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. UV_{270} 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 UV_{270} 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

Ronghua Xu
Huase Ou
Xubiao Yu
Runsheng He
Chong Lin
Chaohai Wei (corresponding author)
School of Environment and Energy, South China University of Technology,
Guangzhou 510006,
China
E-mail: cechwei@scut.edu.cn

Ronghua Xu
Xubiao Yu
Runsheng He
Chong Lin
Chaohai Wei
The Key Lab of Pollution Control and Ecosystem Restoration in Industry Clusters,
Ministry of Education,
Guangzhou 510006,
China

Huase Ou
Key Laboratory of Water/Soil Toxic Pollutants Control and Bioremediation of Guangdong Higher Education Institutions,
School of Environment, Jinan University,
Guangzhou,
510632,
China

LIST OF ABBREVIATIONS AND SYMBOLS

CWW	coking wastewater
CWWTP(s)	coking wastewater treatment plant(s)
DOM	dissolved organic matter
EEM	excitation-emission matrix
PARAFAC	parallel factor analysis
COD	chemical oxygen demand
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

CDOM	chromophoric dissolved organic matter
UV_{254}	ultraviolet absorbance at 254 nm
SUVA	specific UV absorbance at 254 nm
$A_{240-400}$	integral area of UV spectra calculated from 240 to 400 nm
S_R	ratio of the slope of the 275–295 nm region and that of the 350–400 nm region
QSU	quinine sulfate unit

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/aerobic[1]/aerobic[2] (A/O[1]/O[2]) (Ou *et al.* 2014) and anoxic/aerobic[1]/hydrolysis/aerobic[2] (A/O[1]/H/O[2]) (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 O[1] 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/O[1]/H/O[2] 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 m^3 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/O[1]/H/O[2] system coupled with three internal-loop biological fluidized beds. Major operating parameters, functions and main reactions of the A/O[1]/H/O[2] 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/O[1]/H/O[2]. 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/O^1/H/O^2 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	$X–Y + H_2O \rightarrow X–H + Y–OH$
O^1	12	1.02 ± 0.56	2.43	–	Decarbonization and ammoniation	$C_6H_5OH + 7O_2 \rightarrow 6CO_2 + 3H_2O$ SCN$^-$ + $3H_2O \rightarrow CO_2 + H_2S + NH_3 + OH^-$
H	20	0.43 ± 0.09	0.44	NaOH, Na$_2$CO$_3$	Denitrification	$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$
O^2	16	3.46 ± 0.61	0.44	NaOH, Na$_2$CO$_3$	Nitrification	$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + 2H_2O$ $NO_2^- + 0.5O_2 \rightarrow NO_3^-$

X–Y: carboxylic acid esters, nitriles and polysaccharides, etc.
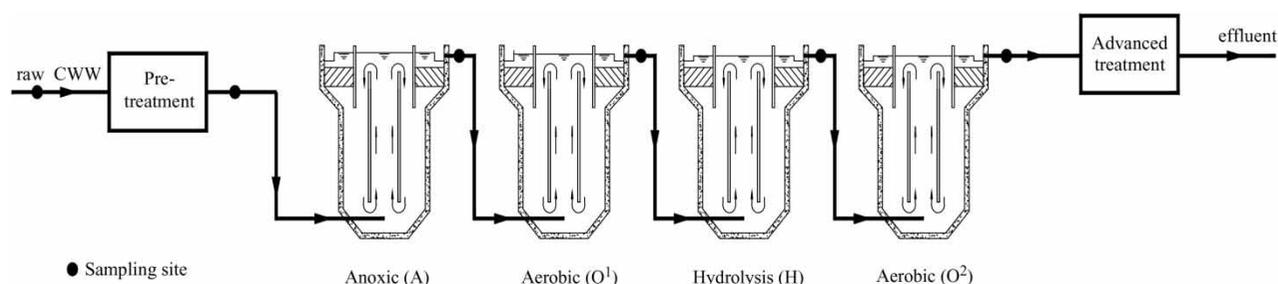
Figure 1 │ Schematic diagram of sampling sites and simplified coking wastewater treatment with an A/O^1/H/O^2 bio-treatment system coupled with three internal-loop biological fluidized beds.

