Bioavailability and characterization of dissolved organic nitrogen and dissolved organic phosphorus in wastewater effluents

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HIGHLIGHTS
- The bioavailability of DON/DOP in wastewater effluents were examined.
- The majority of DON was hydrophilic while more DOP existed in hydrophobic forms.
- Hydrophobic fraction of effluent DOP was more likely to be bioavailable for algae.
- Hydrophilic fraction of effluent DON seemed to exhibit higher bioavailability.
- Fluorescence spectroscopy analysis provided insights of DOM in wastewater effluents.

ABSTRACT
There is still a great knowledge gap in the understanding of characteristics and bioavailability of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) in wastewater effluents, which surmise implications related to both discharge regulation and treatment practice. In this study, we simultaneously investigated the characteristics and bioavailability of both DON and DOP, with separated hydrophilic versus hydrophobic fractions, in highly-treated wastewater effluents for the first time. The tertiary effluents from two wastewater treatment plants were separated into two fractions by XAD-8 resin coupled with anion exchange resin based on the hydrophobicity. Results showed that the majority of DON was present in hydrophilic forms while more DOP existed in hydrophobic forms. Hydrophobic fraction of effluent DOP was more likely to be bioavailable for algae. Hydrophilic fraction of effluent DON seemed to exhibit higher bioavailability. The bioavailable fraction of DON varied widely (28%–61%) for the two plants studied and the hydrophilic fraction with lower C/N ratio seemed to exhibit higher bioavailability than the hydrophobic portion. The differences in bioavailable DON and DOP distributions of effluents from those two plants could be attributed to different receiving effluent compositions and wastewater treatment processes. In addition, fluorescence excitation–emission matrices (EEMs) combined with parallel factor analysis (PARAFAC) were used to provide insights of DOM composition.
Hydrophilic
Hydrophobic fractionation
to characterize the dissolved organic matter (DOM) in wastewater effluent, which provided insights into the nature of organic matter in wastewater samples with different characteristics and originating sources.

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1. Introduction

The effluent discharged from wastewater treatment plants (WWTPs) can be an important source of nutrient loading to aquatic environments and thus contributes to eutrophication. More stringent nitrogen and phosphorus discharge limits have been implemented on wastewater treatment plants in environmentally sensitive areas (Clark et al., 2010). For example, in Chesapeake Bay, more stringent permit limits have been required for plants to achieve effluent quality of 3 mg/L total nitrogen (TN) and 0.3 mg/L total phosphorus (TP). In advanced WWTPs that apply enhanced nutrient removal technologies to meet very low nutrient levels, most of the dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) in wastewater can be removed, then dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) could account for substantial fractions (40–85% of TN, 26–81% of TP) of the remaining TN and TP in effluents (Pagilla et al., 2006; Ragsdale, 2007; Gu et al., 2011, 2014; Liu et al., 2012). Watershed protection plans may therefore have to differentiate the bioavailability of different forms of nitrogen and phosphorus to balance the costs and benefits of removing DON/DOP versus controlling the remaining inorganic nutrient sources (Pehlivanoglu and Sedlak, 2004; Mulholland et al., 2007; Filippino et al., 2011; Simsek et al., 2013).

However, standardized or consented methods to measure the bioavailability of DON and DOP from wastewater effluent are not currently available. And discounting wastewater-effluent DON/DOP from total discharge regulations could substantially reduce construction costs of WWTPs and associated upgrades for advanced nutrient removal (Bromk et al., 2010). For these reasons, it is important to evaluate the potential bioavailability of DON and DOP to effluent receiving water and provide evidence to assist regulatory agencies for determining the necessity in excluding DON/DOP from the nutrient discharge allowance and, whether plants are required to upgrade current nutrient reduction technology for further removal of those organic nutrients in the effluents.

