Quantitative Study on the Structure-bioavailability Relationship of Dissolved Organic Nitrogen in Wastewater Treatment Plant Effluent

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Abstract

This study systematically investigated the relationship between the structure properties and biological characteristics of DON in the effluents from municipal wastewater treatment plants. Ultrafiltration, FTIR spectroscopy, UV spectroscopy and EEM fluorescence spectroscopy were used to characterize the structure of organic matters in the effluent samples, and the bioavailability of DON was determined by algal/bacterial based bioassay. The quantitatively analysis of EEM spectra conducted by fluorescence regional integration method showed that the organic portion of all samples were mainly consistent with fulvic acid and protein. Combined with the bioassay results, a positive correlation between the DON bioavailability and the protein content (sum of region I and region II) ($r=0.80$, $P<0.02$) and soluble microbial byproduct-like materials (region IV) ($r=0.76$, $P<0.03$) were observed. Nevertheless, the humic substances content represented by the region III and V would negatively affect the DON bioavailability. High humification degree (high HIX value) ($r=-0.77$, $P<0.03$) was related to low bioavailability. Furthermore, according to UV spectroscopy results, strong aromaticity (high UV_{254} values) ($r=-0.78$, $P<0.03$) suggested low DON bioavailability. The ultrafiltration experiment showed that the low molecular weight DON (<3 kDa) accounted for 30-73% of the total DON, and no notable relationship was observed for DON molecular weight and its bioavailability.

1. Introduction

Municipal wastewater effluent discharged from urbanized areas is a crucial source of anthropogenic nitrogen load in surface water (Liu et al., 2011; Pehlivanoglu-Mantas and Sedlak, 2006). It is estimated that the wastewater-derived nitrogen accounts for 12-33% of nitrogen input in global river (Howarth, 2004). With the development of advanced nutrient removal system, high inorganic nitrogen removal could be achieved during wastewater treatment process (Kasi et al., 2011). This led to the dissolved organic nitrogen (DON) became a major nitrogen form of the total dissolved nitrogen (TDN) in wastewater effluent.

Previous research suggested that inorganic nitrogen can be readily used by phytoplankton, while the bioavailability of DON varies greatly due to its complicate composition (Pehlivanoglu-Mantas and Sedlak, 2006). Berman's research showed that nucleic acids were taken up readily by planktonic microbiota (Berman and Bronk, 2003). Algae growth could be significantly stimulated when urea was used as nitrogen source, which was even preferred than inorganic nitrogen (Fiedler et al., 2015). On the other hand, humic bound nitrogen substances were often resistant to biodegradation (Liu et al., 2011). However, about 70% of DON in effluent still cannot be identified with currently available methods (Pehlivanoglu-Mantas and Sedlak, 2006; Huo et al., 2013). Therefore, it was difficult to evaluate the contribution of different DON compounds in effluent to eutrophication. Some researchers have studied the relationship between the structure of DON and its bioavailability. The hydrophilic DON was readily assimilated by algal, while the hydrophobic DON was less likely to support algae growth (Liu et al., 2011). The DON bioavailability was also related to its C/N ratio, as it was reported that low C/N ration contained

DON compounds, such as amino acid and protein, had high bioavailability toward algae (Huo et al., 2013).

Above studies suggested that the structure of DON was closely related to its bioavailability, and the variation of DON bioavailability was probably due to the different structures of different DON. Therefore, proper DON structure characterization is the key to understand the relationship between the DON structure and its bioavailability. With the development of spectroscopy technology, some advanced spectroscopy techniques have been widely used in DON structural characterization, including the characterization of functional group composition, aromaticity and degree of humification of DON. For example, EEM fluorescence was used to analyze the humic-like and protein-like materials in eutrophic lakes (Yan et al., 2018). UV spectroscopy could reflect the aromaticity and the degree of substitution on benzene rings of DOM through the detection of absorbance UV_{254} and the absorbance ratio of UV_{240}/UV_{420} and UV_{253}/UV_{203} (Ps. et al., 2003; Hua et al., 2017; Sun et al., 2018). Leenheer used FTIR spectroscopy to characterize the functional groups of DON in effluent from WWTPs (Leenheer et al., 2007).

