogenic, mutagenic and toxic contaminants (Zhang *et al.* 2010). It triggers severe environmental problems to aquatic organisms and human health without reasonable treatment. Therefore, it's essential to efficiently remove these contaminants in CWW to reduce their hazard.

Biological treatment is by far the most widely applied and cost-effective process for CWW treatment plants (CWWTPs). However, good performance in carbon and nitrogen removal is difficult to achieve because of toxic pollutants' inhibition of various biological reactions (Staib & Lant 2007). Many researchers focus on the development of efficient bio-treatment techniques for CWW, but most bio-treatment techniques are just applied at laboratory or pilot scale (Marañón *et al.* 2008; Li *et al.* 2011). To date, few CWWTPs operated successfully at full scale are reported. Anoxic/aerobic¹/aerobic² (A/O¹/O²) (Ou *et al.* 2014) and anoxic/aerobic¹/hydrolysis/aerobic² (A/O¹/H/O²) (Zhang *et al.* 2012) coupled with an internal-loop biological fluidized-bed system, in which the added H process is used to improve the biodegradability of O¹ effluent, were first developed by our research group to treat CWW. They are now successfully applied in a full-scale CWWTP in south China.

Chemical oxygen demand (COD), dissolved organic carbon (DOC) and total nitrogen (TN) are usually chosen to evaluate the performance of carbon and nitrogen removal. However, these variables cannot provide any compositional and structural information of organic substances with complex and heterogeneous nature. Organic matter, especially dissolved organic matter (DOM), which is a heterogeneous mixture of aromatic, amino and aliphatic organic compounds containing oxygen, nitrogen and sulfur functional groups (Chen *et al.* 2003), has been a critical research issue because of its multifunctional role in the aquatic environment (Murphy *et al.* 2011). Therefore, investigating DOM in CWW during bio-treatment could provide much more compositional and structural information as well as removal behaviors of DOM in CWW. Moreover, phenols, PAHs and HCs in CWW (Zhang *et al.* 2013) and soluble microbial byproducts generated from the microbial reactions in wastewater bio-treatments (Li *et al.* 2008) are typical chromophoric dissolved organic matter (CDOM). Spectroscopic analyses, such as UV spectroscopy and fluorescence

excitation-emission matrix (EEM) spectroscopy, can be ideal methods to identify and characterize DOM in CWW. Besides, parallel factor analysis (PARAFAC) can provide quantitative data of EEMs of DOM (Bro 1997). However, most of the reported UV spectroscopy and EEM analysis applied in CWW treatment just focus on DOM removal efficiency in laboratory-scale tests (Sun *et al.* 2008; Wang *et al.* 2008; Zhao *et al.* 2009). Variations of specific UV absorption peaks and fluorescence components of CWW from a full-scale CWWTP have not been fully explored, and the potential application of DOM-specific UV absorption peaks and fluorescence components for real-time monitoring of CWW quality parameters are lacking attention.

Taking a full-scale CWWTP with an A/O¹/H/O² fluidized-bed system as a case study, the objectives of this study are as follows: first, the performance of carbon and nitrogen removal during the bio-treatment processes is examined; second, UV spectroscopy and EEM coupled with PARAFAC are developed to observe the evolution and behaviors that occur in the DOM fraction; then, the correlations between UV indices, fluorescent components and bulk wastewater quality parameters such as COD, DOC and TN are investigated using statistical analysis. These results can provide novel insights into the characteristics and removal behaviors of DOM during CWW bio-treatment and an effective monitoring technique to estimate CWW quality parameters in full-scale CWWTPs.

MATERIALS AND METHODS

Sample collection

The samples were collected from a CWW treatment plant, with a designed treatment capacity of 2,000 m³ day⁻¹, which was located in Bao Steel, Shaoguan, Guangdong Province, China. Some pictures of the CWWTP are shown in Supplementary Figure S1 (available online at <http://www.iwaponline.com/wst/072/333.pdf>).