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. UV$_{254}$ represents the aromatic content of a sample, and the specific ultraviolet absorbance (SUVA) is defined as the ratio of UV$_{254}$ 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 O^1 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 O^2 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 O^1, H and O^2 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,
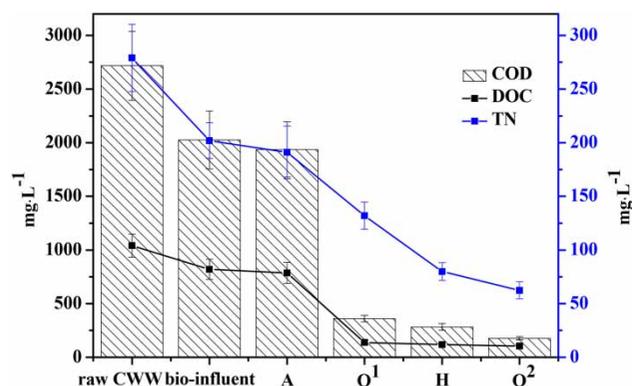

Figure 3 | UV spectra of CWW in the A/O^1/H/O^2 bio-treatment.

Figure 2 | Variations of COD, DOC and TN in A/O^1/H/O^2 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 O^1 stage. No obvious variations were observed in the H and O^2 stages.

To investigate the UV characteristics during CWW bio-treatment in detail, UV$_{254}$, 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), UV$_{270}$ 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 E$_2$ absorption band of PAHs and HCs also makes a contribution to the absorption peak at 270 nm. Therefore, UV$_{270}$ was also measured as a characteristic index.

As shown in Table 2, after bio-treatment, the UV$_{254}$ decreases from 10.06 ± 1.32 cm^{-1} in the bio-influent to 3.03 ± 0.27 cm^{-1} in the O^2 effluent. In contrast to this, the DOC decreases by 87.3%. Because the overall UV$_{254}$ 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 UV_{254}, SUVA, UV_{270}, S_R and $A_{240-400}$ at different treatment stages during $A/O^1/H/O^2$ 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 UV_{254} during bio-treatment suggested that organics which UV_{270} and UV_{254}, 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 O^2 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 O^2 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 ($\lambda_{Ex/Em} = 250/414$ nm), Peak B – tyrosine-like ($\lambda_{Ex/Em} = 270/300$ nm), Peak C – humic-like ($\lambda_{Ex/Em} = 350/434$ nm), Peak T1 – tryptophan-like ($\lambda_{Ex/Em} = 285/346$ nm) and Peak T2 – tryptophan-like ($\lambda_{Ex/Em} = 255/348$ 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 $\lambda_{Ex/Em} = 270/298$ nm, component 2 (C2) showed primary and secondary peaks with the $\lambda_{Ex/Em} = 245$ and $285/356$ nm, and component 3 (C3) also included two peaks with the $\lambda_{Ex/Em} = 250$ and $325/432$ nm. Based on the previous studies, C1 was
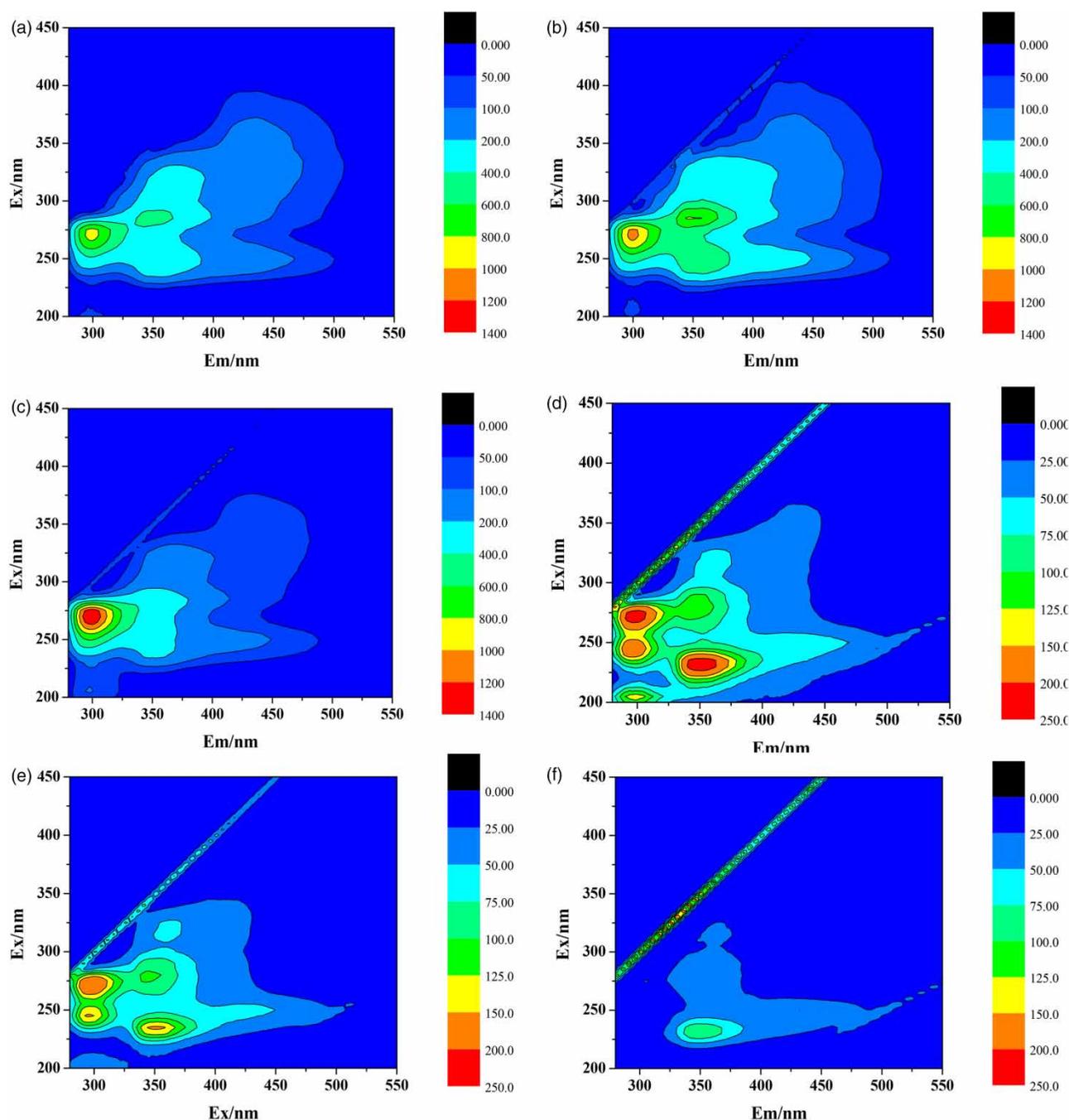
Figure 4 │ EEM spectra of CWW in the A/O^1/H/O^2 bio-treatment ((a) raw CWW; (b) bio-influent; (c) A; (d) O^1; (e) H; (f) O^2).