There are a limited number of studies on wastewater effluent DON bioavailability. In those studies, bioavailability of DON is determined by cultivating defined cultures or microbial communities from natural receiving waters in wastewater final effluent, where monitored net DON decrease was defined as bioavailable DON (Pehlivanoglu and Sedlak, 2004; Urgun-Demirats et al., 2008; Bromn et al., 2010; Filippino et al., 2011; Liu et al., 2012; Simsek et al., 2013). These studies showed a wide range (18–96%) of the initial DON in low-total-nitrogen wastewater effluents that was bioavailable to algae and/or bacteria. However, the determination of true bioavailable DON only from the net changes of DON might not be accurate as the algae would both consume and release organic nutrients during the bioassay. Most studies mentioned above recognized that the increased DON caused by production from microbial community would mask the true utilization of effluent DON. Using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) analysis, Mesfoui et al. (2012) showed that 79% to 100% of the organic nitrogen compounds present at the start of the incubation were removed during the incubation with new nitrogen-containing compounds produced, which indicated that the effluent DON pool is much more dynamic than the net DON changes could reveal.

The characteristics and bioavailability of wastewater-derived DON have rarely been studied (Gu et al., 2011, 2014; Li and Brett, 2012, 2013) and less reported than that of DON. The few studies evaluated the bioavailability of soluble reactive phosphorus (SRP) fraction and DOP, and showed that the classic assumption that SRP approximately equals bioavailable P (BAP) was not true for most of the cases (Li and Brett, 2013). They suggested that most organic forms of dissolved phosphorus (P) seemed highly bioavailable and could be utilized as a supplemental P source in the absence of inorganic P. The P fractions in the humic substances did not support algal growth, indicating that the organic P in these humic-Al-Fe complexes had very low bioavailability to algae.

In this study, we simultaneously investigated the bioavailability of both DON and DOP, with separated hydrophilic versus hydrophobic fractions, in highly-treated wastewater effluents for the first time. The bioavailable DON and DOP were determined by algae growth yield since previous studies have shown that using algae growth to assess organic nutrient bioavailability is feasible (Pehlivanoglu and Sedlak, 2004; Li and Brett, 2012). Due to the dynamic changes of the DON and DOP pool in the bioassay as mentioned earlier, we believe that bioavailability determined based on algal growth is more accurate than simply tracking the net changes in the total amount of DON or DOP. Effluents from two wastewater treatment plants were obtained and separated into two fractions based on the hydrophobicity, so as to examine the bioavailability of hydrophobic versus hydrophilic DON and DOP. Moreover, fluorescence excitation–emission matrices (EEMs) combined with parallel factor analysis (PARAFAC) were also applied here to characterize the dissolved organic matter (DOM) in wastewater effluent and monitor the changes in the composition or nature of the complex and dynamic DON and DOP pool during the bioassay.

2. Materials and methods

2.1. Effluent sampling

The wastewater treatment effluents from two enhanced nutrient removal plants were chosen in this study because their final effluents contained higher fraction of DON and DOP in relation to the dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP). The Broad Run Water Reclamation Facility in Loudoun County of Virginia (Loudoun Plant) consists of primary clarifier, fine screen, BNR-Membrane Biological Reactor (MBR) (5-stage Bardenpho process with membrane filtration) and a Granular Activated Carbon (GAC) adsorption process. The Pinery Wastewater Reclamation Facility (Pinery Plant) treats domestic wastewater from a service area located south of Parker Colorado, and the treatment processes include primary treatment, BNR secondary process (5-stage Bardenpho), followed by a multi-stage tertiary filtration process. The two effluents (grab samples taken at one time) were designated as Loudoun and Pinery effluent samples in this paper, respectively. All the effluent samples in this study were collected prior to UV disinfection. The wastewater effluent samples were filtered through a polycarbonate membrane filter (Millipore, 47 mm, 0.45 µm) immediately once shipped to the lab in coolers and then stored at 4 °C prior to use.

2.2. Separation of hydrophobic and hydrophilic fraction in wastewater effluent

The scheme of resin separation for effluent fractionation was shown in Fig. S1 and more details of the separation protocol were given by Liu et al. (2012). All the wastewater effluent samples were first acidified to pH of 2.0 with HCl and then pumped through Amberlite XAD-8 resin (now Supelite XAD-8 resin, Sigma-Aldrich) with flow rate of 1 mL/min. The eluents were collected as hydrophilic fractions that contained hydrophilic DON, DOP, inorganic nitrogen species (NH₄⁺, NO₂⁻, NO₃⁻) and phosphates. The hydrophobic fraction retained on XAD-8 resin was later eluted by 0.1 M NaOH at flow rate of 1 mL/min in reverse direction. To
remove dissolved inorganic nitrogen (DIN) species and dissolved inorganic phosphate (DIP) from the hydrophilic eluents that passed through XAD-8 resin, the samples were passed again through anion exchange resin (Dowex Chloride form resin, Sigma-Aldrich) to obtain the hydrophilic DIN-free and DIP-free fraction. No DIN or DIP was detected in these fractions.