In this paper, the structure properties of DOM in wastewater effluent, including the molecular weight, functional groups, aromaticity, humification degree and humic substances category, were characterized by ultrafiltration experiments, FTIR spectroscopy, UV spectroscopy and EEM fluorescence spectroscopy, respectively. Combined with the DON bioavailability results obtained by 14-day nitrogen uptake bioassay, the relationship between the bioavailability and structure properties of DON were systematically investigated.

2. Materials And Methods

2.1 Sampling

24 Effluent samples collected from 8 WWTPs in Chengdu, China were used in this study (Table S1 and Figure S1 of the supporting information, SI). After collection, all samples were filtered through 1.0 μm and 0.45 μm Millipore filters to remove suspended particulate and then stored at 4°C prior to use. The effluent samples were labeled E₁-E₈ in this paper.

2.2 Chemical analysis

The concentration of total nitrogen (TN) and total dissolved nitrogen (TDN) were analyzed using the persulfate digestion-ultraviolet spectrophotometric method. This method converts all nitrogenous compounds to nitrates (Clescerl et al., 1998). The concentration of nitrate nitrogen ($\text{NO}_3\text{-N}$) and nitrite nitrogen ($\text{NO}_2\text{-N}$) were determined using UV spectrophotometry. The determination of Ammonia Nitrogen ($\text{NH}_4\text{-N}$) in water by Nessler's Reagent Spectrophotometry (Table S2 of SI). DON was calculated as the difference between measured TDN and sum of measured dissolved inorganic nitrogen (DIN) species using following equation (Wadhawan et al., 2014):

$$\text{DON} = \text{TDN} - (\text{NO}_3^- - \text{N} + \text{NO}_2^- - \text{N} + \text{NH}_4^+ - \text{N})$$

2.3 Resin separation

High inorganic nitrogen concentration might negatively affect the calculation accuracy of the DON concentration. Thus, resin separation was used as pretreatment to reduce the DIN concentration. Prior to use, the 100 g ECA90 anion exchange resin packed in a borosilicate glass column was cleaned with 10% sodium chloride and then twice with ultrapure water. After cleaning, the effluent was slowly passed through the resin with 5 mL/min for inorganic nitrogen extraction. After treating the samples with ion exchange, the nitrate and ammonia nitrogen concentration were detected to be less than 0.5 mg N/L and 0.1 mg N/L, respectively. The Detailed information about the DON concentration before and after ion exchange was provided in Table S4.

2.4 Algal bioassay

Selenastrum capricornutum was used as the test algae, which can be easily cultured in the laboratory and has been used as a standard test organism for eutrophication studies for over 45 years (Pehlivanoglu and Sedlak, 2004; Fan et al., 2017). The freshwater algae culture was maintained using the nutrient medium suggested by Miller (Fogg, 1979). Nutrient stress was induced by incubating algae cultures with nitrogen free medium 7-10 days prior to the bioassay (Fan et al., 2017).

The experiment was initiated by adding nitrogen starved algae (initial chlorophyll-a concentration 30 ug/L of) to the samples (Fan et al., 2018; Fan et al., 2020). Control samples with incubated with algae and bacteria were also prepared to understand the influence of bacteria during DON uptake. Specifically, 3 L of mixed sample from each treatment plants were filtered through 1 µm filter to remove large particulate pollutants and then through a 0.2 µm membrane filter. The retentate on the 0.2 µm filter was resuspended in 100 mL of 0.2 µm filtered wastewater effluent and used as the bacterial inoculum (Liu et al., 2011). Triplicates of each sample were incubated and the results were averaged for the final calculations.

During the algal bioassay experiments, all samples were incubated at the temperature of $25 \pm 1^\circ\text{C}$ under continuous fluorescent lighting of 1300 Lm in a horizontal shaker at 110 rpm for 14 days. During the bioassay, all nutrients (except N) were added twice a week to avoid nutrient co-limitation. To ensure the maximum algae potential growth rate, the optimum incubation environment was maintained for all samples with abundant nutrient addition. 30 mL samples were collected and analyzed for TDN, $\text{NO}_2^- - \text{N}$, $\text{NO}_3^- - \text{N}$, $\text{NH}_4^+ - \text{N}$ and chlorophyll-a on day 0, 0.33, 1, 2, 4, 7, 10, and 14.

