Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.811115

52 (2014) 5840–5847
September

Taylor & Francis
Taylor & Francis Group

Role of a constructed wetland to humify effluent organic matter from a wastewater treatment plant

Dongjin Lee[a], Jaeweon Cho[b], Kangmin Chon[c], Byungjin Lim[a], Sudip Chakraborty[d], Kyongmi Chon[e,*]

[a]*Youngsan River Environment Research Center, 1110-8 Oryong-dong, Buk-gu, Gwangju 500-480, Korea*
[b]*School of Civil and Environmental Engineering, Yonsei University, Yonsei-ro 50, Seoul 120-749, Korea*
[c]*Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse, 133, 8600 Dübendorf, Switzerland*
[d]*Department of Chemical Engineering and Materials, University of Calabria, Cubo-44a, Via-P.Bucci, 87036 Rende (CS), Italy*
[e]*School of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 123 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Korea*
Tel. +82-62-715-2449; Fax: +82-62-715-2434; email: kyongmi@gist.ac.kr

Received 28 February 2013; Accepted 24 May 2013

ABSTRACT

The degree of aromaticity or hydrophobicity in wastewater effluent organic matter (EfOM) increases during flow-through constructed wetlands connected directly to a wastewater treatment plant (WWTP), as identified using fractionation followed by analysis methods, with respect to major biopolymers (polysaccharides, amino sugars, protein, polyhydroxy aromatics, and lignins). In this study, WWTP effluent and wetland EfOM were fractionated using preparative high-performance liquid chromatography (prep-HPLC) with both UV and RI detectors, and then, their physical and chemical properties were characterized using UV/Vis, high-performance size exclusion chromatography (HPSEC), 3D fluorescence, and pyrolysis-GC/MS (Py-GC/MS). WWTP and wetland EfOM were separated into three fractions (peak #1–3), using prep-HPLC, through C-18 and size exclusion mechanisms. Results of specific UV absorbance (SUVA), 3D fluorescence, and Py-GC/MS analyses indicate that relative aromaticity/hydrophobicity of organic matter are in the order of peak #3 > peak #2 > peak #1, which also represents order of molecular weight (MW) (peak #1 > peak #2 > peak #3).

Keywords: Constructed wetland; Effluent organic matter; Preparative HPLC; Pyrolysis GC/MS; Humification

1. Introduction

Natural organic matter (NOM) present in ground and surface waters consists of a heterogeneous mixture of humic and fulvic acids, carbohydrates, proteins (PRs), and lignins (LG), which are chemical and biological products of plant and animal residues [1–4]. NOM is known to play an important role in global carbon/nutrient cycling, and in the outcome and transport of many toxic organic or inorganic chemicals [5–8]. The structural and functional characterization of

*Corresponding author.

NOM has been extremely challenging because of its heterogeneous and ill-defined nature.

Effluent organic matter (EfOM) is mixture of structurally complex, heterogeneous organic compounds derived from raw wastewater and microbial activities in biological treatment processes. When investigating the composition of EfOM, only 15–20% of effluent organic carbon matter was identified, although a few major components of EfOM have been identified, such as aquatic humic substances (AHS), extracellular polymeric substances (EPS) or soluble microbial products (SMP), lipids and organic acids [9,10]. The chemical composition of EfOM has been compared with and is thought to be similar to NOM [11].

In order to understand the structural and functional properties of NOM better, various NOM fractionation methods have been used, which are either based on charge characteristics (ion-exchange, electrophoresis), on chemical and physical properties, on adsorption behavior (XAD resin methods), or on molecular size (size-exclusion chromatography, flow-field-flow fractionation, ultrafiltration) [12–14].

Preparative high-performance liquid chromatography (prep-HPLC) has been used for the isolation and purification of valuable products in the chemical and pharmaceutical industry, as well as in biotechnology and biochemistry [15–18]. However, it was not until Piccolo et al. [19] used this system to fractionate humic acid (HA) based on molecular weight (MW) that prep-HPLC was applied to NOM. Piccolo et al. [19] and Peuravuori and Pihlaja [20] later proposed an alternative method of NOM fractionation using high-performance size exclusion chromatography (HPSEC) in preparative mode. They collected samples up to eight fractions for further analyses. Unlike analytical HPLC, the prep-HPLC system requires a much larger amount of sample for the column as well as a higher flow rate, which allows for the collection of a target amount of fractionated NOM samples by repeat injections.