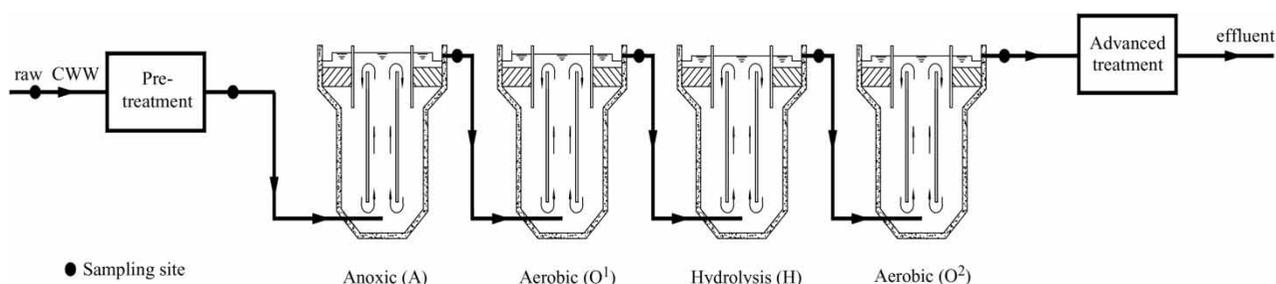
The treatment processes consisted of pretreatment, bio-treatment and advanced treatment. The bio-treatment process was an A/O¹/H/O² system coupled with three internal-loop biological fluidized beds. Major operating parameters, functions and main reactions of the A/O¹/H/O² three internal-loop biological fluidized beds are shown in Table 1.

The water samples were collected from raw CWW, bio-influent and effluent of each stage in A/O¹/H/O². Figure 1 is a schematic diagram of the sampling sites. At each site, 2 L of water sample was collected using glass bottles which were

Table 1 | Major operating parameters of the A/O¹/H/O² bio-treatment processes

	HRT (h)	DO (mg·L ⁻¹)	COD volume loading (kg·m ⁻³ ·day ⁻¹)	Addition	Function	Representative reactions
A	28	0.15 ± 0.08	1.60	NaH ₂ PO ₄	Anaerobic hydrolysis	X-Y + H ₂ O → X-H + Y-OH
O ¹	12	1.02 ± 0.56	2.43	-	Decarbonization and ammoniation	C ₆ H ₅ OH + 7O ₂ → 6CO ₂ + 3H ₂ O SCN ⁻ + 3H ₂ O → CO ₂ + H ₂ S + NH ₃ + OH ⁻
H	20	0.43 ± 0.09	0.44	NaOH, Na ₂ CO ₃	Denitrification	NH ₄ ⁺ + NO ₂ ⁻ → N ₂ + 2H ₂ O
O ²	16	3.46 ± 0.61	0.44	NaOH, Na ₂ CO ₃	Nitrification	NH ₄ ⁺ + 1.5O ₂ → NO ₂ ⁻ + 2H ⁺ + 2H ₂ O NO ₂ ⁻ + 0.5O ₂ → NO ₃ ⁻

X-Y: carboxylic acid esters, nitriles and polysaccharides, etc.

**Figure 1** | Schematic diagram of sampling sites and simplified coking wastewater treatment with an A/O¹/H/O² bio-treatment system coupled with three internal-loop biological fluidized beds.

acid-washed and pre-rinsed with sample water. Sampling was conducted monthly from March to December 2012. All samples were stored at 2–4 °C prior to analysis.

Analytical method

All samples were filtered through glass fiber filters (Whatman GF/F, 0.45 μm, pre-combusted at 450 °C for 4 h) prior to analysis. The DOC concentrations were measured by a total organic carbon analyzer (TOC-V_{CPN}, Shimadzu, Japan). COD and TN were determined according to standard methods (SEPA 2002).

For optical measurements, the water samples were diluted 10 times with Milli-Q water and the pH values of diluted solutions were adjusted to 7.0 ± 0.2. UV spectra were measured by a UV-Vis spectrophotometer (Shanghai Unico Instrument Co., Ltd) and measurements were baseline corrected using Milli-Q water. UV₂₅₄ represents the aromatic content of a sample, and the specific ultraviolet absorbance (SUVA) is defined as the ratio of UV₂₅₄ and DOC of the sample, which is an index of aromaticity of the organic matter. A_{240–400} is the integral area of UV spectra calculated from 240 to 400 nm. S_R is the ratio of the slope of the 275–295 nm region (S_{275–295}) and that of the 350–400 nm region (S_{350–400}) (Helms *et al.* 2008).

EEM fluorescence spectra were recorded using a fluorescence spectrometer (F-7000, Hitachi High-Technologies, Japan). The fluorescence intensity values were standardized in quinine sulfate units (QSU) (Yamashita & Tanoue 2003). Prior to analysis, the Raman scatter and Rayleigh scatter effects should be removed; the removal method refers to a previous study (Yao *et al.* 2011).

PARAFAC modeling

EEM is modeled using PARAFAC, which uses an alternating least squares algorithm to minimize the sum of squared residuals across the dataset and estimate the underlying structure of the EEM (Bro 1997). The data signal was decomposed into a set of trilinear terms and a residual array. In the current study, EEM spectrograms were combined into a three-dimensional data array: 60 samples × 51 excitations × 136 emissions. After removing effects of the scatter effects, the PARAFAC analysis was performed in MATLAB 7.7 using the DOMFlour toolbox (Stedmon & Bro 2008). In this study, PARAFAC modeling with two to seven components was conducted, and the number of fluorescent components was determined according to split half analysis, residual analysis and visual inspection.