associated with tyrosine-containing matter, which might be related to phenols ($\lambda_{Ex/Em} = 265–270/298$ 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 ($\lambda_{Ex/Em} = 245, 275/340$ 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 ($\lambda_{Ex/Em} = 310–340/380–440$ nm), and PAHs, such as benzo[g,h,i]perylene ($\lambda_{Ex/Em} = 300/420$ 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 $A/O^1/H/O^2$ 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 O^2 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 O^1 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
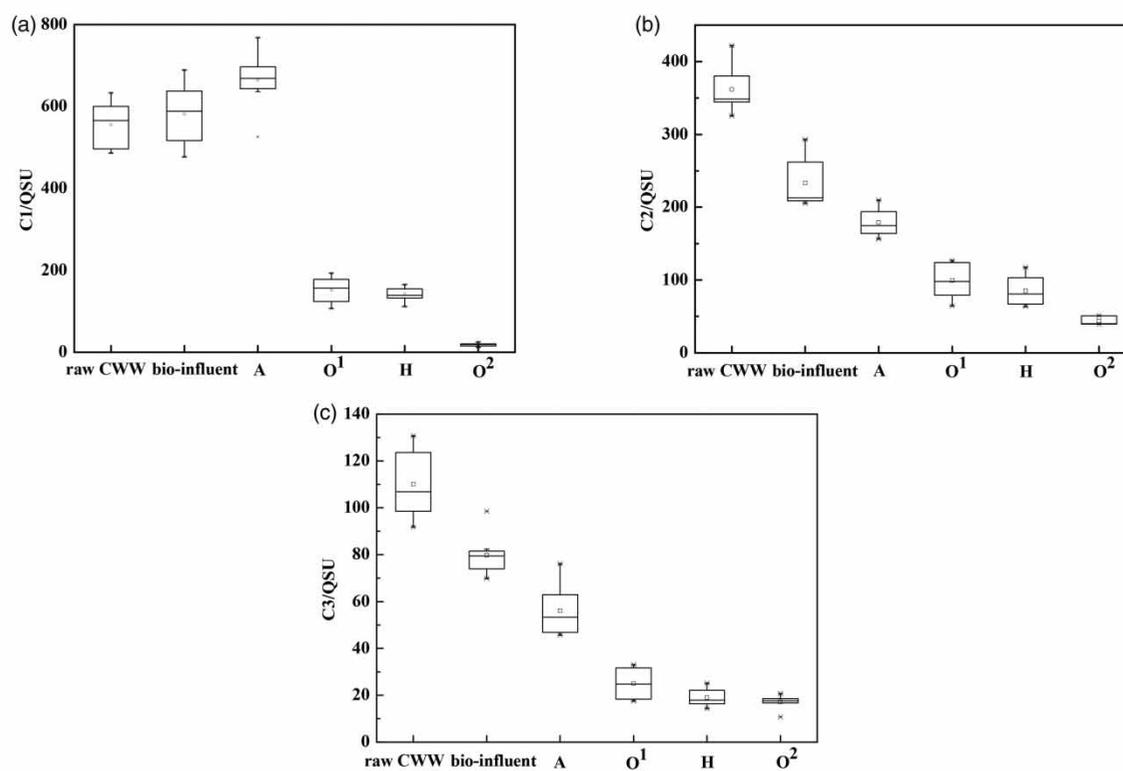
Figure 5 | Fluorescence intensities of PARAFAC components of CWW in the $A/O^1/H/O^2$ bio-treatment ((a) C1; (b) C2; (c) C3).