In this study, the fractionation of NOM samples was conducted using a prep-HPLC system with UV and RI detectors. This system allowed relatively large volume injection up to 10 mL, as it was equipped with a specialized preparation column; further analyses of fractionated samples were also performed. The objective of this study was to fractionate the bulk NOM from a wastewater treatment plant (WWTP) and wetland effluent into well-defined subcomponents by using prep-HPLC, and then characterize their physical and chemical properties using UV/Vis, HPSEC, 3D fluorescence, and pyrolysis-GC/MS (Py-GC/MS). We focused on the characterization of fractionated organic matter with respect to major biopolymers (polysaccharides (PS), amino sugars (AS), PR, polyhydroxy aromatics (PHA), and LG) using Py-GC/MS and understanding the degree of aromaticity/hydrophobicity in those samples.

2. Materials and methods

2.1. Sampling collection and measurement

WWTP effluent, treated by secondary (activated sludge process) and tertiary process for the removal of nitrogen and phosphorus, and wetland effluent were collected from the discharge points of the Damyang wastewater treatment plant (DY WWTP) in Korea and the adjacent free surface-flow constructed wetlands (35˚18′N, 126˚58′E), on 24 May 2011. The wetlands involved two different ponds, containing *Acorus* followed by *Typha* plants (i.e. WWTP → *Acorus* pond → *Typha* pond → wetland effluent). The wetland effluent flows to the Youngsan River, Korea. The entire wetland was designed to have a hydraulic retention time (HRT) of 6 h and a flow rate of 1,800 m^3/day. It was 220 m in length and 30 m in width, with an average depth of 0.13 m [21]. The samples were filtered through 0.45 μm microfilters (Mixed cellulose ester, Advantec, Japan) and then stored at 4˚C until further analyses.

The concentrations of dissolved organic carbon (DOC) and total nitrogen (TN) in the wastewater and wetland effluent samples were determined by a total organic carbon analyzer (TOC-V CPH, Shimadzu, Japan) equipped with a TN analyzer (TNM-1, Shimadzu, Japan). The UV absorbance at wavelength 254 nm (UV_{254}) was measured by a UV–vis spectrophotometer (UV-1601, Shimadzu, Japan). The specific UV absorbance (SUVA) value (an indicator of aromaticity) was calculated by dividing the UV_{254} by the DOC concentration. Both nitrate and nitrite in the samples were quantified using an ion chromatography (IC) apparatus (DX-120, Dionex, CA, US), equipped with an AS14 column (4 × 250 mm, Dionex, CA, US). 1.5 L of sample was concentrated to 50 mL with a rotary evaporator (Eyela, Japan) prior to fractionation by prep-HPLC. 15 mL of concentrated samples were freeze-dried using a freeze dryer (Ilshin, Korea) for Py-GC/MS analysis.

2.2. Preparative HPLC (prep-HPLC)

Fractions of NOM from DY WWTP effluent and wetland effluent were obtained by using prep-HPLC (JAI-LC-9201, Japan Analytical Industry Co. Ltd., Tokyo, Japan) with both UV (JAI UV3702) and RI (JAI RI RI-50) detectors. A gel permeation chromatography

(GPC) column (Jaigel GS310, 21.5 mm I.D. × 500 mm length, Japan Analytical Industry Co. Ltd.,) was used, and deionized (DI) water was used as elution solvent at a flow rate of 5 mL/min. According to the manufacturer, separation by the column is based on a combination of hydrophobic interaction and size exclusion. The injection volume of the concentrated samples was 3 mL. The experimental data were acquired and processed with Multichro 2000 V4.2 (JAI, Japan).

2.3. High-performance size exclusion chromatography (HPSEC)

The MW distribution analyses of samples were performed by high-performance size exclusion chromatography (HPSEC) using UV (SPD-10Avp, Shimadzu, Japan) and fluorescence (RF-10A XL, Shimadzu, Japan) detectors with a SEC column (PR pak 125, 7.8 × 300 mm, Part No. WAT084601). The UV wavelength was 254 nm for detecting aromatic compounds. The fluorescence detector at excitation wavelength of 279 nm, and emission wavelength of 353 nm was used to identify PR-like substances. The HPSEC mobile phase was prepared from a phosphate buffer (2.4 mM NaH_2PO_4 and 1.6 mM Na_2HPO_4 at pH 6.8) and 96 mM NaCl. The flow rate of the buffer solution was 0.70 mL/min, with a sample injection volume of 200 µl. A MW calibration curve was constructed by using standards of polystyrene sulfonates (PSS) with different MWs of 210 (Fluka, Switzerland), 1,000, 4,600, 8,000, and 18,000 Da (Polysciences, USA).

2.4. Fluorescence spectroscopy

Fluorescence measurements were conducted using a fluorescence spectrophotometer (model F-2500, Hitachi, Japan) with a 400-W xenon lamp (Tokyo, Japan). A 3D excitation-emission matrix was obtained by measuring the excitation and emission spectra in the range 220–500 nm at 10 nm intervals. The excitation and emission slit widths were set to 5 nm with a scan speed of 3,000 nm/min. The 3D fluorescence spectra data were plotted by the SigmaPlot 10 program (Systat Software, Inc., San Jose, CA, USA).