RESULTS AND DISCUSSION

Removal of carbon and nitrogen

Figure 2 represents the removal profile of COD, DOC and TN in CWW during bio-treatment. The COD presents a decreasing trend during the whole bio-treatment stage. The largest decline happens in the O¹ stage, where it decreases from $1,937 \pm 259$ to 361 ± 30.7 mg L⁻¹. The degradation of phenols and reducing inorganic matter results in this phenomenon (Ou et al. 2014). Negligible decreases of COD are observed in the A and H stages. It could be concluded that the anoxic stage only changed the structures of organic matter in CWW. After the O² stage, COD decreases from 283 ± 31.4 to 176 ± 15.9 mg L⁻¹. Meanwhile, the variation tendency of DOC is similar to that of COD. After bio-treatment, 91.3% COD and 87.3% DOC were removed. Slight decreases of TN are observed in the A stage, where it decreases from 202 ± 16.5 to 191 ± 24.6 mg L⁻¹. And it reduces by about 60 mg L⁻¹, 55 mg L⁻¹ and 20 mg L⁻¹ in the O¹, H and O² stages, respectively. The reduction of TN in the aerobic stages and the hydrolysis stage is mainly due to nitrogenous compounds as an N source utilized by aerobic micro-organisms according to the ratio of biochemical oxygen demand (BOD): N: P = 100: 5: 1 and denitrification process, respectively. In short, 69.1% TN in CWW was removed during the whole bio-treatment processes.

UV spectra analysis

The UV spectrum of CWW during bio-treatment is shown in Figure 3. An intense absorption band from 200 nm to 400 nm is observed, and two absorption peaks occur in the range of 200–250 nm and 250–300 nm in raw CWW,

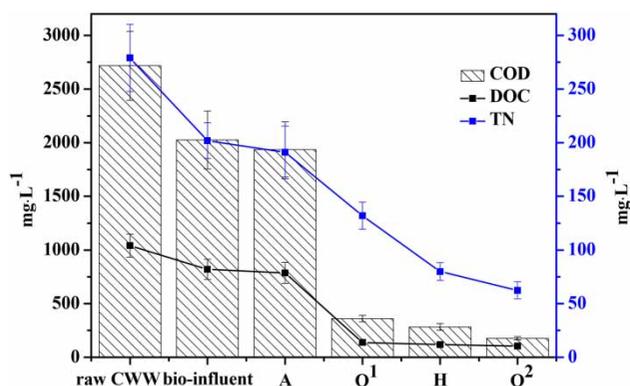


Figure 2 | Variations of COD, DOC and TN in A/O¹/H/O² bio-treatment.

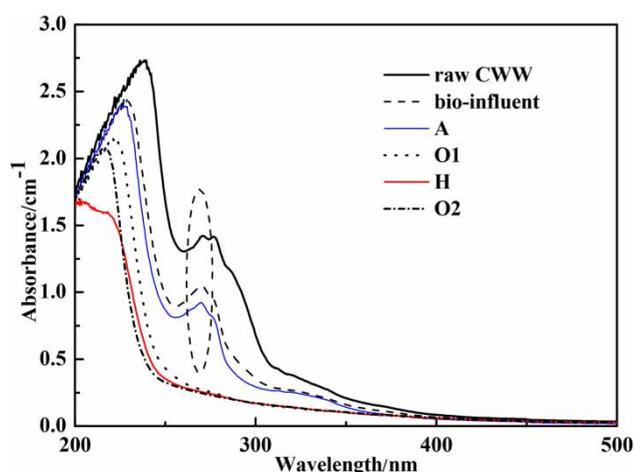


Figure 3 | UV spectra of CWW in the A/O¹/H/O² bio-treatment.

respectively. According to previous studies and UV spectroscopy laws (Wang et al. 2012), it can be concluded that considerable amounts of single-ring aromatic compounds and relatively fewer PAHs and HCs existed in raw CWW. The absorbance decreased slightly after the A stage and it reduced significantly in the O¹ stage. No obvious variations were observed in the H and O² stages.

To investigate the UV characteristics during CWW bio-treatment in detail, UV₂₅₄, SUVA, S_R, and A_{240–400} were measured (Table 2). Of note, the absorption peak between 200 and 250 nm showed a blue shift during bio-treatment, while the absorption peak presenting in the region of 250–300 nm, which appeared around 270 nm, had a relatively fixed position. Furthermore, according to our long-term monitoring of CWW and evidence from some related literature (Wang et al. 2008; Zhang et al. 2010), UV₂₇₀ is found as a stable absorption peak existing in the UV spectra of CWW, and it is regarded as an alternative indicator of phenolic compounds (Wang et al. 2008). Moreover, the E₂ absorption band of PAHs and HCs also makes a contribution to the absorption peak at 270 nm. Therefore, UV₂₇₀ was also measured as a characteristic index.