2.5 UV

UV absorbance measurements were conducted on a Lambda950 UV spectrophotometer (PerkinElmer, USA) between 200 and 800 nm with 1 nm as the scanning interval. One cm light path was provided with a quartz and the Milli-Q water was used as blank sample during UV absorbance measurements. The UV_{254} value was defined as UV absorbance measured at the wavelengths of 254 nm. $\text{UV}_{240}/\text{UV}_{420}$ and

UV₂₅₃ /UV₂₀₃ were the absorbance ratio at the wavelengths of 240 nm, 420 nm, 253 nm and 203 nm, respectively.

2.6 EEM

Fluorescence spectra of effluents samples were recorded using a HORIBA/FM4PC fluorescence spectrophotometer (PerkinElmer, USA), which was equipped with double monochromators both at the excitation and the emission sides. The fluorescence EEM spectra were collected by subsequent scanning emission from 240 to 800 nm at sequential 2 nm increments; for each excitation wavelength, the emission wavelength from 240 to 800 nm was detected at sequential 3 nm increments. The water Raman scattering and second order Rayleigh scattering are eliminated by using Milli-Q water as blank.

Based on the fluorescence regional integration (FRI) method, the excitation and emission boundaries could be classified into five regions, and each region represented materials like aromatic protein, fulvic acid, soluble microbial byproduct and humic acid. The volume (ϕ_i) beneath region i of an EEM can be calculated with:

$$\phi_i = \int_{ex} \int_{em} I(\lambda_{ex} \lambda_{em}) d\lambda_{ex} d\lambda_{em}$$

where $I(\lambda_{ex} \lambda_{em})$ is the fluorescence intensity at each excitation–emission wavelength pair, $d\lambda_{ex}$ is the differential of the excitation wavelength and $d\lambda_{em}$ is the differential of the emission wavelength. For discrete data, the volume (ϕ_i) is expressed by:

$$\phi_i = \sum_{ex} \sum_{em} I(\lambda_{ex} \lambda_{em}) \Delta\lambda_{ex} \Delta\lambda_{em}$$

Where $\Delta\lambda_{ex}$ is the excitation wavelength increment, $\Delta\lambda_{em}$ is the emission wavelength increment.

The normalized excitation-emission area volumes (ϕ_{in}) and normalized percentage (P_{in}) were calculated according to following equation:

$$\phi_{in} = MF_i \phi_i$$

$$\phi_{Tn} = \sum_{i=1}^5 \phi_{in}$$

$$P_{in} = \frac{\phi_{in}}{\phi_{Tn}} \times 100\%$$

2.7 FTIR

FTIR spectra of the dried DON samples was obtained on a VERTEX 70 Fourier-transform infrared (SHIMADZU, Japan). The pellets were made from the mixture of dried DON samples and KBr in a 1:100 ratio. The instrument was set up to scan from 4,000 to 400 cm^{-1} averaging 10 scans at 1.0 cm^{-1} intervals with a resolution of 4.0 cm^{-1} .

2.8 Ultrafiltration experiment

For ultrafiltration experiment, pre-treated effluent samples were fractionated using 400 mL commercial stirred cell units. The samples were fractionated into five groups using a series of cellulose-derivative UF membranes with MW cut-offs of 100 kDa, 30 kDa, 10 kDa and 3 kDa, respectively, under high purity nitrogen gas at 0-0.15 MPa. The membrane was soaked overnight in advance and filtered with Milli-Q water for half an hour before each filtration to remove any organic matter attached to the membrane.

3. Results And Discussion

3.1 DON Bioavailability

In order to effectively remove DIN and improve the calculation accuracy of DON, the effluent samples were pretreated with ion exchange before the N composition analysis. The obtained results showed that the DON concentration of the effluent samples was ranged from 0.78-1.52 mg/L and the DIN concentration was less than 0.5 mg/L. The DON bioavailability of effluents was assessed using algal/bacterial based bioassay, and the obtained results showed that the bioavailable nitrogen (BAN) concentration of E₁-E₈ samples of algae group accounted for 35.01%, 20.24%, 35.80%, 27.18%, 24.85%, 29.29%, 29.36% and 32.83% of the DON, respectively (Fig. 1). The chlorophyll-a concentration increased by 112.48-760.60 $\mu\text{g/L}$ after 14 days of incubation. The concentration of BAN increased 6.04-17.43% and the chlorophyll-a concentration increased 13.10-85.18% with the presence of bacteria. Similar to previous study, the addition of bacteria could convert the high molecular weight DON into low molecular weight DON, which favored the algal growth stimulation (Pehlivanoglu and Sedlak, 2004). On the other hand, for control sample, the added $\text{NO}_3^-/\text{NH}_4^+$ (TN concentration of 3 mg L^{-1}) was completed used up during the 14 days bioassay and it showed much higher Chl-a value by the end of the incubation. This indicated that the DON from effluent and was generally low the its ability to stimulate algal growth is also low.