2.5. Pyrolysis-GC/MS (Py-GC/MS)

Curie-point Py-GC/MS was performed on an Agilent 7890A gas chromatograph coupled to a 5975C quadrupole mass spectrometer (ion source temperature 220˚C, scanning from 40 to 500 amu, electron energy 70 eV). About 0.5–1.0 mg of freeze-dried sample powder was tightly wrapped in a pyrofoil (Pyrofoil F590, Japan Analytical Industry, Japan) and inserted into a quartz sample tube and inductively heated to their Curie temperature of 590˚C by a Curie point injector JCI-22 (Japan Analytical Industries, Japan). Pyrolysis fragments were separated by a GC equipped with a 30-m DB-5MS column (0.25 mm i.d., 0.50 µm film thickness, Agilent Technologies, USA) and identified using a mass spectrometer. Helium was used as the carrier gas. The temperature program of the GC oven was initially kept at 40˚C for 5 min, then raised at a rate of 7˚C/min up to 300˚C, and kept there for 10 min, giving a total run time of 52.14 min. The pyrochromatograms were interpreted by the methods described by Bruchet et al. [22]. Each peak compound was identified by the comparison of its mass spectra with library spectra and literature data. Each sample could be fractionated into specific biopolymer components, including PS, PR, AS, PHA, and LG.

3. Results and discussion

WWTP effluent and wetland effluent were fractionated into three fractions by using prep-HPLC, as shown in Fig. 1. The RI peak #3 of the WWTP

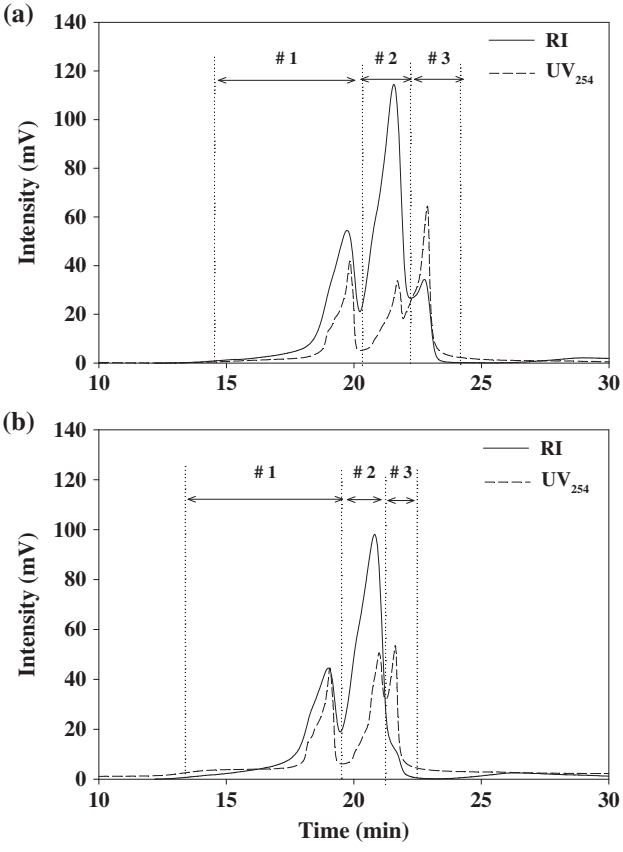

Fig. 1. Fractionation of organic matter from (a) WWTP effluent and (b) wetland effluent by prep-HPLC.

effluents disappeared in the wetland effluent sample. UV peaks were separated into three fractions in both WWTP and wetland effluent samples.

As listed in Table 1, nitrate was efficiently removed throughout the wetland. However, the constructed wetlands exhibited relatively negative performance for the removal of wastewater EfOM.

Water characteristics of each peak of WWTP and wetland effluent fractionated by prep-HPLC were summarized in Table 2. The recovery of collected fractions from WWTP and wetland effluents was 93.8 and 89.2%, respectively. The level of DOC, TN, and SUVA (index of aromaticity) increased from peak #1 to peak #3 in both WWTP effluent and wetland effluent samples. TN was mainly composed of nitrate as N. This result corresponded with the separation by the GPC column, based on a combination of hydrophobic interaction and size exclusion.

MW distribution of the aromatic and PR-like substances in WWTP effluent and wetland effluent are shown in Fig. 2. The MW of aromatic substances ranged from about 1,278–2,300 Da in both WWTP and wetland effluents. In the case of PR-like substances, both low MW (555 and 893 Da) and high MW (29,233 Da) were found to be major fractions of organic matter in WWTP and wetland effluents. Both WWTP effluent and wetland effluent had similar MW distributions of aromatic and PR-like substances.