As shown in Table 2, after bio-treatment, the UV₂₅₄ decreases from 10.06 ± 1.32 cm⁻¹ in the bio-influent to 3.03 ± 0.27 cm⁻¹ in the O² effluent. In contrast to this, the DOC decreases by 87.3%. Because the overall UV₂₅₄ removal rate is lower than the DOC removal rate, the biological treatment (except H) stage resulted in an increase of the SUVA from 1.23 ± 0.14 L mg⁻¹ m⁻¹ to 2.91 ± 0.24 L mg⁻¹ m⁻¹, indicating that the less UV-absorbing fraction of the DOC was removed preferentially during biological treatment. The result is consistent with the previous studies (Krasner et al. 2009). Some HCs and PAHs

Table 2 | The values of UV₂₅₄, SUVA, UV₂₇₀, S_R and A_{240–400} at different treatment stages during A/O¹/H/O² bio-treatment

	UV ₂₅₄ (cm ⁻¹)	SUVA (L·mg ⁻¹ ·m ⁻¹)	UV ₂₇₀ (cm ⁻¹)	S _R	A _{240–400}
Raw CWW	13.65 ± 1.50	1.31 ± 0.13	13.92 ± 1.35	3.02 ± 0.18	110 ± 16
Bio-influent	10.06 ± 1.32	1.23 ± 0.14	10.51 ± 1.21	3.32 ± 0.23	87 ± 12
A	9.95 ± 1.04	1.27 ± 0.12	9.20 ± 1.08	2.85 ± 0.14	76 ± 8
O ¹	3.82 ± 0.45	2.75 ± 0.27	2.73 ± 0.41	1.24 ± 0.10	31 ± 5
H	3.21 ± 0.21	2.72 ± 0.16	2.55 ± 0.24	1.26 ± 0.10	29 ± 3
O ²	3.03 ± 0.27	2.91 ± 0.24	2.49 ± 0.20	0.96 ± 0.12	26 ± 4

degraded by ring-opening reaction may contribute to the slight reduction of SUVA in the H stage. A similar variation trend with UV₂₅₄ during bio-treatment suggested that organics which UV₂₇₀ and UV₂₅₄, respectively, represented in CWW may have the same origin.

S_R can be used to track changes in the relative size of DOM, and the value is inversely proportional to molecular weight (MW) (Helms *et al.* 2008). The S_R decreased from 3.32 ± 0.23 in the bio-influent to 0.96 ± 0.12 in the O² effluent. It meant that MW of CWW showed an obvious increasing trend during bio-treatment except in the H stage. Our results agreed with some previous studies (Park *et al.* 2010; Esparza-Soto *et al.* 2011). This phenomenon may be caused by the following reasons. On the one hand, lower MW fractions were degraded, whereas relatively high MW components remained during the process; on the other hand, high MW substances were formed by micro-organisms during the biological degradation process. Reduction of MW in the H stage is attributed to hydrolysis of high MW organics. This was exactly consistent with the previous explanation on SUVA.

As A_{240–400} value is largely affected by the presence of polar functional groups, it can represent the content of polar functional groups, such as hydroxyl, carbonyl, carboxyl and ester groups (He *et al.* 2011). A_{240–400} decreased from 110 ± 16 in the bio-influent to 26 ± 4 in the O² effluent. This indicated that the content of oxygen-containing polar functional groups had been greatly reduced after the bio-treatment.

EEM spectra analysis

EEM spectroscopy properties

The EEM spectra of CWW during the whole bio-treatment are presented in Figure 4. Based on the 'peak-picking' method proposed by (Coble 1996), there were five distinct fluorescence peaks observed in the EEM spectra of the raw CWW: Peak A – fulvic-like ($\lambda_{\text{Ex/Em}} = 250/414$ nm), Peak B – tyrosine-like

