Characterization of dissolved organic matter from Australian and Chinese source waters by combined fractionation techniques

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ABSTRACT

The character of dissolved organic matter (DOM) in source waters from two countries (Australia and China) was investigated using an extended fractionation technique by combining resin adsorption, ultrafiltration and high performance size exclusion chromatography. There are distinctive chemical characteristics associated with DOM origins. Australian sourced DOM had higher hydrophobic acid (HoA) content and exhibited a more pronounced humic character, indicating a higher influence from allochthonous organics (decayed plant bodies from vegetated catchments). The higher content of hydrophobic base and neutral components found in Chinese DOM, may be attributed to the effects of increasing pollution caused by the rapid urbanization in China. The molecular weights (MWs) of aquatic HoA are predominantly in the moderate (e.g. 1–10 kDa) or small (e.g. <1 kDa) ranges. This suggests that aquatic HoA should not be assumed as high MW organics without experimental validation. It is also found that some of the low MW compounds in our samples were hydrophobic, which could explain the observation of low MW organic compounds being able to be removed by conventional treatment processes.

Key words | dissolved organic matter, extended fractionation, size exclusion chromatography

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INTRODUCTION

Dissolved organic matter (DOM) plays an important role in water treatment processes. The complete characterization of DOM in environmental samples continues to be a challenge and certain investigators have concluded that humic substances cannot be characterized individually at the molecular level (MacCarthy 2001). Leenheer & Croue (2003) divided DOM research into two categories: whole water studies, in which DOM is characterized in water and its inorganic constituents, and studies of DOM fractions isolated from water. DOM is commonly fractionated on the basis of chemical or physical properties. Three fractionation techniques, namely resin adsorption (RA), ultrafiltration (UF) and high performance size exclusion chromatography (HPSEC), are commonly used.

A hierarchical fractionation procedure developed by the US Geological Survey has been widely used to fractionate DOM (Leenheer 1981). However, it requires extensive preparative steps and large volumes of water, which limits the

use of this technique as a tool to assist water treatment operations. Numerous innovative modifications have been developed (Chow *et al.* 2004; Wei *et al.* 2008a, b) to improve the applicability to allow a more rapid assessment to be performed.

Characterization of the molecular size is also a valuable tool when assessing the effect of DOM on the performance of water treatment systems as well as its geochemical origin (Revchuk & Suffet 2009). Size fractionation is usually accomplished by size exclusion chromatography (SEC), separation based on chromatographic effect and dead-end or cross flow UF separation based on using membranes with different nominal molecular weight cutoffs (MWCO) (e.g. 1, 3 and 10 kDa). SEC has been widely used to investigate the molecular size distribution of DOM from marine, freshwater and wastewater samples (Perminova *et al.* 2003; Chow *et al.* 2008).

Unfortunately, each of these methods has certain disadvantages and limitations. RA requires extensive preparative

steps and is time-consuming. UF membrane manufacturing generates a range of pore sizes, and MWCO are subjective and depend on the shape and charge properties of the standard solutes used (Revchuk & Suffet 2009). SEC MW determination is limited by the relatively few representative standard chemicals for MW calibration and similarly it can be affected by the interactions between DOM and the gel phase in the column (Schafer *et al.* 2002). Therefore, in our opinion, combining several methods to get a more detailed and accurate assessment of DOM character is the best approach. Up to the present, most of the studies for characterizing DOM used one of these fractionation methods (i.e. RA, UF and SEC). In some cases, two or more methods were reported in published studies (Kitis *et al.* 2002; Fabris *et al.* 2008; Wei *et al.* 2008a), but each of the methods was still generally used separately (parallel experiments being run).

