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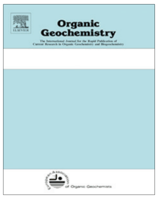

Characterization of size fractionated dissolved organic matter from river water and wastewater effluent using preparative high performance size exclusion chromatography

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ABSTRACT

Structural and functional characterization of dissolved organic matter (DOM) is challenging due to its inherent heterogeneity and coherent complexity. Here, a relatively new method based on preparative high performance size exclusion chromatography using deionized water as mobile phase (recovery rate of total carbon mass > 97%) was developed to fractionate DOM depending on molecular size and identify molecular weight (MW)-dependent DOM characteristics presumably associated with its potential for formation of complexes with inorganic species (i.e. metals and metalloids), membrane fouling and disinfection byproducts. Size fractionated DOM from different types of source (river water vs. wastewater effluent) provided similar patterns in water quality, fluorescence properties and biopolymer composition. This suggested that discharge of municipal wastewater had an impact on the river water. However, the propensity for complexation between each size fractionated DOM sample and various inorganic species was substantially different, due to the differences in fluorescence spectral properties and associated biopolymer composition. Among three distinct size fractionated DOM samples, the fraction with medium MW and the main biopolymers polyhydroxy aromatics exhibited a relatively higher potential for metal complexation and the normalized ratio of metals and metalloids to dissolved organic carbon (DOC) [metal/DOC (fraction i, i = I, II, III)] to [metal/DOC (fraction I)] than the other size fractionated DOMs. The results indicate that the biopolymer composition of DOM plays a critical role in its complexation with inorganic species.

1. Introduction

Owing to different sources and mechanisms of decomposition, the structure of dissolved organic matter (DOM) may exhibit varying characteristics with respect to chemical composition, molecular size, functional groups/charge density, aromaticity and hydrophobicity (Thurman, 1985; Leenheer and Croué, 2003; Karanfil et al., 2008; Matilainen et al., 2011). Therefore, various analytical methods, such as UV and fluorescence spectroscopy, high performance size exclusion chromatography (HPSEC), nuclear magnetic resonance (NMR) spectroscopy, Amberlite XAD-8/4 resin, Fourier-transform infrared (FTIR) spectroscopy and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS), have been widely used to identify the fate and reactivity of DOM in both engineered (i.e. water treatment processes) and natural systems (Thurman, 1985; Piccolo et al., 2002; Leenheer and Croué, 2003; Karanfil et al., 2008).

In aquatic systems, DOM may provide C and energy sources for microorganisms, affect the levels of dissolved O_2, C, N and P, as well as acidity and mediating photochemical reactions, especially with humic substances (Leenheer and Croué, 2003; Weishaar et al., 2003). Furthermore, it plays an important role in the speciation of trace metals, which is closely associated with their bioavailability, mobility and toxicity (Kinniburgh et al., 1999). In spite of the importance of DOM in the biogeochemical processes in aquatic systems, it has also been considered as a major water pollutant since it can pose a serious threat both directly and indirectly for drinking water: (i) esthetic concerns (e.g. taste, color and odor), (ii) promotion of bacterial regrowth in water distribution systems, (iii) contribution to flux decline in membrane processes, (iv) increase in demand for coagulants and oxidants and (v) formation

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of disinfection byproducts (e.g. trihalomethanes, haloacetic acids, bromate and *N*-nitrosodimethylamine), which may cause undesirable health effects because of their carcinogenicity and mutagenicity (Westerhoff et al., 1999; Leenheer and Croué, 2003; Richardson et al., 2007; Chon et al., 2012a, 2013a).

The inherent heterogeneity of DOM is a major obstacle to its structural and functional characterization (Piccolo et al., 2002). Therefore, many researchers have attempted to establish protocols to separate and concentrate it from surface water and wastewater for reducing its heterogeneity, prior to its characterizations (Ma et al., 2001; Maurice et al., 2002; Kim et al., 2003; Schwede-Thomas et al., 2005). Amberlite XAD-8/4 resin and/or C_{18} solid phase extraction (SPE) cartridges have been commonly used to isolate and concentrate DOM from water samples (Maurice et al., 2002; Kim et al., 2003). However, these methods may not be adequate for isolating hydrophilic DOM fractions (i.e. polysaccharides and proteins) as the separation of DOM with Amberlite XAD-8/4 resin and/or C_{18} SPE cartridges is based mainly on the hydrophobic interaction between hydrophobic DOM fractions (i.e. humic substances) and polymeric adsorbents (Croué, 2004; Williams et al., 2016). Furthermore, structural alteration may occur during the extraction procedure (Wu et al., 2007). Ultrafiltration (UF) has been used to fractionate DOM depending on the molecular weight (MW) but is not useful for isolating relatively low MW fractions of DOM [MW < 1000 Daltons (Da)] from water samples since the lowest MW cutoff for UF membranes is > 1000 Da (Croué, 2004). For these reasons, there is a considerable need to develop a new approach for extending knowledge on the fate and reactivity of DOM in either engineered systems or natural systems.