2.3. Chemical analysis

Concentrations of nitrate and nitrite were determined by Dionex ICS-5000 ion chromatography system along with known standards. Concentrations of ammonium were measured in a UV mini-1240 spectrophotometer following the manual phenate method (Clescerl et al., 1998). Total dissolved nitrogen was measured with the persulfate oxidation method (Clescerl et al., 1998) and DON concentrations were obtained by the difference between total dissolved nitrogen and dissolved inorganic nitrogen according to other studies (Arnaldos and Pagilla, 2010; Liu et al., 2012). Concentrations of dissolved organic carbon (DOC) in various fractionated effluent samples were measured using a carbon analyzer (Shimadzu TOC-5000). The DON in wastewater effluent was determined according to Standard Methods (4500-P) which subtracts dissolved acid hydrolysable P (sAHP) and dissolved reactive P (sRP) from total dissolved acid-digested P (sTP) (Clescerl et al., 1998; Gu et al., 2014). Accuracy and reproducibility of low DON and DOC measurements were discussed in the previous reports (Gu et al., 2011, 2014; Li and Brett, 2013) and the detection limits of the DON and DOC in this study were 0.05 mg N/L and 0.005 mg P/L, respectively. The organic carbon-to-nitrogen (C:N) and carbon-to-phosphorus (C:P) ratio of the samples was calculated as DOC/DON and DOC/DOP, respectively.

2.4. Bioassay protocols

To examine the bioavailability of different effluent fractions to algae growth, the hydrophobic and hydrophilic fractions of wastewater effluent samples were investigated using algal bioassay (Clescerl et al., 1998). *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*, obtained from the Culture Collection of Algae at The University of Texas at Austin) were chosen as test algae culture in this study (Clescerl et al., 1998). The bioavailability of DON and DOP was assessed in two groups, respectively. In the first group, the algae were pre-suspended in N-free medium (supplied with NaCl instead of NaNO₃) for a week before the DON bioavailability bioassays. The nutrient medium described by Miller and Greene (1978) except phosphorous. Samples were incubated in flasks in horizontal shaker (speed of 11 rpm) at 25 °C under continuous fluorescent lighting of 4300 Lm for 14 days. Phosphorus free medium was used as the control and hydrophobic fractions spiked with 0.1 mg/L of phosphate were used as the positive control. All the treatments were conducted in triplicates in this study. Standard media with known concentration series of KH₂PO₄ (0, 25, 50, 100, 150, 200 μg P L⁻¹) were also incubated in triplicates to create the linear standard curve of algal growth yield as a function of available phosphorus (Fig. S2B, R² = 0.981). The bioavailable DOP was finally calculated based on the algal cell density in effluent samples at day 14 according to the algal cell count versus phosphorus concentration standard curve (Fig. S2B) similar to the approach used by Li and Brett (2012).

2.5. Characterization of wastewater effluent DOM with fluorescence spectrometry

Wastewater effluents before bioassay as well as the effluent filtrates at the end of bioassay tests were subjected to fluorescence analysis using a Varian Cary Eclipse Fluorescence Spectrometer. The Bandwidths were set to 5 nm for both excitation and emission scans. A series of emission scans (240–600 nm in 1 nm increments) were collected over excitation wavelengths ranging from 220 to 450 nm by 5 nm increments.

The fluorescence spectra were then Raman calibrated by normalizing to the area under the Raman scatter peak (excitation wavelength of 350 nm) of a Milli-Q water sample in Raman units (R.U., nm⁻¹). To remove Raman signal, Raman normalized fluorescence spectra were then Raman subtracted from sample EEM. Rayleigh scatter effects were removed from the data set where emission equals excitation wavelength and emission equals twice the excitation wavelength. The parallel factor analysis (PARAFAC) was carried out in Matlab R2009 with the DOMFluor toolbox (www.models.life.ku.dk; Stedmon and Bro, 2008). The PARAFAC model with 2–6 components was computed here and the best component numbers were determined based on residual analysis and split half analysis (Stedmon et al., 2003; Stedmon and Bro, 2008). After determining the optimal component numbers for the data set in this study, the fluorescence intensities of each component in each sample could be calculated by DOMFluor toolbox to reveal the qualitative differences of component fluorescence among samples.