In this study, source waters chosen for this investigation were previously well characterized by the authors (Chow *et al.* 2004, 2008; Fabris *et al.* 2008; Wei *et al.* 2008b). They were selected to provide a good basis for water quality comparison, particularly the Australian catchments are surrounded by natural vegetation and the Chinese catchments are more urbanized with industries. The objectives of this study were to (1) combine the fractionation methods of RA, UF and SEC for rapid and extended characterization of DOM, to obtain more detailed information on DOM character than provided by one method alone, (2) compare the character of DOM in source waters from two countries (Australia and China) using an extended fractionation technique (EFT) and (3) gain further understanding of the applicable conditions and limitations of these methods.

METHODS AND MATERIALS

Water sources

The Chinese water samples were collected from the intakes of two drinking water treatment plants: the Tianjin (TJ) water was obtained from the Yellow River, north of China and the Shenzhen (SZ) water was collected from the East-Lake Reservoir near Shenzhen City, south of China. Both rivers flow across several cities and are potentially polluted by sewage and urban runoff.

The Australian water samples were collected from the Myponga (MYP) Reservoir, South Australia and Woronora (WOR) Reservoir, New South Wales. The MYP water is sourced from the local enclosed catchment and it is a high

colour and DOC water. WOR water is sourced from a protected catchment and it is a low colour and low DOC water. The pH of TJ, SZ, MYP and WOR is 8.3, 7.2, 7.6 and 7.2, respectively.

Resin fractionation of DOM

Quantification of characterized fractions by DOC mass balance

The method reported by Wei *et al.* (2008a, b) was adopted with slight modification. Filtered (0.45 μm) water sample was first passed through DAX-8 resin without any pH adjustment, resulting in the hydrophobic base and neutral fraction (HoBN) being retained on the resin. The effluent from the DAX-8 column was then adjusted to pH 2 and loaded onto another DAX-8 resin column to adsorb the hydrophobic acid (HoA) fraction. Then, the effluent from the second DAX-8 column was passed through XAD-4 resin. The fraction adsorbed by the XAD-4 resin can be described as weakly hydrophobic acid (WHoA). Organics contained in the last effluent (not retained on either DAX-8 or XAD-4) were named as hydrophilic matter (HiM). Each DOM fraction was quantified by the organic carbon concentration difference between the samples before and after each RA.

Dissolved organic carbon (DOC) concentrations were determined by the same UV/Persulfate oxidation method using a Sievers 820 total organic carbon analyzer (GE Analytical Instruments, USA) in Australia and a Phoenix 8000 TOC Analyzer (Tekmar Dohrmann, USA) in China.

HoA fraction recovery

To compare the results obtained by the method described above, the HoA was isolated and recovered by a traditional preparative method (Leenheer 1981). After the acidified sample was passed through the second DAX-8 column, the HoA was recovered by loading 10 bed-volumes of 0.1 mol/L NaOH solution through the column. The recovered HoA fraction (effluent) was then normalized to the concentration in the natural water by dilution. The HoA sample was then fractionated by UF and HPSEC directly.

UF fractionation procedures

UF was performed by using a stirred UF cell (Millipore, 8200) with YM disc membranes (Amicon, nominal MWCOs are 1, 3 and 10 kDa), as previously described (Wei *et al.* 2008b). The schematic diagram of the UF procedure is shown in

(a)