3.2 UV spectroscopy

The UV spectrum characteristic parameters of DON in the effluent were shown in Table 1. The UV_{254} value (ranged from 0.052-0.179) that was often used to characterize the aromaticity of DOM, and according to the bioassay, the UV_{254} was showed to be negatively correlated with DON bioavailability ($r=-0.78$, $P<0.03$). This result indicated that the increased of UV_{254} value could results in the decrease of DON bioavailability. Similar conclusion was also proposed by Nishijima and Speitel (Nishijima and Speitel, 2004). The $\text{UV}_{240}/\text{UV}_{420}$ values for all effluent samples ranged from 9.82 to 16.11, with an average value of 12.90, suggesting that the humus in the samples was mainly fulvic acid (Sun et al.,

2018). High UV_{253}/UV_{203} value result indicated that there were more substituents with carboxyl, carbonyl, hydroxyl and ester content on the aromatic ring. These functional groups could easily provide sites for heavy metal coordination complexation, and adsorb heavy metal ions in water to form macromolecular complexes, which would greatly reduce the DON bioavailability (Velazquez-Jimenez et al., 2013). This was in agreement of the bioassay results, which showed that the DON in sample E₂ and E₇ with the highest UV_{253}/UV_{203} value was highly recalcitrant compared to the rest of the samples.

3.3 EEM fluorescence spectroscopy

The EEM spectrum of all effluent samples are shown in Fig. 2. Three fluorescence indices FI, HIX and BIX were calculated to characterize the DOM in samples (Table 1). The FI value was used to estimate the source of DOM. The DOM was considered to be originated from microbial sources when the FI value was > 1.90, while it might be from terrestrial and soil sources when the FI value was < 1.40 (Huguet et al., 2008). The HIX value often characterize the humification degree of DOM. HIX higher than 10 suggested high humification degree of the fluorescent component, while HIX lower than 4 indicated low humification degree (Yan et al., 2018). For BIX value, when it was observed to be greater than 1, the DOM was believed to be newly released into the water. When the BIX was 0.60-0.70, the DOM was considered to be composed with less autogenic matters (Yan et al., 2018). As shown in Table 1, the FI values for tested effluent samples were ranged from 1.41 to 1.83. For samples E₁, E₃, E₄, E₅ and E₈, the FI values were close to 1.90, indicating that the DON from these samples was originated from microbial sources (Maie et al., 2006). Similar phenomenon was observed by Yang (Yang et al., 2017), who reported that the DOM in secondary effluent was mainly from a biological source. The HIX values less than 4 were detected for all effluent samples, indicating that the humification degree of DON were relatively low. The HIX and the BAN/DON ratios were negatively correlated ($r=-0.77$, $P<0.03$), which indicated that the lower humification degree, the higher the bioavailability of DON. The BIX value was observed between 0.88 and 0.97, implying that the DON in water was mainly from autogenous sources. This result was in consistent with the conclusion obtained from the FI value, and similar results were also proposed by other researchers (Cory and McKnight, 2005; Yang et al., 2018).

According to Chen's method, the EEM spectra could be divided into five consecutive regions (Chen et al., 2015). As shown in Table 2, the proportion of the sum of fluorescent protein region I and region II ranged from 33.53-55.77%. The proportion range of fulvic acids in the region III and humic acids in the region V were 36.94-61.37% and 1.72-2.06%, respectively. In region IV, the proportion of soluble microbial byproduct-like materials in the fluorescence region ranged from 3.08-5.64%. These results showed that the effluent samples were mainly contained with humic-like substances and protein-like substances. This is similar to Wu's study, which showed that the urban streams were enriched with anthropogenic and aquagenic fulvic acid and protein-like DOM (Zhipeng et al., 2019). Besides, this was also in consistent with the result of the UV_{240}/UV_{420} values. Combined with the bioassay results, it can be concluded that the protein-like content was positively correlated with the BAN/DON ratios ($r=0.80$, $P<0.02$), while the humic substances content was negatively correlated to the BAN/DON ratio ($r=-0.86$, $P<0.01$). This was in agreement with the previously reported results, that the protein compounds were highly bioavailable due