2.6. Statistical analysis

Student’s T-test was performed to determine whether there are significant differences in algae growth between hydrophobic or hydrophilic DON/DOP fractions and the N-free or P-free medium blank controls. Differences were considered statistically significant if the resultant p value was < 0.05.

3. Results & discussion

3.1. Quantification of hydrophobic and hydrophilic fractions of DON and DOP in wastewater effluents

Fig. 1 shows the distribution of hydrophobic and hydrophilic DON and DOP fractions, as well as the total DON and DOP measured in the
forms. 31P-NMR spectroscopy from Gigliotti et al. (2002) suggested that the hydrophilic fraction is rich in larger series of nitrogenous molecules by FTICR-MS analysis (Reemtsma et al., 2008). These consistent findings support our finding and suggest that the dominant hydrophobic DOP in these effluent samples is likely comprised of phosphate monoester and phosphate diester.

3.2. Bioavailability assessment of DON and DOP in effluents

An exemplary algae growth profiles with different fractionated effluent samples during 14-day bioavailability assay are shown in Figs. S3 and S4. Algal growth in the hydrophobic fraction was assumed to be associated with available hydrophobic DON or DOP, and the growth in the ion exchange treated DIN or DIP-free hydrophilic fraction was assumed to be stimulated by the bioavailable hydrophilic DON or DOP.

For DON bioavailability assay, similar results were observed for effluents from the two plants and shown in Fig. S3A and B. Algae grew the most in both untreated effluent samples and the hydrophilic fractions due to the relatively abundant bioavailable nitrogen in the form of nitrate. The similar algae growth in hydrophobic fraction and the N-free media, both amended with 0.7 mg N/L of nitrate, suggested that there was no observable toxicity in fractionated hydrophilic DON samples, ruling out the possibility that the fractionation process may introduce some toxicants.

The algae growth with both hydrophobic and ionic exchange treated DIN-free hydrophobic effluent fractions exhibited slightly but statistically significant higher rates ($p < 0.05$) than the N-free medium control group (Fig. S3, Table S1). These results showed that both the hydrophobic and hydrophilic DOP stimulated algae growth relative to N-free medium control and similar patterns were observed for effluents from both plants. Similarly, stimulated algal growth was also observed with both hydrophilic and hydrophobic effluent DOP fractions from the two plants (Fig. S4, Table S1). Compared to P-free medium control treatment, algae grew significantly faster in both hydrophobic and hydrophilic DOP fractions. Unlike the DON bioavailability results, the hydrophobic DOP fraction was more prone to stimulate algae growth than hydrophilic DOP fraction during the 14-day assay.

3.3. Distributions of bioavailable DON and DOP

The amount of bioavailable hydrophobic and hydrophilic effluent DON fractions was determined based on the algae growth at day 14 according to the biomass versus nitrogen concentration standard curve (Fig. S2), and the results are shown in Fig. 2. The non-bioavailable fractions were calculated as the difference between the measured DON fraction concentrations and the bioavailable DON concentrations. As shown in Fig. 2, approximately 60.5% and 27.9% of the effluent DON was bioavailable for Loudoun and Pinery effluents. About 78.2% and 5.6% of the hydrophobic fraction of effluent DON were bioavailable, and about 48.7% and 36.3% of the hydrophilic DON fraction were bioavailable for the effluents in Loudoun and Pinery plants, respectively.

Liu et al. (2012) studied the bioavailability of DON in wastewater effluents using the same bioassay but with the presence of bacteria, and found that 40–85% hydrophobic DON of effluents was consumed, indicating that hydrophobic DON fraction is more bioavailable than hydrophilic DON fraction. While in this work, both hydrophobic and hydrophilic DON stimulated a relatively low level of algal growth with wastewater effluent from the two plants studied. Note that the activated carbon adsorption process employed at Loudoun might remove a fraction of hydrophobic organic substances, which consequently affected the fraction composition. Given the fact that similar bioassay algae, TN, and DON-level effluent were used, the introduction of bacteria to the bioassay in their study (Liu et al., 2012) likely contributed to the enhancement of hydrophilic DON bioavailability, because there are great