(b)
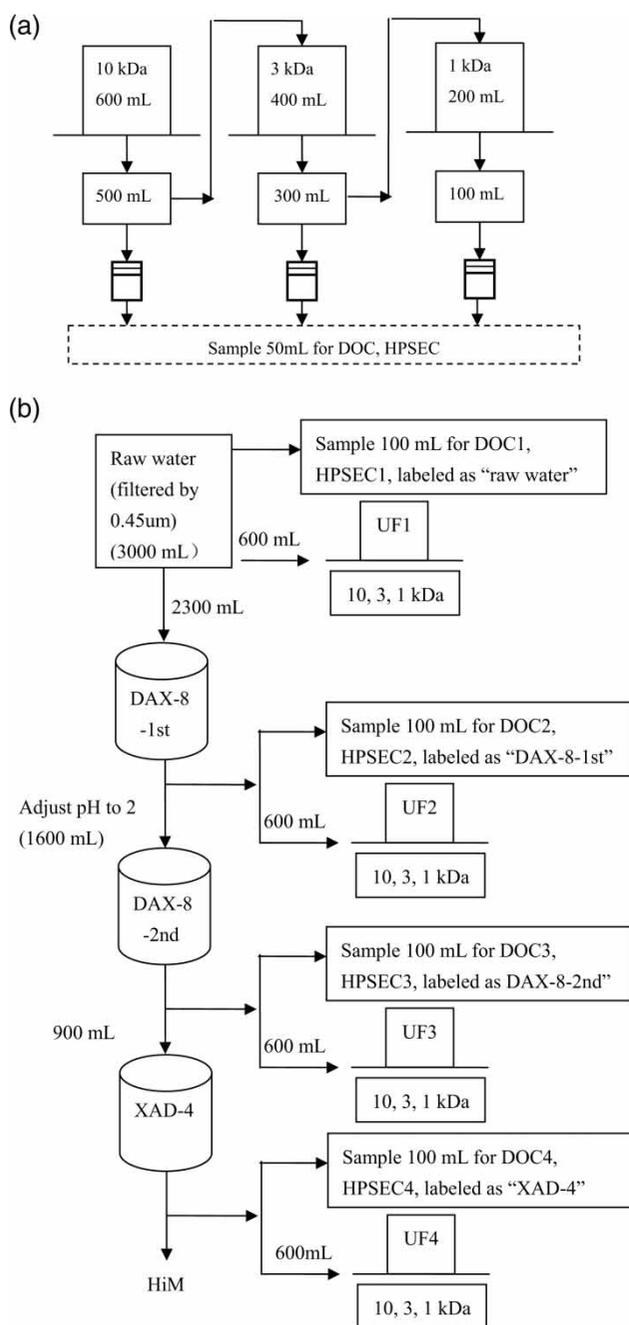
Figure 1 | Schematic diagram of DOM fractionation using (a) UF and (b) extended frac-tionation by RA, UF and HPSEC.

Figure 1(a). The concentration of each fraction was based on the difference between the DOC concentration of the feed and permeate in each filtration step.

MW distribution by HPSEC

HPSEC was performed using a Waters Alliance 2690 HPLC with a 996 photodiode array detector (Waters, USA) and a

Shodex KW-802.5 column (Shoko Co., Japan). The carrier consisted of a 0.02 mol/L phosphate buffer (pH 6.8) adjusted to an ionic strength of 0.1 mol/L with NaCl. The flow rate was 1 mL/min and the absorbance at 260 nm was measured (Chow et al. 2008). Calibration was performed using poly-styrene sulfonate (PSS) standards (Polysciences, USA) of MWs 35,000, 18,000, 8,000 and 4,600 Daltons.

Extended fractionation of DOM by RA, UF and HPSEC

Step 1. Fractionation of DOM sample by UF and HPSEC before RA

A 600 mL filtered (0.45 μm) water sample was fractionated by UF using 10, 3 and 1 kDa membranes. The DOC of each size fraction (>10, 10–3, 3–1 and <1 kDa) was obtained as described in the UF Procedures Section. A 100 mL water sample was collected for HPSEC analysis in parallel. The detailed procedures are shown in Figure 1(b).

Step 2. Fractionation of DOM sample by UF and HPSEC after RA

The same water mentioned above (without pH adjustment) was added onto a DAX-8 column and the HoBN adsorbed first. Following this, the effluent solution from the DAX-8 column was fractionated by the same UF or HPSEC pro-cedure as mentioned above. Then, the DOC of each size fraction in the first effluent from DAX-8 was obtained (Figure 1(b)).