MW distributions of each peak from WWTP effluent and wetland effluent separated by prep-HPLC are shown in Fig. 3. From peak #1 to peak #3, the MW of aromatic and PR-like substances of WWTP effluent and wetland effluent decreased. HPSEC analysis of EfOM for fractionated samples revealed that the column separated organic matter based on size exclusion. The highest intensity of UV detection were at 2,242, 1,625, and 1,282 Da in peak #1–#3 WWTP effluent samples, respectively. The highest intensity of fluorescence detection corresponded to the 1,672, 889, and 573 Da fraction in peak #1–#3 WWTP effluent samples, respectively. Wetland effluent fractionated samples had similar MW distribution of aromatic and PR-like substances as the WWTP effluent fractionated samples.

The fluorescence characteristics of WWTP and wetland effluents are shown in Fig. 4. The maximum peak of PR-like substances was found at Ex = 280/Em = 310 nm, and humic-like fluorescence had two maximum peaks at Ex = 330 nm/Em = 410 nm and Ex = 270 nm/Em = 420 nm in WWTP effluent. Wetland effluent sample had two strong maximum peaks at Ex = 330 nm/Em = 410 nm and Ex = 250 nm/Em = 420 nm, and one maximum peak of

Table 1
Water characteristics of WWTP effluent and wetland effluent

	NOM	WWTP effluent	Wetland effluent
pH	1	6.40	6.03
Conductivity (μS/cm)	1	567	589
DOC (mg C/L)	2	7.10 (±0.02)	7.95 (±0.33)
UVA_{254} (cm^{-1})	2	0.1528 (±0.0003)	0.1778 (±0.0002)
SUVA (L mg^{-1} m^{-1})	2	2.15 (±0.01)	2.23 (±0.21)
TN (mg N/L)	2	9.48 (±0.09)	3.77 (±0.02)
Nitrate (mg N/L)	2	8.90 (±0.36)	3.28 (±0.10)

Note: NOM: number of measurements.

Table 2
Water characteristics of WWTP effluent and wetland effluent separated by prep-HPLC

Sample	DOC (mg C/L)	TN (mg N/L)	UVA_{254} (cm^{-1})	SUVA (L mg^{-1} m^{-1})	Nitrite (mg N/l)	Nitrate (mg N/l)	Recovery (%)
WWTP effluent #1	5.73	0.73	0.107	1.88	N.D.	0.03	
WWTP effluent #2	11.20	26.59	0.236	2.11	0.66	25.38	
WWTP effluent #3	12.57	58.32	0.293	2.33	N.D.	57.81	93.8
Wetland effluent #1	6.81	0.87	0.131	1.92	N.D.	0.02	
Wetland effluent #2	11.90	15.04	0.270	2.27	N.D.	13.84	
Wetland effluent #3	13.27	19.01	0.332	2.50	N.D.	18.61	89.2

Note: N.D.: not detected.

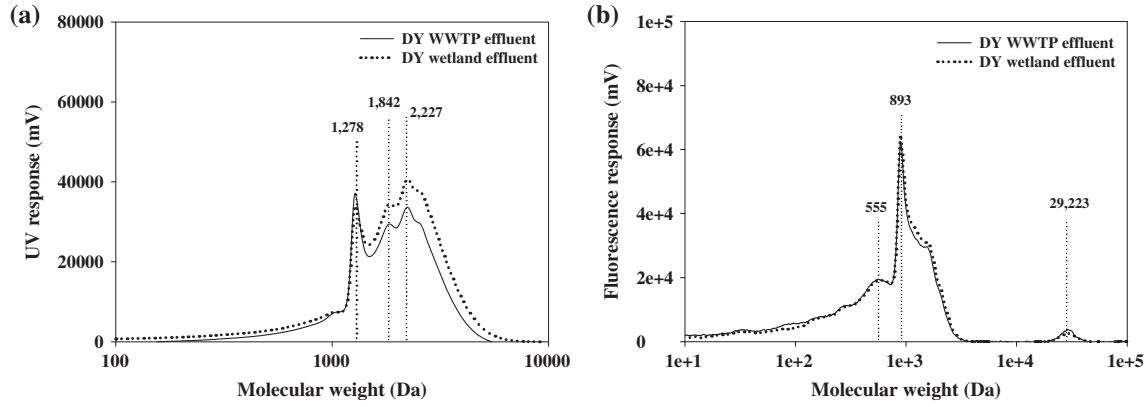

Fig. 2. MW distribution of WWTP effluent and wetland effluent: (a) aromatic substances and (b) PR-like substances.