![Fig. 1. Distribution and recovery of hydrophilic and hydrophobic DON and DOP fractions in wastewater effluents. (A) DON fractions, (B) DOP fractions.](image-url)
The differences in the DON utilization mechanisms between algae and bacteria. The phytoplankton could either break down large DON polymers into smaller molecules for easier utilization or take up DON molecules as whole through enzymatic release, pinocytosis and phagocytosis process (Bronk et al., 2007); while natural indigenous bacteria separated from river water can help to break down DON via extracellular enzymes and thus enhance algae uptake of DON (Pehlivanoglu and Sedlak, 2004). The processes and factors in the natural environment that may affect the bioavailability of effluents are therefore of interests, but beyond the scope of this study.

In the second group of bioassays, the DOP bioavailability was determined in the same manner as that for DON bioavailability assessment. Similarly, the amounts of bioavailable hydrophobic and hydrophilic effluent DOP fractions were determined according to the biomass versus phosphate concentration standard curve (Fig. S2) based on algae growth on day 14 in different effluent samples. As shown in Fig. 3, about 75.4% of the total effluent DOP from Loudoun plant was bioavailable (0.043 mg/L) and 53.5% of which is of hydrophobic nature; about 65.7% of the hydrophobic fraction and 90.9% of the hydrophilic fraction of the DOP were bioavailable. For Pinery effluent, approximately 73.7% of the effluent DOP was bioavailable, of which most was distributed to hydrophobic fraction (0.035 mg P/L, 76.1% of hydrophobic DOP fraction); and only a small amount of hydrophilic DOP (0.007 mg P/L, 63.6% of hydrophilic DOP fraction) was bioavailable. These results were consistent with the algae growth shown in Fig. S4, where the hydrophobic DOP fraction dominates the algae growth during the 14-day assay.

According to the results shown above, the effluent DOP seems more labile in nature than the DON components. About 60.5% and 27.9% of the effluent DON were bioavailable for Loudoun and Pinery effluents respectively, whereas, 75.4% and 73.7% of the effluent DOP were bioavailable for the two effluents we studied.

3.4. C/N and C/P ratios associated with DON and DOP fractions

The C:N ratio is a good indicator to identify the sources of organic matter in the environment. Usually, high C:N ratio indicates allochthonous input of organic matter and more humic-like DON while low C:N ratio suggests autochthonous input of organic matter and more proteinaceous DON (Huang et al., 2005; Liu et al., 2012). Fig. 4A shows the comparisons of the C:N ratio across different DON fractions, as well as the original whole effluent sample. The lower C:N ratio of the hydrophilic DON fraction reflects its higher nitrogen richness in relation to the hydrophobic fraction (Fig. 4A). This may explain the differences in the bioavailability between the hydrophobic and hydrophilic fractions, as the hydrophilic DON seems to enrich nitrogen and therefore would be more favored by algae for labile nitrogen use.

C:P ratios in various DOP fractions and the original effluents are shown in Fig. 4B. In contrast to the patterns for DON, the hydrophobic portion of the effluent seems to be more enriched with phosphorus than the hydrophilic proportion, as indicated by its lower C:P ratio. This is consistent with the fact that hydrophobic DOP is more bioavailable, and suggested higher relative abundance of organic phosphorus in the hydrophobic fraction, such as hydrophobic phosphate monoester and phosphate diester. Some studies have demonstrated that phytoplankton could use organic phosphorus compounds as available P source (Cotner and Wetzel, 1992; Huang et al., 2005). However, the utilization mechanism of DOP is still not clear. The algae might utilize DOP after alkaline phosphatase decomposition into inorganic phosphorus (Hong et al., 1995), and another proposed mechanism is that algae uptake DOP directly into algal cell membranes first, then decompose and utilize DOP through alkaline phosphatase located in the membranes (Huang and Hong, 1999).

The differences in bioavailable DON and DOP distributions between the two wastewater effluents might be the results from the differences in effluent compositions. This is reflected by the different C:N or C:P ratios observed for the hydrophilic and hydrophobic DON or DOP fractions.
between the two plants (Fig. 4A, B). As mentioned in section Effluent sampling, Loudon had more advanced treatment than Pinery with membrane filtration followed by GAC. GAC process might remove more hydrophobic organic matters such as humics, and led to different organic compositions of the effluent.