Step 3. Size distribution of each chemical fraction

Subtraction of the UF or HPSEC results of Step 2 from Step 1 provided the size fraction result of HoBN. The concen-tration of HoBN was calculated by DOC1–DOC2, and the size fraction of HoBN was calculated by subtracting the result of UF2 from UF1 or subtracting the result of HPSEC2 from HPSEC1 (Figure 1(b)). The size fractions of other chemical fractions (HoA and WHoA) could be obtained in the same way. HoA is normally the predominant fraction in natural water DOM, the size fraction result of HoA was selected for further investigation.

Statistical analysis

The different fractional characteristics of the DOM between the samples from the two countries were statistically demon-strated by the cluster results processed by SPSS (13.0)

software. The hierarchical cluster method was adopted as follows: cases (waters) cluster with percent of fractions as variables, between-groups linkage and squared Euclidean distance. The shorter the distance (lower coefficients) of the cluster, the more similar are the waters in terms of water quality.

RESULTS AND DISCUSSION

Chemical fractions of DOM

The DOC concentration of TJ, SZ, MYP and WOR is 5.0, 1.8, 10.6 and 4.3 mg/L, respectively (Figure 2(a)). The distribution of DOM chemical fractions of the samples is shown in Figure 2(a), as relative percentages of DOC. The DOM from both Australian waters (MYP and WOR) contained a relatively higher portion of HoA (about 55%) and less HoBN (<10%) than Chinese waters (HoA around 40% and HoBN > 10%). Australian DOM exhibited greater humic character (higher HoA content), indicating a stronger

(a)

(b)

Figure 2 | (a) The distribution of chemical fractions (using RA) with additional UF of the HoA from the two selected sources, TJ and MYP and (b) cluster analysis of the source waters.

influence from allochthonous organic origin (decayed plant matter). The higher HoBN content in Chinese DOM may be accounted for by the effects of pollution caused by the rapid urbanization in China.

The different fractional characteristics of the DOM between the samples from the two countries were statistically compared. Figure 2(b) shows that the two Chinese waters (TJ, SZ) are clustered as one category, and the two Australian waters (MYP, WOR) are clustered as another category. This indicates that there are distinctive chemical characteristics associated with DOM origins. Among these waters, HoA is the predominant fraction in the DOM. A further attempt was made by using RA combined with UF or HPSEC to characterize the HoA fractions.
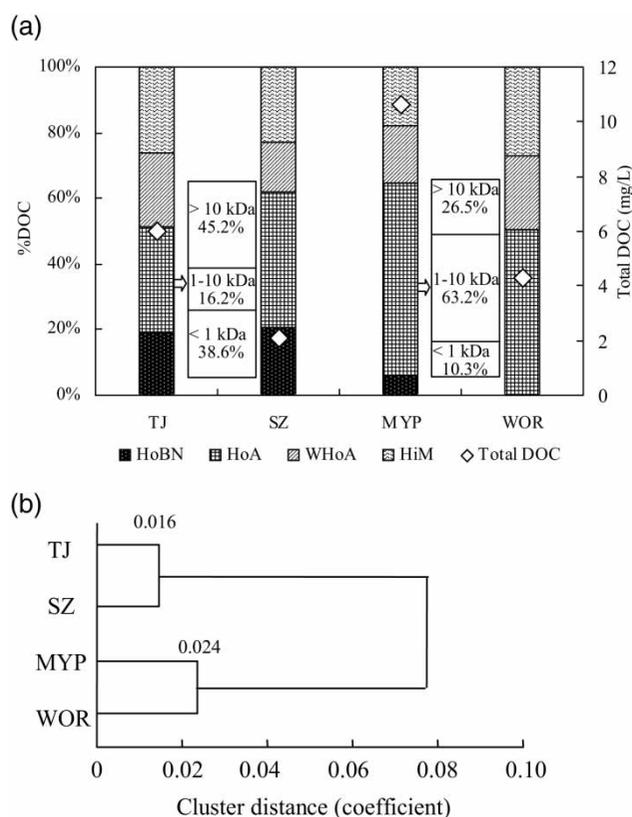
Size fractionation of HoA by UF