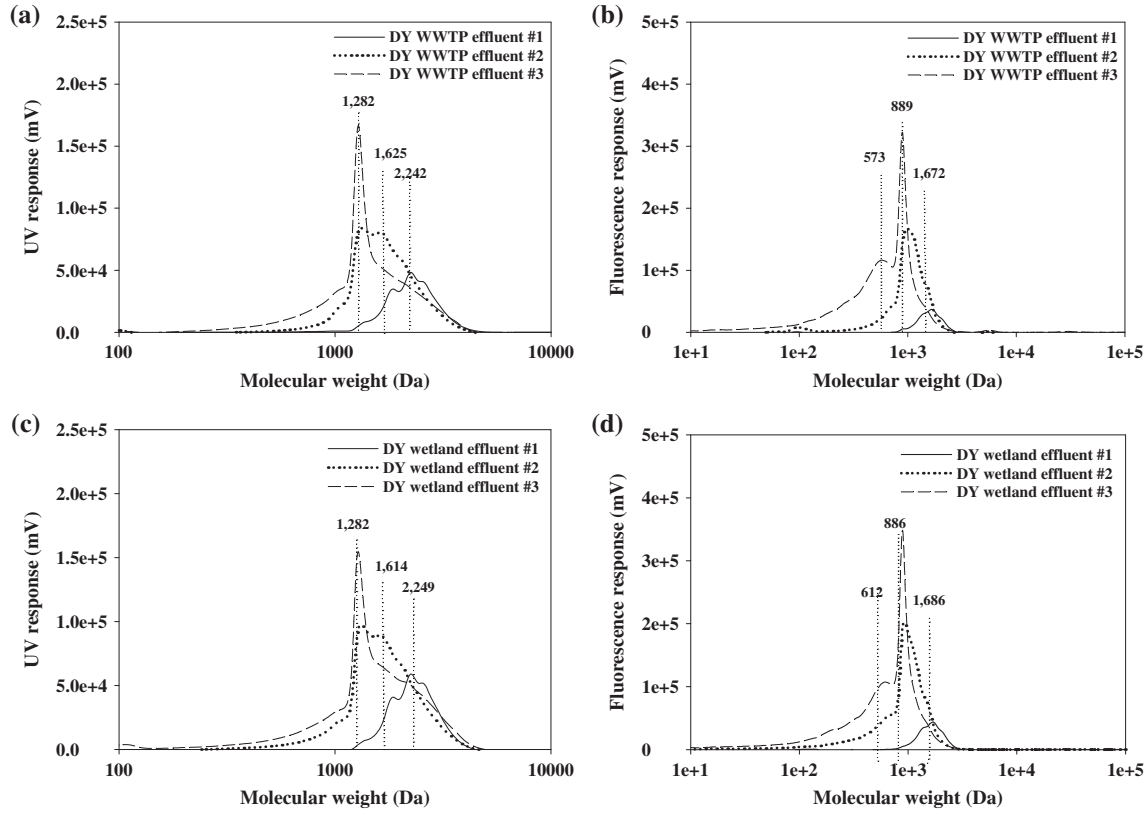

Fig. 3. MW distribution of WWTP effluent and wetland effluent separated by prep-HPLC: (a), (c) aromatic substances and (b), (d) PR-like substances.

PR-like substances at Ex = 280/Em = 310 nm. The maximum peak of samples appeared near the reference material of the humic-like fluorescence (SRHA and SRFA) and PR-like fluorescence [23]. The fluorescence contour plots of each peak of WWTP and wetland effluent separated by prep-HPLC are shown in Fig. 5. Two strong humic-like fluorescence peaks were observed in peak #3 of fractionated WWTP and wetland effluent samples at the same Ex and Em wavelengths (Ex = 330 nm/Em = 410 nm and Ex = 270 nm/Em = 430 nm), but PR-like fluorescence was not observed in peak #3. This indicated that peak #3 sample had relatively high SUVA and aromaticity compared with peak #1 and #2.

Fractions of the fragments from the pyrochromatograms of WWTP and wetland effluents are summarized