($\lambda_{\text{Ex/Em}} = 270/300$ nm), Peak C – humic-like ($\lambda_{\text{Ex/Em}} = 350/434$ nm), Peak T1 – tryptophan-like ($\lambda_{\text{Ex/Em}} = 285/346$ nm) and Peak T2 – tryptophan-like ($\lambda_{\text{Ex/Em}} = 255/348$ nm) (Coble 1996; Chen *et al.* 2003; Hudson *et al.* 2008). The location and intensity of fluorescence peaks of CWW were significantly different from those in a previous study (Ou *et al.* 2014), and this may be attributed to different coal quality, pyrolysis temperature and coking process. Protein-like substances, whose intensity was much higher than humic-like substances, were the main fluorophore in CWW. From the overall bio-treatment, the protein-like removal rate was higher than that of humic-like. Generally, Peaks B and T were common in untreated wastewater, which had a high potential of being oxidized for monitoring the removal efficiency of DOM (Henderson *et al.* 2009). However, fluorescence intensities of Peak B were strengthened in the A stage. It is presumed to be soluble microbial byproduct materials (Chen *et al.* 2003). The contribution of soluble microbial products and extracellular polymers released by anoxic microorganisms could help to understand this phenomenon.

PARAFAC of EEMs

The above-mentioned 'peak-picking' method could only provide a qualitative description of EEM and it could not avoid the spectral overlapping effectively. PARAFAC could realize 'mathematical separation' of the fluorescent components in CWW and analyze them quantitatively. According to split half analysis, residual analysis and visual inspection, PARAFAC modeling of the 60 CWW samples revealed that the EEM of CWW could be characterized by three different fluorescent components (Supplementary Figure S2, available online at <http://www.iwaponline.com/wst/072/333.pdf>). Component 1 (C1) exhibited one peak with the $\lambda_{\text{Ex/Em}} = 270/298$ nm, component 2 (C2) showed primary and secondary peaks with the $\lambda_{\text{Ex/Em}} = 245$ and 285/356 nm, and component 3 (C3) also included two peaks with the $\lambda_{\text{Ex/Em}} = 250$ and 325/432 nm. Based on the previous studies, C1 was