Size separation based on DOC mass balance of RA

The Chinese TJ water and Australian MYP water were selected to compare HoA from different origins. The size separation of HoA was obtained based on the different results from the UF of the sample before and after DAX-8 RA. Figure 2(a) shows the chemical fraction contents of DOM and the MWCO of HoA from the Chinese TJ water and the Australian MYP water, respectively. In Figure 2(a), HoA from different sources have different MW distributions, which shows that the size characteristic of HoA is site-specific, 45.2% of the HoA from the Chinese TJ water were MW > 10 kDa; whereas, 63.2% of HoA from the Australian MYP water were in the MW range of 1–10 kDa.

In addition, it is very interesting that a significant part of the HoA consists of low MW (<1 kDa) molecules, which accounts for 38.6 and 10.3% in the HoA from TJ and MYP water, respectively. This means that some low MW molecules in DOM from natural water are hydrophobic, which could explain the observation in Chow *et al.* (2008) that part of the low MW DOC was removed by conventional coagulation treatment.

UF fractionation of the recovered HoA

In another component, HoA was first recovered from DAX-8 resin using a 0.1 mol/L NaOH solution, then this solution was ultrafiltered after dilution to normalize the HoA concentration of the raw water. The molecular size separations of the recovered HoA from the Australian MYP and WOR waters are shown in Figure 3(a). The result is consistent with Figure 3(a) in that the predominant

(a)

(b)
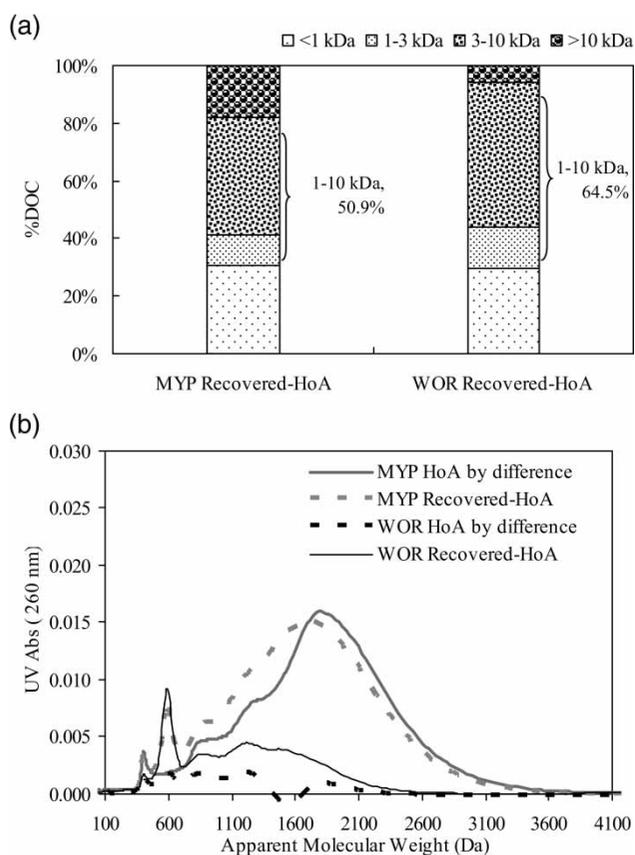
Figure 3 │ (a) Size fractions of recovered HoA from MYP and WOR water by UF; (b) HPSEC of HoA obtained by different RA methods.

size fraction of MYP recovered HoA is still in the range of 1–10 kDa, accounting for 50.9% of HoA as DOC. Furthermore, HoA from both of MYP and WOR waters contained a significant proportion (around 30%) of low MW (<1 kDa) components (Figure 3(a)), which means the MWs of aquatic HoA are with high probability in moderate (e.g. 1–10 kDa) or small (e.g. <1 kDa) ranges.