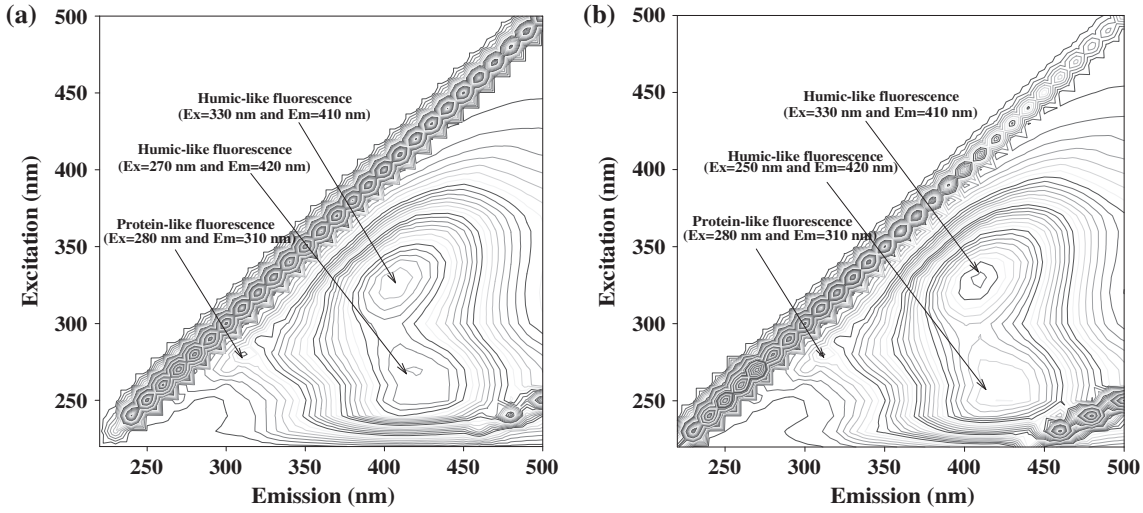

Fig. 4. Fluorescence contour plots: (a) WWTP effluent and (b) wetland effluent.

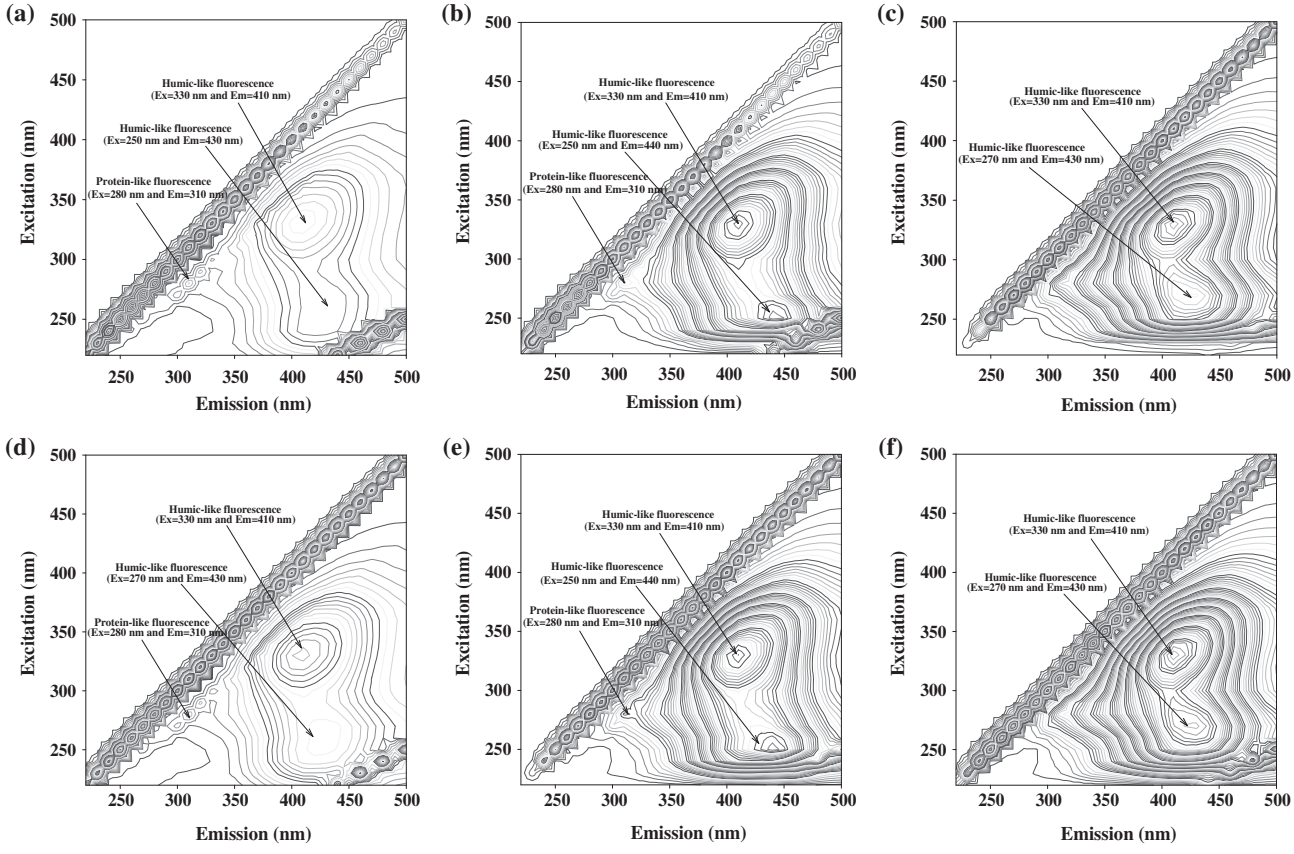

Fig. 5. Fluorescence contour plots of WWTP effluent and wetland effluent separated by prep-HPLC: (a)–(c) WWTP effluent #1–#3, (d)–(f) wetland effluent #1–#3.