to its low C/N ratios, while humic substances tended to be resistant to biodegradation (Liu et al., 2011; Huo et al., 2013). Thus, high value of region I + region II indicated high DON bioavailability, while high value of region II + region V reflected low DON bioavailability. Moreover, the generated results also exhibited a positive correlation between the content of soluble microbial byproduct-like materials (proportion of region IV) and BAN/DON ratios ($r=0.76$, $P=0.03$), because microorganism-derived DON was a nitrogen source to algae and bacteria (Liao et al., 2020).

3.4 FTIR spectroscopy

FTIR was used to characterize the DOM functional groups of all samples, and the obtained spectra are showed in Fig. 3. Due to the overlap of functional groups, the spectra of all effluent samples showed similar patterns in the position of principal absorption bands (Abdulla et al., 2010). All effluent samples formed a broad peak around 3420 cm^{-1} , which can be assigned to the O-H stretching of carbohydrate, carboxylic acid and phenol compounds. The relative wide breadth of this peak was probably due to the intra-molecular and inter-molecular hydrogen bond interactions of carboxylic acids (Pavia et al., 2009; Abdulla et al., 2010; Yang et al., 2017). The sharp peaks around $2970\text{--}2930\text{ cm}^{-1}$ and $2870\text{--}2850\text{ cm}^{-1}$ were attributed to asymmetric stretching of aliphatic C-H, and related to aliphatic compounds methylene ($-\text{CH}_2$) groups and methyl ($-\text{CH}_3$) groups (Gandois et al., 2013). These two peaks were more pronounced for sample E_2 , illustrating the presence of more aliphatic compounds in sample E_2 compared to other samples. The absorption peak near 1720 cm^{-1} was attributed to the C=O stretching of the conjugated carbonyl groups in carboxylic acids, ketones and aldehydes. The absorption peak observed at around 1640 cm^{-1} was related to the C=O stretching of the amide groups in the protein-like component (Giovanela et al., 2004). Sample E_1 showed the highest peak area of 206 at 1640 cm^{-1} , while the peak area at this peak for sample E_2 was only 105. The bioassay also indicated that the bioavailability of DON in sample E_1 was much higher than that of sample E_2 , since previous studies showed that protein could be taken up readily by algae (Liu et al., 2011; Fan et al., 2018). The two absorption peaks around 1170 cm^{-1} and 1070 cm^{-1} were ascribed to the C-O stretching of polysaccharides (Gandois et al., 2013).

3.5 Molecular weight distribution

Figure 4 presented the DON molecular weight distribution of all effluent samples. The results showed that the low molecular weight DON accounted for 30-73% of the total DON concentration, which indicated that DON in effluent was mainly contained with low molecular weight DON. However, unlike conclusions proposed by other researchers, in this study, the low molecular weight DON content was not evidently correlated with its bioavailability. This was probably because that some high molecular weight DON compounds, such as certain type of humic acid can also be decomposed by algae. While some low molecular weight DON compounds, such as Uracil and Aminobenzoic acid, tend to be recalcitrant to biodegradation (Fan et al., 2018). Besides, it was reported that some low molecular weight nitrogenous compounds, such as amino acids, purines and pyrimidine might be adsorbed onto the humic core structure, and these adsorbed compounds could not be identified as low molecular DON using ultrafiltration experiment (Antia et al., 1991; Zhai et al., 2016; Zhang et al., 2016).

Conclusion

The present study investigated the relationship between the structure and bioavailability of DON in the effluent of WWTPs using spectroscopy characterization techniques and algae/bacterial based bioassay. The EEM spectrum and bioassay results showed that the protein content (sum of region I and region II) and soluble microbial byproduct-like materials content (region IV) in DON were positively correlated with bioavailability, while the humic substance content (region III and region V) was negatively correlated. The high humification degree (high HIX values) corresponded to low DON bioavailability. The UV spectroscopy analysis results showed that low bioavailability DON had stronger aromaticity with higher UV_{254} values. Molecular weight distribution experiments showed that there was no significant correlation between molecular weight and bioavailability.