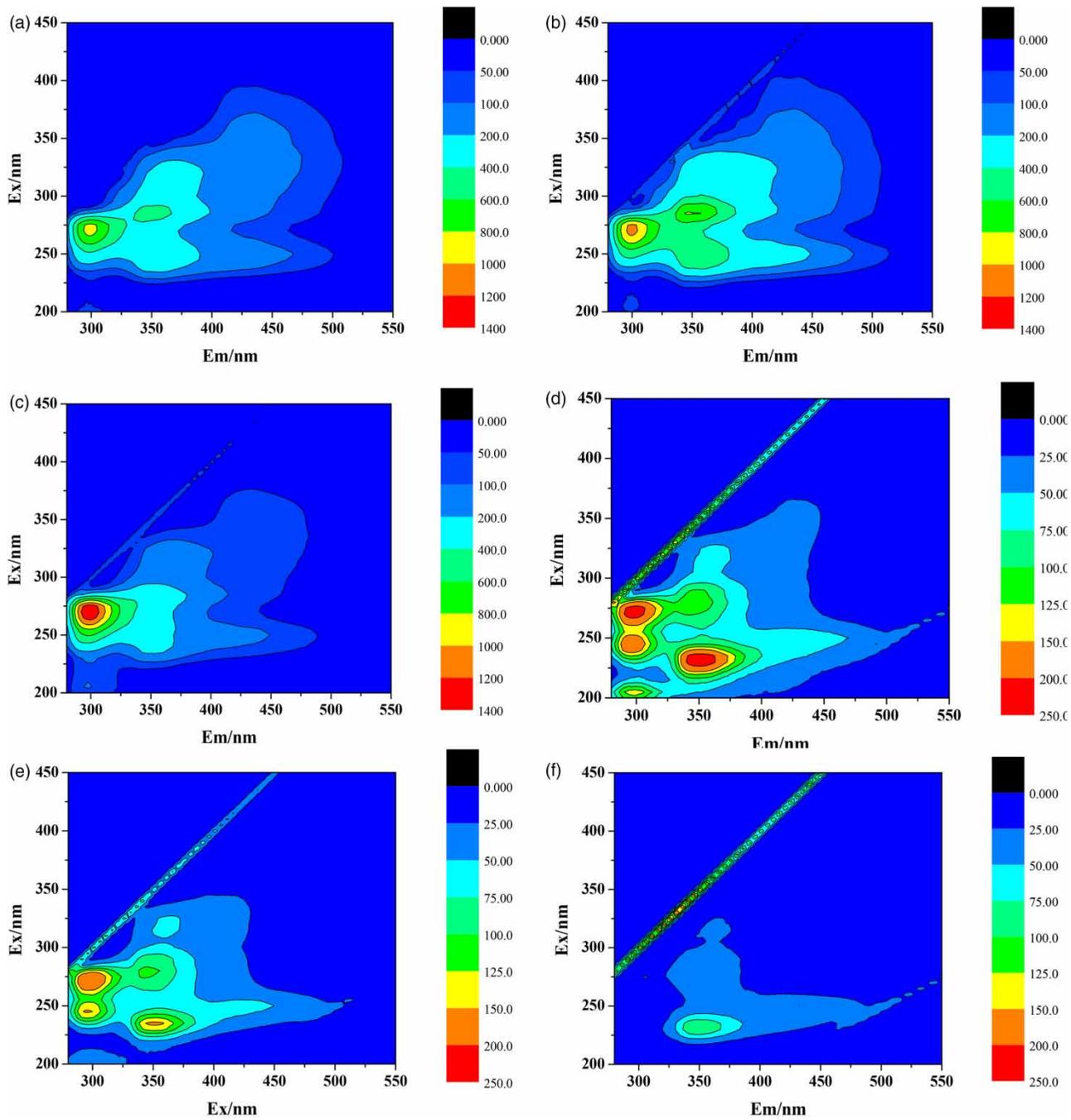


Figure 4 | EEM spectra of CWW in the A/O¹/H/O² bio-treatment ((a) raw CWW; (b) bio-influent; (c) A; (d) O¹; (e) H; (f) O²).

associated with tyrosine-containing matter, which might be related to phenols ($\lambda_{\text{Ex/Em}} = 265\text{--}270/298\text{ nm}$) (Lu *et al.* 2009; Ou *et al.* 2014), PAHs (Alarcón *et al.* 2013) and soluble microbial byproduct materials (Chen *et al.* 2003). C2 was likely related to tryptophan-like material. The characteristic fluorescent peaks of aniline ($\lambda_{\text{Ex/Em}} = 245, 275/340\text{ nm}$) and some PAHs, such as naphthalene (265/336 nm), were all located in this region. C3 was termed humic-like material.

Fluorescence emitted by HCs, such as quinolone ($\lambda_{\text{Ex/Em}} = 310\text{--}340/380\text{--}440\text{ nm}$), and PAHs, such as benzo[g,h,i]perylene ($\lambda_{\text{Ex/Em}} = 300/420\text{ nm}$) (Burich *et al.* 1974), were considered to contribute to C3. The EEMs of phenols, aniline and quinolone are shown in Supplementary Figure S3 (available online at <http://www.iwaponline.com/wst/072/333.pdf>). Therefore, C2 and C3 reflected the mixture of HCs, PAHs, amines and their derivatives in CWW.

The behaviors and fates of C1, C2 and C3 extracted by PARAFAC were tracked by their maximum fluorescence intensities (Fmax) during bio-treatment. Fmax was considered to be proportional to the relative concentrations of different components (Henderson *et al.* 2009). Figure 5 shows the Fmax changes of C1, C2 and C3 during the A/O¹/H/O² bio-treatment. In raw CWW, the Fmax of C1 (556 ± 57 QSU) and C2 (362 ± 34 QSU) were much higher than that of humic-like (Fmax of C3: 110 ± 22 QSU), which indicated that C1 and C2 were considered as the main fluorescent components in CWW. Fmax of C2 and C3 reduced continuously from 233 ± 36 QSU to 82 ± 10 QSU in bio-influent to 43 ± 6 QSU and 18 ± 3 QSU in O² effluent, respectively. However, Fmax of C1 increased from 582 ± 77 QSU in bio-influent to 664 ± 69 QSU after the A stage. Fluorescence of soluble microbial byproduct released by anoxic microorganisms contributed to the variation of C1 in the A stage, and it exactly coincided with the explanation on Peak B mentioned above. During the bio-treatment, the removal rate of C1, C2 and C3 was 96.9%, 81.5% and 78.0%, respectively, suggesting that the protein-like material was degraded more easily than the humic-like material. These results were also consistent with

research on municipal wastewater (Yu *et al.* 2013). Moreover, the three different fluorescent components were removed to the greatest degree at the O¹ stage. Of note, the C1/C3 decreased from 7.10 to 1.00 during the bio-treatment, while the C2/C3 decreased from 2.84 to 2.39. Although both of them decreased, the decline of C1 was more remarkable than that of C2, suggesting that the substances which C1 represented were easier to biodegrade. The result showed that a tyrosine-like component, rather than a tryptophan-like component as proposed by previous studies (Reynolds & Ahmad 1997; Hudson *et al.* 2008), could be an alternative bioavailable organic matter in CWW.