in Table 3. Wetland effluent revealed stronger PHA characteristics than WWTP effluent. The proportion of PHA increased throughout the constructed wetland. This result agrees with previous findings that wastewater EfOM becomes more aromatic during wetland treatment [24,25]. The composition of the organic matters in the EfOM shifted from PS/PRs to PHA (i.e. through humification), which is a good news, with

Table 3
Fractions of biopolymers for WWTP effluent and wetland effluent

	Fractions (%)					
	PS[a]	AS	PR	PHA	LG	Sum
WWTP effluent	9.9	5.4	24.0	38.6	22.1	100
Wetland effluent	3.1	N.D.	32.6	60.5	3.8	100

[a]PS = polysaccharides; AS = amino sugars; PR = protein; PHA = polyhydroxy aromatics; LG = lignins. N.D.: not detected.

Table 4
Fractions of biopolymers for WWTP effluent and wetland effluent separated by prep-HPLC

	Fractions (%)					
	PS[a]	AS	PR	PHA	LG	Sum
WWTP effluent #1	9.7	3.2	32.3	34.3	20.5	100
WWTP effluent #2	7.5	15.9	21.8	54.8	N.D.	100
WWTP effluent #3	1.8	0.7	19.2	64.2	14.1	100
Wetland effluent #1	48.6	N.D.	29.5	15.4	6.5	100
Wetland effluent #2	17.4	16.9	15.5	25.8	24.4	100
Wetland effluent #3	1.2	N.D.	10.5	80.7	7.6	100

[a]PS = polysaccharides; AS = amino sugars; PR = protein; PHA = polyhydroxy aromatics; LG = lignins. N.D.: not detected.

respect to the water management of discharging river since it could provide more biologically stable forms of organic matter.

Fractions of biopolymers for each peak of WWTP and wetland effluents separated by prep-HPLC are shown in Table 4. Peak #3 of WWTP and wetland effluents exhibited strong PHA characteristics to a greater extent than peak #1 and #2 samples, while peak #1 of both WWTP and wetland effluents showed higher PS and PR characteristics than other peak samples.

The proportion of PHA increased from peak #1 to #3 of WWTP and wetland effluent, but the percentage of PS and PR decreased. With the SUVA, 3D fluorescence, and Py-GC/MS results, it can be concluded that the peak comes off the column later had more aromaticity/hydrophobicity of organic matter. We suggest that in conjunction with fractionation of NOM using prep-HPLC, Py-GC/MS data can provide useful insights into the origin of organic matter from WWTP and wetland effluents.

4. Conclusions

Relatively large volume of organic sample was attempted to be fractionated based on both hydrophobic interaction and size exclusion for further analyses, including the revelation of fundamental biopolymers using Py-GC/MS. This type of organic sample pretreatment with a large volume column with HPLC was firstly developed by our group, to our knowledge. By comparing the WWTP and constructed wetland effluents, it was found peaks that come off the column of the prep-HPLC later had greater aromaticity and portion of poly-aromatic carbon, identified using 3D fluorescence and Py-GC/MS, and lower MW identified using HPSEC. With this, the constructed wetland is believed to transform some portions of the WWTP EfOM into organic matters exhibiting greater humic (aromatic) properties, providing a notion that the wastewater stabilizing treatment wetland plays an important role in humification of wastewater EfOM. In this study, fundamental biopolymers were only tried to be identified using the developed pretreatment protocols, however, other analyses can be further conducted with fractionated organic samples, with respect to biodegradability, disinfection by-products formation potential, and even comprising organic structures.

Acknowledgements

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012047029), and also by National Research Foundation of Korea Grant funded by the Korean Government Ministry of Education, Science and Technology (NRF-2011-355-D00030).

References

[1] G.R. Aiken, D.M. McKnight, R.L. Wershaw, P. MacCarthy, Humic Substances in Soil, Sediment, and Water, John Wiley, New York, NY, 1985.

[2] I.H. Suffet, P. MacCarthy, Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants, American Chemical Society, Washington, DC, 1989.

[3] M. Nystrom, K. Ruohomaki, L. Kaipia, Humic acid as a fouling agent in filtration, Desalination 106 (1996) 79–87.

[4] W. Yuan, A.L. Zydney, Humic acid fouling during microfiltration, J. Membr. Sci. 157 (1999) 1–12.

[5] M. Wolf, G. Teichmann, E. Hoque, W. Szymczak, W. Schimmack, Copper speciation in aqueous solutions of fulvic acid and related molecular weight distributions, Fresen. J. Anal. Chem. 363 (1999) 596–599.