3.5. Characterization of dissolved organic matter in wastewater effluents via fluorescence spectroscopy

EEM fluorescence spectroscopy has become the novel emerging technique to characterize organic matter in aquatic ecosystems (Stedmon and Bro, 2008; Gray et al., 2011). Coupled with PARAFAC, fluorescence spectroscopy could decompose the complex fluorescence matrices into different fluorescent components and reveal the origin of resolved fluorescent components by the position of fluorescence maxima. In this study, we used fluorescence spectroscopy method to (1) characterize wastewater untreated effluent and fractionated samples, and (2) identify individual fluorescent components with PARAFAC models and monitor the component intensity during algae bioassay.

First, the appropriate number of components for the model was determined by the comparison of the sum of squared differences for 2–6 components. The results suggested that 4 components are sufficient (the variability explained > 95.5%) for interpretation of the data set in this study (Figs. S5 and S6). Fig. S7 shows the representative contour plots of identified components in the 4-component PARAFAC model using all the samples in this study. By comparing the positions of fluorescence peaks with those in other reported studies, the organic nature of the four determined components was identified (Table 1).

The identified fluorescent components allowed for the characterization and comparison of the wastewater effluents and different fractions. Wastewater effluent from different WWTPs and their hydrophilic or hydrophobic fractions exhibited distinct fluorescence patterns (Fig. S8). As shown in Fig. 5, the relative abundance of each identified component in whole and fractionated wastewater effluents varied for different WWTPs and they provided more insights into their molecular composition. Compared to hydrophobic fraction, relatively higher abundance of WWTPs and they provided more insights into their molecular composition and comparison of the wastewater effluent using all the samples in this study. By comparing the positions of fluorescence peaks with those in other reported studies, the organic nature of the four determined components was identified (Table 1).

In summary, 27.9–60.5% of DON and 73.7–75.4% of DOP from wastewater treatment effluent were found to be potentially bioavailable using algae bioassay test. Considering the coexistence of bacteria in the wastewater effluent receiving water, which will enhance DON utilization due to symbiotic effect (Pehlivanoglu and Sedlak, 2004; Urgun-Demirtas et al., 2008; Liu et al., 2012), the effluent DON and DOP could be more bioavailable than what we previously expect in natural aquatic environment. Therefore, these effluent-derived DON and DOP should be counted towards the nutrient allocations for WWTP discharge and not be excluded from wastewater effluent TN and TP regulations. However, then the WWTPs with stringent nutrient discharge limits will have to adopt more advanced treatment processes (e.g., reverse osmosis, advanced oxidation, adsorption technique) to reduce DON and DOP levels in final discharged effluent (Krasner et al., 2009; Liu et al., 2012). Note that these observations are based on samples from a limited number of WWTPs, further investigation with larger and more varied studies may be desired to obtain more comprehensive understanding.

Furthermore, when evaluating or implementing technologies for dissolved organic nutrient removal, the high and added capital and O&M cost associated with multi-stage and more complex treatment processes for removing effluent residual DON and DOP must be balanced with environmental and economic assessments. In other words,

Table 1. Fluorescence spectroscopy identified components in the effluent samples.

<table>
<thead>
<tr>
<th>Component</th>
<th>Excitation and emission maxima</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ex: 254 nm &amp; 360 nm; Em: 450 nm</td>
<td>Terrestrial humic-like materials</td>
<td>Stedmon et al. (2003)</td>
</tr>
<tr>
<td>2</td>
<td>Ex: 235 nm &amp; 275 nm; Em: 354 nm</td>
<td>Protein-like (tryptophan) fluorophores</td>
<td>Determann et al. (1994, 1996), Coble et al. (1998)</td>
</tr>
<tr>
<td>3</td>
<td>Ex &lt; 240 nm; Em = 450 nm</td>
<td>Not previously reported, not discussed in this study</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ex: 235 nm &amp; 220 nm; Em: 411 nm</td>
<td>Humic fluorophore with anthropogenic origin and microbial humic-like compounds</td>
<td>Stedmon and Markager (2005), Murphy et al. (2011)</td>
</tr>
</tbody>
</table>
the benefits in potential eutrophication reduction must be weighed against both the direct cost and other unintended indirect costs associated with various environmental and health impacts (i.e. carbon footprint, greenhouse gas emission, and toxicity) during the life cycle.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.11.005.

References


