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Short Communication

Application of a new combined fractionation technique (CFT) to detect fluorophores in size-fractionated hydrophobic acid of DOM as indicators of urban pollution

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ABSTRACT

For pollution monitoring, we developed a new combined fractionation technique (CFT) to characterize dissolved organic matter (DOM) in natural water by combining resin adsorption (RA) and ultrafiltration (UF) with 3D-fluorescence measurement. We tested the new technique on 4 polluted and 4 unpolluted samples. The 3D-fluorescence characteristics of size sub-components in the hydrophobic acid (HPOA) fraction could distinguish unpolluted from polluted DOM. The unpolluted HPOA fraction was composed of a single dominant size component—peak A (fulvic-like, around Ex240/Em410 nm) material with relatively large molecular weight (MW) (>10 kDa). In comparison, the HPOA in polluted DOM contained another predominant size component with lower MW (<5 kDa)—peak T material (tryptophan-like protein, around Ex230/Em340 nm). The fluorescence of peak T material with lower MW (<5 kDa) in HPOA would be a good indicator of pollution or deterioration of source water quality. The application of this new CFT could yield more detailed and scientific information on the size and chemical character of the fluorophores in DOM sub-fractions.

1. Introduction

One of the major roles of water utilities is to ensure the supply of safe drinking water to consumers. This continues to be a challenge, especially as the number and severity of water pollution incidents are increasing, especially in China (Yuan et al., 2009). Most of the pollution incidents arise from the spillage of wastewater due to process failure or environmental events such as storm runoff or illegal discharge (Bourgeois et al., 2003; Yuan et al., 2009). Therefore, there is a strong need to develop rapid analytical procedures, and/or surrogate parameters, for quality assessment of source water, including targeted monitoring after emergency situations involving wastewater treatment plant failure and mains breakages in sewage networks (Stadler et al., 2010). However, sensor development for monitoring water pollution continues to be challenging, because it is very difficult to have a specific sensor or sensors which can cover the broad range of chemicals present in water.

An important substance among the broad range of chemicals of environmental concern in aquatic systems is dissolved organic matter (DOM), consisting of organic components such as humic and fulvic acids, polysaccharides, proteins, and extracellular products (DOM is commonly considered to be aquatic organic matter <0.45 μm (Chow et al., 2009; Shi et al., 2007; Wei et al., 2010)). Many of these components are considered as pollutants in natural waters (Leenheer and Croue, 2003; Xu et al., 2011).

From a more positive perspective, some DOM components could be used as indicators for pollution assessment, and the utilization of surrogate aquatic chemicals such as DOM, for monitoring or assessment of water pollution, has generated a lot of interest in recent literature (Bieroza et al., 2009; Henderson et al., 2009; Standley et al., 2000; Takahashi and Kawamura, 2007). For example, Standley et al. (2000) developed a molecular tracer method for identifying organic matter sources to surface waters, using gas chromatography/mass spectrometry to analyze the tracers including fecal steroids, caffeine, and petroleum. However, the analytical procedure was time-consuming and therefore not appropriate for rapid water quality monitoring.

The use of 3D fluorescence characterization of DOM by fluorescence excitation-emission matrix spectrophotometry (EEMS) has great potential for on-line monitoring of various pollutants entering river systems (Baker, 2001, 2005; Baker et al., 2003; Baker and Spencer, 2004; Henderson et al., 2009; Liu et al., 2007; Wu et al., 2003). In these earlier studies, the analytical information was generated indirectly by establishing relationships between the fluorophores in bulk DOM and parameters such as biochemical oxygen demand and nitrate (Baker and Inverarity, 2004; Hudson et al., 2008). However, a key limitation of this approach was that important analytical information about the physical character (size) and chemical character (hydrophobic/hydrophilic) of the fluorophores was not available.

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Therefore, the broad objective of our present study was to extend our knowledge of fluorophores in DOM fractions and to achieve this by: (i) developing a new combined fractionation technique (CFT) to isolate organic fractions in DOM, (ii) using multiple detection systems, including EEMS, to characterize the DOM fractions, and (iii) using this information to assess the feasibility of using characteristics of the organic fractions as indicators of water pollution.

2. Methods and materials

2.1. Source water and sampling procedures

In July 2010, four kinds of water samples, (two river waters and two reservoir waters), were collected in Guangdong Province, southern China. The two river water samples (Dongjiang river and Sheweicun river) were representative of urban polluted water sources such as wastewater, sewage or runoff. They were sampled downstream of Huizhou city (N22°53.688′, E114°30.434′, H 20.0 m), and downstream of Santou town (N23°05.065′, E114°09.885′, H 15.2 m), respectively. The two reservoir waters (Futian reservoir, N22°45.521′, E114°17.802′, H 28.2 m and Wanyao reservoir, N22°55.521′, E114°17.802′, H 77.4 m) were presumed uncontaminated by urban pollution; both locations were on a mountain with good environmental protection, and without industry or farming activities. The polluted waters and their DOM were labeled as PoL1 (Dongjiang river) and PoL2 (Sheweicun river), and the unpolluted waters and their DOM were labeled as UnP1 (Futian reservoir) and UnP2 (Wanyao reservoir).

In January 2011, four additional source water samples were collected in Guangdong Province. The two urban polluted sampling positions were N23°00.919′, E114°43.915′ (Xiaobu-Xizhi river, PoL3) and N23°02.376′, E114°29.942′ (Maan-Xizhi river, PoL4), and the two unpolluted sampling positions on mountaintop were N22°51.495′, E114°32.702′ (Shatian reservoir, UnP3) and N22°51.974′, E114°25.629′ (Jixinshi reservoir, UnP4).