Declarations

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Author contribution

Cihang Yan: Conceptualization, Sampling, Investigation, Interpretation, Writing, Review and Editing.

Zhiyu Wei: Sampling, Investigation, Interpretation.

Jiayin Liu: Sampling, Investigation, Interpretation.

Jie Chen: Review and Editing.

Lu Fan: Initial idea, Sampling, Project manager, Project leader, Laboratory activity, Conceptualization, Methodology, Investigation, Interpretation, Writing, Review and Editing.

Ethics declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Tables

Table1. Fluorescence parameters and UV parameters of DON in the effluent samples.

	FI	HIX	BIX	UV ₂₅₄	UV ₂₄₀ /UV ₄₂₀	UV ₂₅₃ /UV ₂₀₃
E ₁	1.833	1.370	0.882	0.052	12.439	0.010
E ₂	1.413	2.564	0.947	0.179	16.106	0.029
E ₃	1.805	1.532	0.967	0.059	10.820	0.012
E ₄	1.525	1.669	0.910	0.086	13.323	0.026
E ₅	1.600	2.798	0.834	0.079	13.439	0.022
E ₆	1.465	2.308	0.930	0.065	13.232	0.016
E ₇	1.431	1.832	0.943	0.145	14.398	0.027
E ₈	1.733	1.705	0.936	0.063	12.424	0.020

Table2. FRI parameters for operationally defined EEM regions.

Sample					
E ₁	Region I	1.036	41.7	43.201	17.160
	Region II	2.331	41.7	97.203	38.609
	Region III	4.366	21.3	92.996	36.938
	Region IV	1.776	21.3	14.030	5.573
	Region V	3.330	1.1	4.329	1.720
E ₂	Region I	0.530	41.7	22.101	11.148
	Region II	1.064	41.7	44.369	22.381
	Region III	5.712	21.3	121.666	61.371
	Region IV	0.773	21.3	6.105	3.080
	Region V	3.080	1.1	4.004	2.020
E ₃	Region I	0.851	41.7	35.487	15.009
	Region II	2.014	41.7	83.973	35.517
	Region III	4.650	21.3	99.045	41.892
	Region IV	1.688	21.3	13.331	5.639
	Region V	3.533	1.1	4.592	1.942
E ₄	Region I	0.747	41.7	31.150	15.304
	Region II	1.593	41.7	66.428	32.635
	Region III	4.368	21.3	93.038	45.709
	Region IV	1.176	21.3	9.290	4.564
	Region V	2.800	1.3	3.640	1.788
E ₅	Region I	0.634	41.7	26.438	12.452
	Region II	1.370	41.7	57.108	26.897
	Region III	5.416	21.3	115.361	54.332
	Region IV	1.188	21.3	9.385	4.420
	Region V	3.102	1.3	4.033	1.899

	Region I	0.693	41.7	28.898	13.244
	Region II	1.641	41.7	68.439	31.366
E ₆	Region III	5.135	21.3	109.376	50.127
	Region IV	0.923	21.3	7.292	3.342
	Region V	3.224	1.3	4.191	1.921
	Region I	0.568	41.7	23.686	12.121
	Region II	1.177	41.7	49.060	25.106
E ₇	Region III	5.629	21.3	111.378	56.997
	Region IV	0.897	21.3	7.086	3.626
	Region V	3.231	1.3	4.200	2.149
	Region I	0.780	41.7	32.526	14.243
	Region II	1.853	41.7	77.249	33.827
E ₈	Region III	4.875	21.3	103.838	45.469
	Region IV	1.365	21.3	10.784	4.722
	Region V	3.055	1.3	3.972	1.739

Figures

Figure 1

(a) The DON bioavailability of all effluent samples; (2) Chlorophyll-a concentration for all samples after 14 days of incubation.

Figure 2

EEM spectra of effluent samples (figure a-h for sample E₁-E₈).

Figure 3

FTIR spectra of effluent samples (figure a-h for sample E₁-E₈).

Figure 4

Molecular size fractionation of DON in the effluents samples.

Supplementary Files

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