Correlation analysis

The correlations between spectroscopic indices and bulk water quality parameters had been investigated by some researchers aiming at developing rapid and reliable monitoring techniques. For example, prediction of COD, BOD and TN of a typical urban river is determined by EEM-PARAFAC combined with UV spectra analysis (Hur & Cho 2012); fluorescent components provide indications for reduction in COD and the total microbial activity in wastewater (Cohen

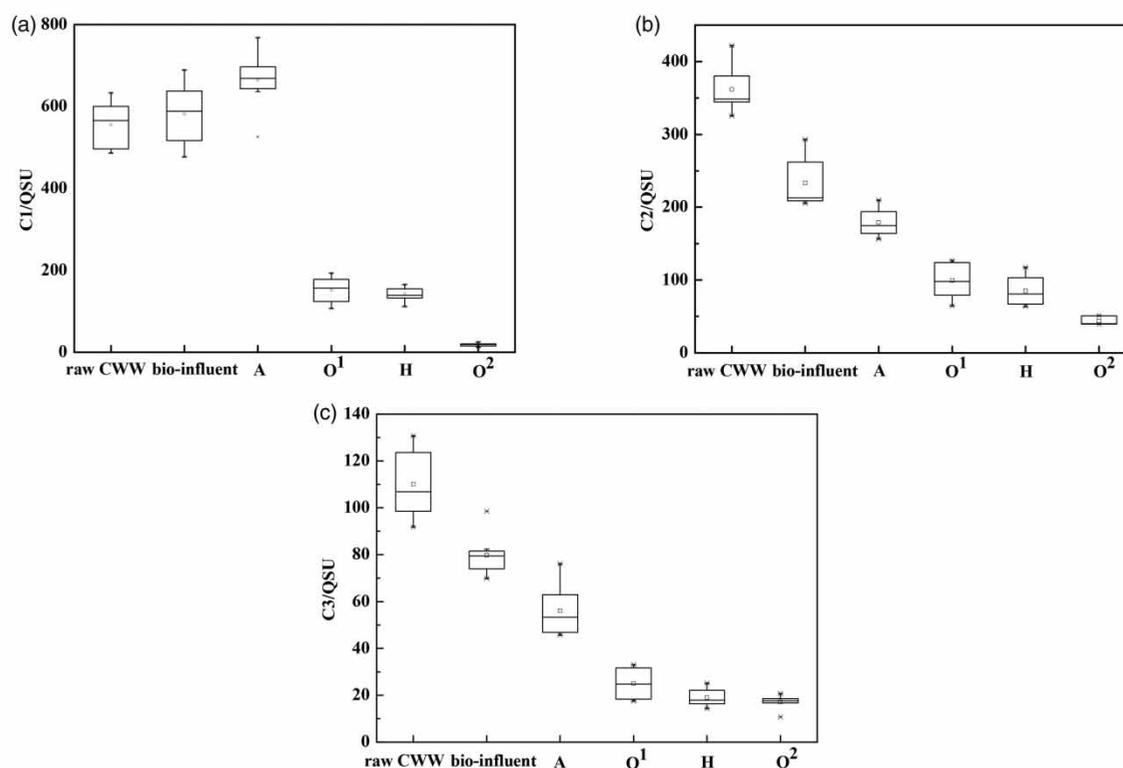


Figure 5 | Fluorescence intensities of PARAFAC components of CWW in the A/O¹/H/O² bio-treatment ((a) C1; (b) C2; (c) C3).

et al. 2014); also absorption and fluorescence properties of CDOM were applied for monitoring water quality such as TN, total phosphorus, COD and DOC in a large subtropical reservoir (Liu *et al.* 2014). To exploit the potential of rapid and continuous monitoring of contaminant removal efficiency in CWWTPs by spectroscopic technology, the Pearson's coefficients between the spectroscopic indices and the water quality parameters (COD, DOC and TN) of all the CWW samples were calculated (Supplementary Table S1, available online at <http://www.iwaponline.com/wst/072/333.pdf>).

In general, UV indices showed a better correlation with organics content than fluorescence components. UV₂₇₀ exhibited the highest correlation with COD and DOC, for which the Pearson's correlation coefficient r was 0.921 and 0.959 (Figure 6(a), (b)), respectively. The result verified that UV₂₇₀ could be used to indicate changes of organic content and monitor variations of COD and DOC. Besides, significant positive correlation between organics content and UV₂₅₄ illustrated that organic matter decreases were accompanied by aromatics content reduction. However, A_{240–400}, which was the integral area of UV spectra calculated from 240 to 400 nm, did not enhance the estimation

capability for COD and DOC compared with UV₂₇₀ and UV₂₅₄. The Pearson's coefficients r between A_{240–400} with COD and DOC were 0.876 and 0.892 (Supplementary Table S1), respectively. It meant the removal of organic matter was not just a reduction process of polar functional groups in the A/O¹/H/O² bio-treatment.