Piccolo et al. (2002) developed a preparative HPSEC method for isolating humic acids (HAs) from water samples. Unlike conventional DOM fractionation methods, such as Amberlite XAD-8/4 resin, C_{18} SPE cartridges and UF membranes, preparative HPSEC can allow systematic collection of different MW fractions of HAs, with distinctive chemical characteristics (Egeberg and Alberts, 2003; Peuravuori and Pihlaja, 2004). Moreover, changes in the structure of HAs during separation and the homogeneity of the size fractionated DOM, with regard to the original DOM, can be confirmed using analytical HPSEC with the size fractionated DOM (Piccolo et al., 2002). Some studies have demonstrated that preparative HPSEC is also effective for reducing the heterogeneity of DOM in surface waters, and subsequent characterization of the size fractionated DOM using Py-GC-MS and FTIR may provide valuable insight into MW-dependent structural characteristics of DOM in surface waters (Piccolo et al., 2002; Peuravuori and Pihlaja, 2004; Allpike et al., 2005). Nevertheless, preparative HPSEC has not been used to identify the molecular and functional composition of size fractionated DOM from wastewater effluent. Fundamental knowledge of the physicochemical characteristics of the size fractionated DOM samples from wastewater effluent is therefore limited.

The main objectives of this study were to (i) reduce the heterogeneity of DOM in river water and wastewater effluent using preparative HPSEC, (ii) collect fractionated DOM samples having different molecular size in relatively large sample volume and (iii) characterize the size fractionated DOM, with respect to similarities and differences in physicochemical properties, as opposed to bulk DOM in both river water and wastewater effluent samples.

2. Material and methods

2.1. Preparation of samples

A surface water sample from the midstream of the Yeongsan River (Gwangju, Korea) and a secondary effluent sample from the Gwangju wastewater treatment plant (WWTP; Gwangju, Korea) were used as primary sources of DOM. Both samples were filtered using 0.45 μm cellulose ester microfiltration filters (Advantec, Tokyo, Japan) and the filtered samples (1.5 l each) were concentrated [concentration factor 30; recovery > 97% based on total organic carbon (TOC) mass balance] using a rotary vacuum evaporator at 40 °C (N-1001S-W; Eyela, Tokyo, Japan). The bulk and size fractionated DOM samples were freeze dried using a freeze dryer [Ilshin Biobase, Gyeonggi-do, Korea] for Py-GC-MS analysis.

2.2. Preparative HPSEC

Each concentrated water sample (3 ml) was injected into a preparative HPSEC system [LC-9201; Japan Analytical industry (JAI), Tokyo, Japan] equipped with a gel permeation chromatography (GPC) column packed with a polyvinyl alcohol type polymer [7.6 mm inner diameter (ID), 500 mm length; Jaigel GS 310, JAI, Tokyo, Japan] using deionized water (DI water) as mobile phase at 5 ml/min, with UV detection at 254 nm (UV 3072; JAI, Tokyo, Japan) for aromatic substances and refractive index (RI) detection (RI-50; JAI, Tokyo, Japan) for polysaccharides and amino sugars. JAI provided the information on the GPC column, which was designed to fractionate DOM chromatographically using water as mobile phase, depending on molecular size and hydrophobicity, and is specialized for large volume injection of samples, with a maximum injection volume of 10 ml. The size fractionated DOM samples were collected manually, as monitored using both the UV and RI detection, into vials for further analysis. The recovery rate values of DOM after preparative HPSEC were calculated from TOC mass [DOC (mg/l) × sample volume (l)] of bulk and size fractionated DOM samples using the following equation: recovery rate of DOM = [sum of TOC mass of each size fractionated DOM (fraction i, i = I, II, III)/total carbon mass of bulk DOM].

2.3. Analytical HPSEC

The homogeneity and uniformity of the size fractionated DOM using preparative HPSEC with DI water as mobile phase, with respect to molecular size of the bulk DOM in the river water and secondary effluent (i.e. aromatic and protein-like substances), were investigated by way of an analytical HPSEC system equipped with a Protein-Pak 125 separation column (7.8 mm ID, 300 mm length; Waters, Milford, MA, USA), with UV detection at 254 nm (SPD-$10A_{VP}$; Shimadzu, Kyoto, Japan), fluorescence detection at excitation (Ex) wavelength 278 nm and emission (Em) wavelength 353 nm (RF-$10A_{XL}$; Shimadzu, Kyoto, Japan) and phosphate buffer (96 mM NaCl + 2.4 mM NaH_2PO_4 + 1.6 mM Na_2HPO_4, ionic strength 0.1 M, pH 6.8), with a flow rate of 0.7 ml/min and injection volume 200 μl (Chon et al., 2012b). The calibration curve was plotted using polystyrene sulfonate standards of 210 (Fluka, Buchs, Switzerland), 1800, 4600, 8000 and 18,000 Da (Polysciences, Warrington, PA, USA) to determine the relationship between retention time and MW.

2.4. Fluorescence spectroscopy

Fluorescent chromophores for both the bulk and size fractionated DOM were identified using a fluorescence spectrophotometer equipped with a 400 W Xe lamp (F-2500; Hitachi, Tokyo, Japan). A three dimensional fluorescence excitation-emission matrix of the bulk and size fractionated DOM was obtained by collecting the Ex and Em spectra over the range 220–500 nm at a photomultiplier tube voltage of 700 V with a scan speed of 3000 nm/min, Ex and Em slit width 5 nm and data collection interval of 10 nm. Subtraction of Raman scattering peaks in the samples was conducted using a DI water blank (Coble et al., 1993; Chon et al., 2013a).

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