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. UV_{270} 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 UV_{270} could be used to indicate changes of organic content and monitor variations of COD and DOC. Besides, significant positive correlation between organics content and UV_{254} 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 UV_{270} and UV_{254}. 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 $A/O^1/H/O^2$ 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 NH_4^+ and NO_3^- 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 ($\lambda_{Ex/Em} = 245/340$) was exactly located in the region of C2 ($\lambda_{Ex/Em} = 245,285/356$). 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
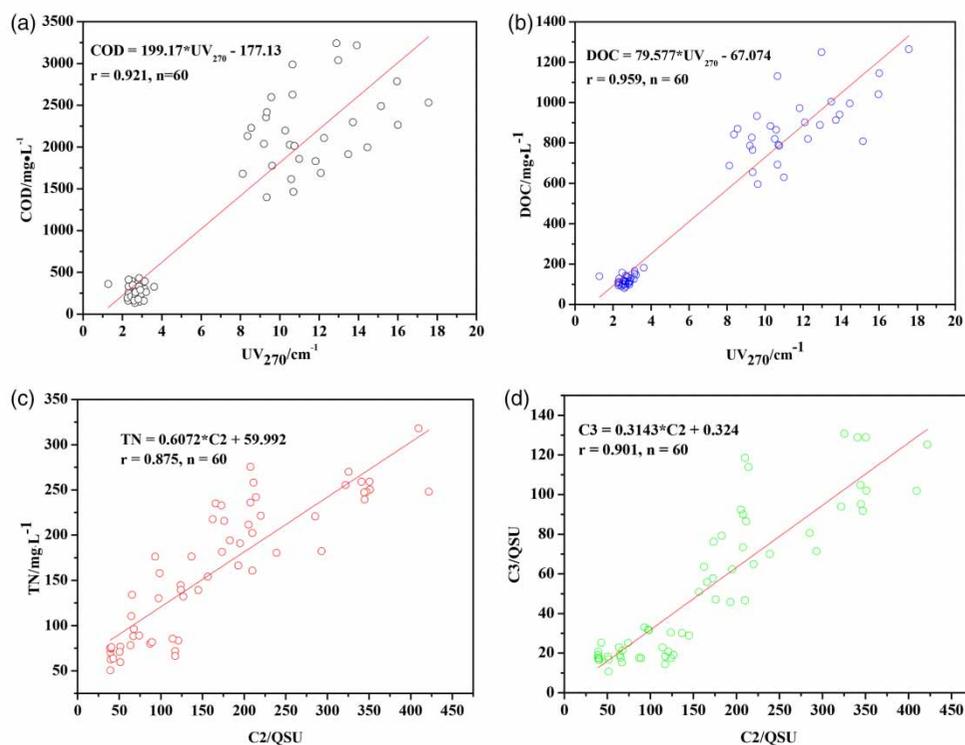