However, there is still a significant difference between the results of Figures 2(a) and 3(a), which relates to the content of the <1 kDa fraction in MYP HoA. The percentage of the <1 kDa fraction obtained from the recovered HoA is obviously higher than that based on the difference of the UF results of sample before and after DAX-8 RA. This could be due to some 'soft' molecules (molecules with various shapes and three-dimensional positions) larger than 1 kDa in the recovered HoA that may still possibly pass through the UF membrane with 1 kDa MWCO (Rosario-Ortiz *et al.* 2009). This phenomenon of 'soft' molecules should be covered by the difference between the two step UF results. In addition, it is noteworthy in Figure 3(a) that the 1–10 kDa fraction of recovered HoA

from both MYP and WOR waters are mostly in the size range of 3–10 kDa.

Size distribution of HoA obtained by HPSEC

Delta-HPSEC

Figure 3(b) shows the molecular size (HPSEC) profiles of HoA from Australian MYP and WOR waters. The solid lines represent the results based on the difference between the HPSEC profiles (delta) of samples before and after DAX-8 RA. This result is consistent with Figure 2a in that the prominent molecular size fraction in MYP HoA is in the range 1–10 kDa, and more specifically in the range 1–3 kDa, which holds true for WOR HoA. Additionally, a smaller proportion of size fraction <1 kDa in MYP HoA and the MW of this fraction is in fact around 400 Da. This is also shown in Figure 2(a), the content of this fraction with MW around 400 Da accounts for about 10% of MYP HoA. However, there is still a difference between the result of Figures 2(a) and 3(b) determined using two different characterization methods. The Figure 2(a) UF result shows that the MYP HoA contains a relatively high percentage (26.5%) of size fraction with high MW (>10 kDa), which is not apparent in Figure 3(b) of the HPSEC result. That difference between UF and HPSEC results, related to the high MW fraction in DOM, will be further discussed.

Direct HPSEC of recovered HoA

In contrast to using delta HPSEC to obtain the HoA MW profile by subtraction, the recovered HoA was measured directly by HPSEC. The dashed lines in Figure 3(b) are the HPSEC results of the recovered HoA (the adsorbed HoA was eluted using NaOH) from MYP and WOR waters isolated by the recovery procedure described earlier. These confirm that the HoA from both waters mainly consists of the 1–3 kDa fraction. In addition, the dashed line results show that there is another prominent size fraction contained in the recovered HoA, which is in the range of 500–700 kDa. This is different from the results based on the difference between the samples before and after RA. It is consistent with the finding detailed in the Section 'UF fractionation of the recovered HoA' that the content of low size fraction (<1 kDa) of HoA determined by the recovery method is always higher than that determined from the difference between the samples before and after RA.

However, there is a difference in MW distribution results within the 1–10 kDa fraction of HoA, as shown in

Figure 3(a) and 3(b). In Figure 3(a), the main components of the 1–10 kDa size fraction of MYP or WOR recovered HoA is in the range of 3–10 kDa, whereas, in Figure 3(b) the predominant components of 1–10 kDa fraction of the recovered HoA are in the range of 1–3 kDa. Another interesting result is related to the content of the >10 kDa fraction: MYP HoA obviously has a proportion of components with MW > 10 kDa, as shown in the UF results (Figure 3(a)), but this is not evident in the HPSEC results (Figure 3(b)).