For each sampling site, 4 L water sample was collected in a brown color borate glass bottle that had been pre-cleaned with 10% HCl, 1% NaOH, and distilled water sequentially. Samples were filtered (0.45 μm) in the field and then refrigerated at 4 °C immediately.

2.2. Analytical fractionation of DOM by resin adsorption (RA)

The volume of water samples for the RA fractionation was at least 500 mL, and the ratio of water volume to wet resin volume was 30:1. A filtered (0.45 μm) water sample was first passed through DAX-8 resin (Supelco, USA) without any pH adjustment, resulting in the hydrophobic base and neutral (HPOBN) fraction being retained on the resin. The effluent from this first DAX-8 column was then adjusted to pH 2, and loaded onto another DAX-8 resin column to adsorb the hydrophobic acid (HPOA) fraction. Subsequently, the effluent from the second DAX-8 column was passed through XAD-4 resin. The fraction adsorbed by the XAD-4 resin was weakly hydrophobic acid (WHPOA).

The organics contained in the last effluent (not retained on either DAX-8 or XAD-4) were identified as hydrophilic matter (HiM). The dissolved organic carbon (DOC), ultraviolet absorbance at 254 nm (UV_{254}), and 3D-fluorescence of all influent and effluent samples were measured, and then the DOC, UV_{254} and fluorescence (the more detailed subtraction procedure is described below) of each DOM fraction were quantified by the difference between the influent and effluent samples.

The amount of each fraction was calculated by subtraction as follows, and the result for each fraction was labeled "subtracted": (i) HPOBN-subtracted = bulk DOM—1st effluent of DAX-8, (ii) HPOA-subtracted = 1st effluent of DAX-8—2nd effluent of DAX-8, (iii) WHPOA-subtracted = 2nd effluent of DAX-8—effluent of XAD-4, and (iv) HiM = effluent of XAD-4.

The primary distribution and percentage (%) of the chemical fractions of each DOM (e.g. % DOC) could be easily calculated by fraction-subtracted/bulk DOM.

2.3. Ultrafiltration (UF)

Ultrafiltration was performed by using a stirred UF cell (Millipore, 8200) with YM disk membranes (Amicon, nominal molecular weight cutoffs (MWCOs) of 5 and 10 kDa), as previously described (Wei et al., 2008a, 2008b). The parameter (DOC or UV_{254}) of each physical (size) MW fraction was also based on the difference between the feed and permeate samples in each filtration step. The concentration factor (CF) of UF was set at 6 for each filtration step.

2.4. Combined fractionation technique (CFT) of RA-UF

To obtain the size subcomponents of a chemical fraction, the fraction (e.g. HPOA) was first isolated and recovered by a traditional preparative fractionation method (Leenheer, 1981). The HPOA was recovered by loading 10 bed-volumes of 0.01 mol L^{-1} NaOH solution through the column after the acidified sample (2 L) was passed through the second DAX-8 column. The recovered HPOA fraction in the effluent was then normalized to the DOC concentration of 7 mg L^{-1}, by dilution with Milipore-Q (Milli-Q) pure water and its pH was adjusted to 7 (±0.05). This solution was then fractionated by UF and the size subcomponents were obtained as described in Section 2.3. The combination of RA with UF may be defined as a combined fractionation technique (CFT) (Wei et al., 2011) which provides further detailed information about the size subcomponents of the primary chemical fraction.

2.5. Chemical analysis

Reagent-grade chemicals and Milli-Q water were used. The filtered solution (0.45 μm pore size of membrane) was analyzed for dissolved organic carbon (DOC) (TOC-VCPH, Shimadzu, Japan) and UV_{254} (UV-9600 spectrophotometer, Beijing Rayleigh). The SUVA is the specific UV absorbance calculated by $UV_{254}/DOC \times 100$ ($L\,mg^{-1}\,m^{-1}$).

The 3D-fluorescence measurements were undertaken using an F-4600 FL spectrophotometer (Hitachi, Japan), and methods of Baker (2001) and Liu et al. (2007) were adopted. The spectrometer used a Xenon excitation source, and the slits were set to 5 nm for both excitation and emission. To obtain 3D-fluorescence EEMS, excitation wavelengths were incremented from 200 to 400 nm at 5 nm steps; for each excitation wavelength, the emission was detected from 280 to 500 nm in 2 nm steps. The temperature of the analysis and PMT voltage were set at 23 ± 1.5 °C and 770 V, respectively. Quinine sulfate (QS) solution (1 μg QS L^{-1} in 0.1 mol L^{-1} H_2SO_4) was used to monitor the stability of the energy emitted by the Xenon lamp in the fluorometer, and no change in QS fluorescence was observed. The maximum relative standard deviation for EEMS measurement was lower than 2.5%. All samples were adjusted by HCl or NaOH to around pH 7 before fluorescence measurement.

2.6. Data processing

The 3D contour graphs were plotted by OriginPro (7.5) software based on matrix data from the F-4600 instrument. The cluster analysis was done by SPSS (13.0) software using "Between-Groups Linkage and Squared Euclidean Distance" options.

3. Results and discussion

3.1. Primary chemical fraction distribution of DOM

The percentage of DOC (%DOC) and SUVA in the chemical fractions of each water sample is shown in Fig. 1A and B, respectively.