Figure 6 | Correlations between selected spectroscopic indices with water quality parameters ((a) correlation between UV_{270} and COD; (b) correlation between UV_{270} and DOC; (c) correlation between C2 and TN; (d) correlation between C2 and C3).

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 $A/O^1/H/O^2$ 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 $A/O^1/H/O^2$ bio-treatment, overall lower UV_{254} 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. UV_{270}, 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.

ACKNOWLEDGEMENT

REFERENCES

Alarcón, F., Báez, M. E., Bravo, M., Richter, P., Escandar, G. M., Olivieri, A. C. & Fuentes, E. 2013 Feasibility of the determination of polycyclic aromatic hydrocarbons in edible oils via unfolded partial least-squares/residual bilinearization and parallel factor analysis of fluorescence excitation emission matrices. *Talanta* **103**, 361–370.

Bro, R. 1997 PARAFAC. Tutorial and applications. *Chemometr. Intell. Lab.* **38** (2), 149–171.

Burich, I., Nikolich, K. & Aleksich, A. 1974 Fluorescence of the hydrochlorides of some quinoline derivatives. *J. Appl. Spectrosc.* **21** (3), 1200–1204.

Chen, W., Westerhoff, P., Leenheer, J. A. & Booksh, K. 2003 Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. *Environ. Sci. Technol.* **37** (24), 5701–5710.

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

Cohen, E., Levy, G. J. & Borisover, M. 2014 Fluorescent components of organic matter in wastewater: efficacy and selectivity of the water treatment. *Water Res.* **55**, 323–334.

Esparza-Soto, M., Núñez-Hernández, S. & Fall, C. 2011 Spectrometric characterization of effluent organic matter of a sequencing batch reactor operated at three sludge retention times. *Water Res.* **45** (19), 6555–6563.

He, X., Xi, B., Wei, Z., Guo, X., Li, M., An, D. & Liu, H. 2011 Spectroscopic characterization of water extractable organic matter during composting of municipal solid waste. *Chemosphere* **82** (4), 541–548.

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. *Limnol. Oceanogr.* **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 Res.* **43** (4), 863–881.

Hudson, N., Baker, A., Ward, D., Reynolds, D. M., Brunsdon, C., Carliell-Marquet, C. & Browning, S. 2008 Can fluorescence spectrometry be used as a surrogate for the biochemical oxygen demand (BOD) test in water quality assessment? An example from South West England. *Sci. Total Environ.* **391** (1), 149–158.

Hur, J. & Cho, J. 2012 Prediction of BOD, COD, and total nitrogen concentrations in a typical urban river using a fluorescence excitation-emission matrix with PARAFAC and UV absorption indices. *Sensors* **12** (1), 972–986.

Krasner, S. W., Westerhoff, P., Chen, B., Rittmann, B. E., Nam, S. & Amy, G. 2009 Impact of wastewater treatment processes on organic carbon, organic nitrogen, and DBP precursors in effluent organic matter. *Environ. Sci. Technol.* **43** (8), 2911–2918.

Li, W., Sheng, G., Liu, X. & Yu, H. 2008 Characterizing the extracellular and intracellular fluorescent products of activated sludge in a sequencing batch reactor. *Water Res.* **42** (12), 3173–3181.

Li, H., Han, H., Du, M. & Wang, W. 2011 Removal of phenols, thiocyanate and ammonium from coal gasification wastewater using moving bed biofilm reactor. *Bioresour. Technol.* **102** (7), 4667–4673.

Liu, X., Zhang, Y., Shi, K., Zhu, G., Xu, H. & Zhu, M. 2014 Absorption and fluorescence properties of chromophoric dissolved organic matter: implications for the monitoring of

water quality in a large subtropical reservoir. *Environ. Sci. Pollut. R.* **21** (24), 14078–14090.