Analysis of UF size fractions of HoA by HPSEC

To further reveal the differences between DOM fractionation results obtained by HPSEC and UF, the size fractions after UF were taken and analyzed directly by HPSEC, and the results are shown in Figure 4. Because of the different results observed in the components with MW of 1–10 kDa, as mentioned in the 'Direct HPSEC of recovered HoA' Section, only the HPSEC results for MW fractions in this range are shown in Figure 4.
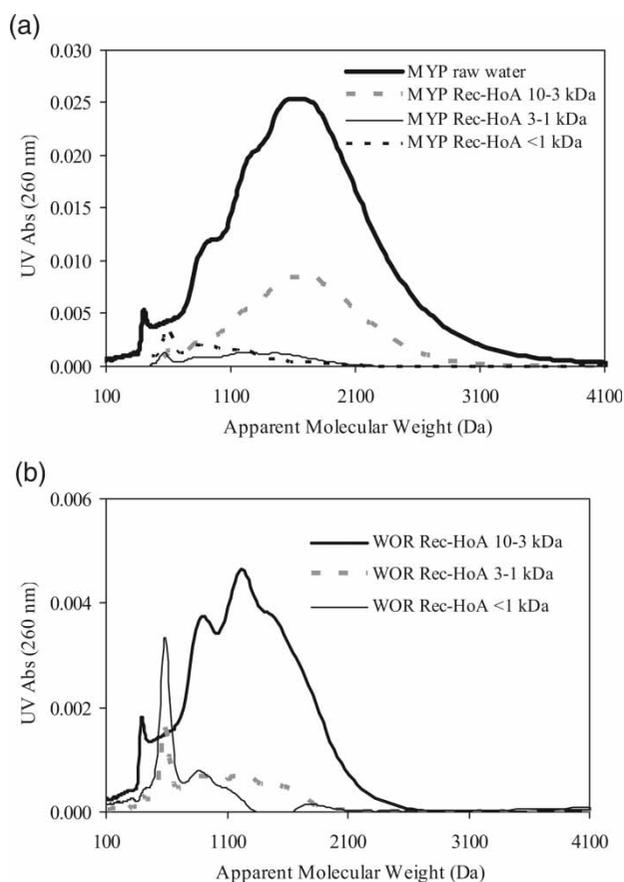

Figure 4 | Size distribution of recovered HoA from (a) MYP and (b) WOR water based on HPSEC combined with UF.

From Figure 4(a), several key findings are worth further discussion. First, the HPSEC scan of the recovered HoA (isolated by the 3–10 kDa UF membrane) indicated the DOM was in the HPSEC MW range of 1–3 kDa (mainly around 2 kDa). This suggested that the MW values from UF may be appear higher than those of HPSEC when characterizing the MW or size of DOM. Secondly, there was no detection of compounds in the >10 kDa range of the HPSEC scan, even for the raw water DOM, which also confirms that the size distribution result of DOM obtained by HPSEC often appears lower than that by UF.

One of the reasons may be related to the 'nominal' MWCO of the membranes as defined by manufacturers, i.e. it is based on a 90% retention of a given MW solute after a specified duration of filtration (Revchuk & Suffet 2009). Specifically, if the content of UF size fractions is gained based on the DOC difference between samples before and after membrane filtration, the components of the membrane MWCO size would be partly regarded as those of larger MWs.

CONCLUSIONS

The fractional characters of DOM in source waters from two countries (Australia and China) using an EFT were investigated and compared. Australian DOM exhibited greater humic character indicating a stronger influence from decayed plant bodies. On the other hand, the higher HoBN in Chinese DOM may originate from the increasing pollution associated with the rapid urbanization in China. Some low MW molecules in DOM from natural waters in both countries are hydrophobic, which could explain why a part of DOM with low MW can be removed in some treatment processes.

The MWs of aquatic HoA are probably of moderate (e.g. 1–10 kDa) or small (e.g. <1 kDa) MW ranges. This suggests that aquatic HoA reported in the literature as being entirely high MW should be carefully reviewed.

It is also found that it is important to compare the characteristics of DOM using the same fractionation method under the same conditions. The result obtained by the recovered method (the adsorbed DOM was eluted and analyzed) shows a higher content of low size fraction (<1 kDa) in HoA than by the method based on mass balance (subtraction of organic carbon concentration) between the samples before and after RA. The use of the UF method for MW determination also indicates a relatively higher MW value as compared with the HPSEC method for a given source.

ACKNOWLEDGEMENTS

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