Fluorescent component C2 had the highest correlation with TN compared with other spectroscopic indices; the Pearson's coefficient r was 0.875 (Figure 6(c)). The result showed that variations of C2 were more sensitive to changes of TN concentrations. The phenomenon may be explained by this reason: inorganic nitrogen such as NH₄⁺ and NO₃⁻ as a constituent part of TN had almost no fluorescence quantum yield, but organic nitrogen, such as aniline, had a relatively high fluorescence quantum yield and its fluorescence emission position ($\lambda_{\text{Ex/Em}} = 245/340$) was exactly located in the region of C2 ($\lambda_{\text{Ex/Em}} = 245, 285/356$). The EEM of aniline is as shown in Supplementary Figure S2. Therefore, C2 can be used to trace the content of TN during CWW bio-treatment. Of note, among these three different fluorescent components, the correlation between C2 with C3 was the highest, and the r value was up to 0.901 (Figure 6(d)), demonstrating that these two

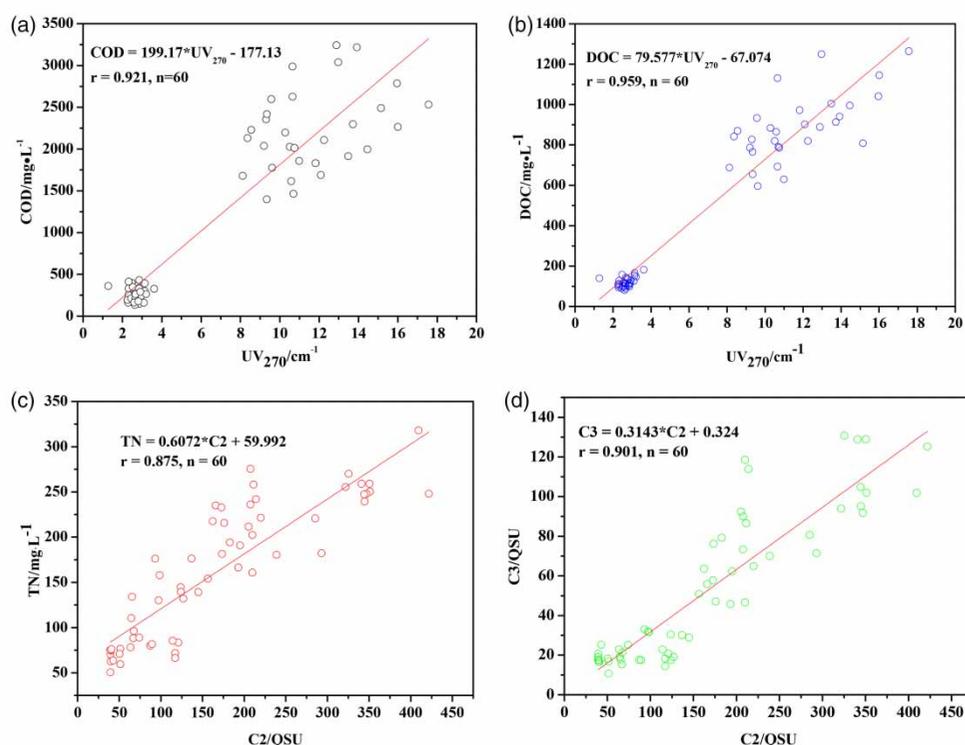


Figure 6 | Correlations between selected spectroscopic indices with water quality parameters ((a) correlation between UV₂₇₀ and COD; (b) correlation between UV₂₇₀ and DOC; (c) correlation between C2 and TN; (d) correlation between C2 and C3).

fluorescence components had a similar origin. Our results suggest that spectroscopic characterization and correlation analysis with water quality parameters can be a reliable method for evaluation of water quality parameters and monitoring the efficiency of bio-treatment in CWWTPs.

CONCLUSIONS

1. The A/O¹/H/O² bio-treatment system coupled with three internal-loop biological fluidized beds was efficient to remove organic matter and nitrogenous compounds in CWW.
2. In the A/O¹/H/O² bio-treatment, overall lower UV₂₅₄ removal rate than DOC removal rate resulted in an increase of aromaticity in effluent; MW of CWW showed an increasing trend; the content of polar functional groups had been greatly reduced.
3. Three main components in CWW were successfully identified by PARAFAC – C1 (tyrosine-like), C2 (tryptophan-like), C3 (humic-like) – and the removal rate of protein-like substance was higher than that of humic-like matter.
4. UV₂₇₀, which was found to be a stable UV absorption peak of CWW that could reflect the mixture of phenols, HCs, PAHs and their derivatives, showed a highly significant linear correlation with COD and DOC; significant correlation was also found between C2 and TN, suggesting C2 could be used to trace variation of TN concentration. Further research can be focused on accurate real-time monitoring CWW quality during bio-treatment based on these identified correlations.

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