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

Marañón, E., Vázquez, I., Rodríguez, J., Castrillón, L., Fernández, Y. & López, H. 2008 Treatment of coke wastewater in a sequential batch reactor (SBR) at pilot plant scale. *Bioresour. Technol.* **99** (10), 4192–4198.

Murphy, K. R., Hambly, A., Singh, S., Henderson, R. K., Baker, A., Stuetz, R. & Khan, S. J. 2011 Organic matter fluorescence in municipal water recycling schemes: toward a unified PARAFAC model. *Environ. Sci. Technol.* **45** (7), 2909–2916.

Ou, H., Wei, C., Mo, C., Wu, H., Ren, Y. & Feng, C. 2014 Novel insights into anoxic/aerobic[1]/aerobic[2] biological fluidized-bed system for coke wastewater treatment by fluorescence excitation – emission matrix spectra coupled with parallel factor analysis. *Chemosphere* **113**, 158–164.

Park, M., Lee, T., Lee, B., Hur, J. & Park, D. 2010 Spectroscopic and chromatographic characterization of wastewater organic matter from a biological treatment plant. *Sensors* **10** (1), 254–265.

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

SEPA 2002 *Water and Wastewater Monitoring Methods*. Chinese Environmental Science Press, Beijing.

Staib, C. & Lant, P. 2007 Thiocyanate degradation during activated sludge treatment of coke-ovens wastewater. *Biochem. Eng. J.* **34** (2), 122–130.

Stedmon, C. A. & Bro, R. 2008 Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. *Limnol. Oceanogr. Meth.* **6** (11), 572–579.

Sun, W., Qu, Y., Yu, Q. & Ni, J. 2008 Adsorption of organic pollutants from coking and papermaking wastewaters by bottom ash. *J. Hazard. Mater.* **154** (1), 595–601.

Wang, B., Chang, X. & Ma, H. 2008 Electrochemical oxidation of refractory organics in the coking wastewater and chemical oxygen demand (COD) removal under extremely mild conditions. *Ind. Eng. Chem. Res.* **47** (21), 8478–8483.

Wang, Y., Sun, S., Ding, G. & Wang, H. 2012 Electrochemical degradation characteristics of refractory organic pollutants in coking wastewater on multiwall carbon nanotube-modified electrode. *J. Nanomater.* **2012**, 1–5.

Yamashita, Y. & Tanoue, E. 2003 Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids. *Mar. Chem.* **82** (3), 255–271.

Yao, X., Zhang, Y., Zhu, G., Qin, B., Feng, L., Cai, L. & Gao, G. 2011 Resolving the variability of CDOM fluorescence to differentiate the sources and fate of DOM in Lake Taihu and its tributaries. *Chemosphere* **82** (2), 145–155.

Yu, H., Song, Y., Tu, X., Du, E., Liu, R. & Peng, J. 2013 Assessing removal efficiency of dissolved organic matter in wastewater treatment using fluorescence excitation emission matrices with parallel factor analysis and second derivative synchronous fluorescence. *Bioresour. Technol.* **144**, 595–601.

Zhang, M. H., Zhao, Q. L., Bai, X. & Ye, Z. F. 2010 Adsorption of organic pollutants from coking wastewater by activated coke. *Colloid Surf. A Physicochem. Eng. Asp.* **362** (1), 140–146.

Zhang, W., Wei, C., Feng, C., Yan, B., Li, N., Peng, P. & Fu, J. 2012 Coking wastewater treatment plant as a source of polycyclic aromatic hydrocarbons (PAHs) to the atmosphere and health-risk assessment for workers. *Sci. Total Environ.* **432**, 396–403.

Zhang, W., Wei, C., Yan, B., Feng, C., Zhao, G., Lin, C., Yuan, M., Wu, C., Ren, Y. & Hu, Y. 2013 Identification and removal of polycyclic aromatic hydrocarbons in wastewater treatment processes from coke production plants. *Environ. Sci. Pollut. R.* **20** (9), 6418–6432.

Zhao, W., Huang, X. & Lee, D. 2009 Enhanced treatment of coke plant wastewater using an anaerobic–anoxic–oxic membrane bioreactor system. *Sep. Purif. Technol.* **66** (2), 279–286.