[6] K. Chon, J. Cho, H.K. Shon, Advanced characterization of algogenic organic matter, bacterial organic matter, humic acids and fulvic acids, Water Sci. Technol. (2013), doi: 10.2166/wst.2013.118

[7] Z. Chen, B. Xing, W.B. McGill, M.J. Dudas, Alphanaphthol sorption as regulated by structural and composition of organic substances in soils and sediments, Can. J. Soil Sci. 76 (1996) 513–522.

[8] W.D. Burgos, N. Pisutpaisal, M. Tuntoolavest, J. Chorover, R. F. Unz, Biodegradation of 1-naphthol in the presence of humic acid, Environ. Eng. Sci. 17 (2000) 343–351.

[9] K. Chon, S.J. Kim, J. Moon, J. Cho, Combined coagulation-disk filtration process as a pretreatment of ultrafiltration and reverse osmosis membrane for wastewater reclamation: An autopsy study of a pilot plant, Water Res. 46 (2012) 1803–1806.

[10] K. Chon, J. Cho, H.K. Shon, Fouling characteristics of a membrane bioreactor and nanofiltration hybrid system for municipal wastewater reclamation, Biores. Technol. 130 (2013) 239–247.

[11] J.E. Drewes, J.P. Croue, New approaches for structural characterization of organic matter in drinking water and wastewater effluents, Water Sci. Technol. Water Supply 2 (2002) 1–10.

[12] R.S. Swift, Fractionation of Soil Humic Substances. Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation, and Characterization, John Wiley, New York, 1985.

[13] M.B. Müller, D. Schmitt, F.H. Frimmel, Fractionation of natural organic matter by size exclusion chromatography properties and stability of fractions, Environ. Sci. Technol. 34 (2000) 4867–4872.

[14] N. Her, L. Amy, M. Mcknight, J. Sohn, Y. Yoon, Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC and fluorescence detection, Water Res. 37 (2003) 4295–4303.

[15] C.M. Grill, L. Miller, T.Q. Yan, Resolution of a racemic pharmaceutical intermediate comparison of preparative HPLC, steady state recycling, and simulated moving bed, J. Chromatogr. A. 2004 (1026) 101–108.

[16] Q.H. Zheng, B.H. Mock, Purification of carbon-11 PET radiotracers from unlabeled precursors by preparative HPLC and SPE, Biomed. Chromatogr. 19 (2005) 671–676.

[17] N. Sharma, U.K. Sharma, S. Malik, S. Bhushan, V. Kumar, S. C. Verma, N. Sharma, M. Sharma, A.K. Sinha, Isolation and purification of acetylshikonin and–acetoxyisovalerylshikonin from cell suspension cultures of *Arnebia euchroma* (Royle) Johnston using rapid preparative HPLC, J. Sep. Sci. 31 (2008) 629–635.

[18] Q. Du, Z. Jiang, D. Wang, Excellent combination of countercurrent chromatography and preparative high-performance liquid chromatography to separate galactolipids from pumpkin, J. Chromatogr. A 1216 (2009) 4176–4180.

[19] A. Piccolo, P. Conte, E. Trivellone, B.V. Lagen, P. Burman, Reduced heterogeneity of a lignite humic acid by preparative HPSEC following interaction with an organic acid. Characterization of size-separates by Pyr-GC–MS and ^{1}H-NMR spectroscopy, Environ. Sci. Technol. 36 (2002) 76–84.

[20] J. Peuravuori, K. Pihlaja, Preliminary study of lake dissolved organic matter in light of nanoscale supramolecular assembly, Environ. Sci. Technol. 38 (2004) 5958–5967.

[21] K. Chon, J. Chang, E. Lee, J. Lee, J. Ryu, J. Cho, Abundance of denitrifying genes coding for nitrate (*narG*), nitrite (*nirS*), and nitrous oxide (*nosZ*) reductases in estuarine versus wastewater effluent-fed constructed wetlands, Ecol. Eng. 37 (2011) 64–69.

[22] A. Bruchet, C. Rousseau, J. Mallevialle, Pyrolysis-GC/MS for investigating high-molecular weight THM precursors and other refractory organics, J. Am. Water Works Assoc. 82 (1990) 66–74.

[23] K. Chon, S. Sarp, S. Lee, J.H. Lee, J.A. Lopez-Ramirez, J. Cho, Evaluation of a membrane bioreactor and nanofiltration for municipal wastewater reclamation: Trace contaminants control and fouling mitigation, Desalination 272 (2011) 128–134.

[24] L.B. Barber, J.A. Leenheer, T.I. Noyes, E.A. Stiles, Nature and transformation of dissolved organic matter in treatment wetlands, Environ. Sci. Technol. 30 (2001) 3501–3507.

[25] D.M. Quanrud, M.M. Karpiscak, K.E. Lansey, R.G. Arnold, Transformation of effluent organic matter during subsurface wetland treatment in the Sonoran Desert, Chemosphere 54 (2004) 777–788.