New approaches for structural characterization of organic matter in drinking water and wastewater effluents

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Abstract The scope if this work was directed to address potential differences or similarities between natural organic matter (NOM) and effluent organic matter (EfOM) by combining operationally defined categorization protocols with state-of-the-art characterization techniques to investigate the bulk of organics in raw drinking water samples (surface and groundwater) and wastewater samples with respect to origin, size, structure, and functionality. Samples of different drinking water and wastewater prior to and after groundwater recharge from France and the U.S. were considered in this study. The physical, chemical, and biological processes that generate and modify organic compounds in natural and engineered systems share many similarities. As a result, the chemical characteristics of effluent derived and naturally derived organic compounds overlap extensively. However, the aromatic moieties present in the EfOM matrix are probably of different origin than the aromatic moieties of the NOM as indicated by a relationship we have established between SUVA and the aromatic carbon content.

Keywords 13C-nuclear magnetic resonance spectroscopy; effluent organic matter; elemental analysis; FT-infrared spectroscopy; natural organic matter; XAD-resin fractionation

Introduction

Considerable effort has been expended over the past two decades to identify and characterize the complex mixture of organic compounds in natural waters and treated wastewaters. In natural systems, humic substances represent a large fraction (40 to 60% in most rivers) of natural organic matter (NOM). However, several extensive studies reported that effluents of wastewater treatment facilities also contain a remarkable percentage of humic and fulvic acids (Manka and Rebhun, 1982; Namour and Mueller, 1998; Drewes and Fox, 1999). Since humic substances do not conform to a unique chemical entity, but represent thousands of chemical species, properties and character of organic matter present in drinking water and wastewater effluents depend on many factors, such as origin, age, pretreatment etc. The character of the watershed usually determines the predominant character of NOM whether it is allochthonously (terrestrial) or autochthonously (algal, bacterial) derived. Both, NOM present in drinking water and soluble microbial products (SMPs) formed during the wastewater treatment process from the decomposition of organic compounds (Link et al., 1989; Barker and Stuckey, 1999) have been demonstrated to significantly affect the amount and character of effluent organic matter (EfOM) (Drewes and Fox, 2000). Researchers have attempted to characterize organic matter by grouping molecules into a limited set of categories or fractions. Although the quality of chemicals and instrumentation for conducting these tasks has improved in recent years, the basic approaches have not changed. Since the assignment of molecules to specific categories is always operational, the categorization is often described in terms of fundamental or conceptual characteristics. Therefore, an implicit expectation of organic matter fractionation is that fractions isolated from independent water sources by the same procedure will have
similar composition and properties, although the concentration of a given fraction may differ (Croué et al., 2000). Peschel et al. (1988) determined the microbial origin of EfOM by comparing XAD resin isolates of EfOM with NOM from natural river water and a peat bog. River water is dominated by authochtonous organic matter whereas humic substances from the peat bog derive from terrigenous (allochthonous) origin. By applying a variety of analytical tools, such as elemental analysis, IR-spectroscopy, carbon-13 nuclear magnetic resonance spectroscopy (13C-NMR) and pyrolysis GC-MS, a high structural similarity between EfOM and river water NOM samples was demonstrated. Jahnel et al. (1998) separated EfOM into humic and fulvic acids and analyzed for specific amino acids and carbohydrates. Some carbohydrates occur in microbial cell walls while others derive from plant components. Hence carbohydrates can identify the origin of aqueous DOC. Accordingly, sewage effluent showed strong microbial influence, whereas bog lake water, groundwater samples and a soil extract reflected higher amounts of plant derived matter. Recent studies have considered hydraulically corresponding samples of drinking water, reclaimed water and reclaimed water after soil-aquifer treatment (SAT) within a local water reuse watershed (Drewes et al., 1999; Drewes and Fox, 1999; Drewes and Fox, 2000). By employing XAD-resin fractionation with subsequent 13C-NMR, structure and composition of XAD-8 and XAD-4 isolates from drinking water and reclaimed water after SAT were similar and representative of natural organic matter. The overall rate of recovery of organic carbon in these studies varied between 66 and 77 percent.

Especially, where effluents are discharged to a river, which potentially serves as drinking water source further downstream, or where treated effluents are re-used to augment in part a drinking water supply source (via groundwater recharge or aquifer injection), the introduction of effluent organic matter is associated with a broad spectrum of potential health concerns. In order to address potential differences or similarities between effluent organic matter (EfOM) and natural organic matter, the scope of this study was directed to combine operationally defined categorization protocols with state-of-the-art characterization techniques to investigate the bulk of organics in raw drinking water samples (surface and groundwater) and wastewater samples with respect to origin, size, structure, and functionality. Samples of different drinking water and wastewater prior to and after groundwater recharge from France and the U.S. were considered in this study. This paper combines results of two large projects on NOM isolation and characterization funded by the American Water Works Association Research Foundation (AWWARF) and results of a tailored collaboration research project funded in part by AWWARF and the U.S. Environmental Protection Agency.

Methods

Samples were analyzed for pH, temperature, conductivity and dissolved oxygen in the field. In the laboratory, all samples were filtered using 0.45 µm cellulose nitrate membrane filters and stored at 4°C pending further analyses. To investigate bulk organics in drinking water and wastewater, total organic carbon (TOC) or dissolved organic carbon (DOC) and specific UV absorbance (SUVA) were measured followed by advanced characterization techniques including XAD-8/-4 resin fractionation. The sequential use of XAD-8 and XAD-4 resins provide a separation of organic molecules according to polarity (Aiken 1988; Aiken and Leenheer, 1993; Croué et al., 1999, Leenheer et al., 2000). The result is three operationally-defined “fractions” corresponding to a range of polarities and, consequently, hydrophobicities/hydrophilicities. The hydrophobic fraction (HPO) adsorbed at pH 2 onto XAD-8 resin and the transphilic fraction (TPI) adsorbed at the same pH onto XAD-4 resin are desorbed by elution by an organic solvent (75 percent acetonitrile/25 percent milli-Q-water) or by changing the pH (NaOH at pH 13) and further purified.
(removal of salts) before final lyophilization. The hydrophilic carbon corresponds to the fraction that does not adsorb on either resin. Isolates generated following this approach were used for solid-state carbon-13 nuclear magnetic resonance spectroscopy ($^{13}$C-NMR) to determine the distribution of carbon functional groups. Additionally, the isolates were used for elemental analysis (C, H, N), isotopic composition using $\delta^{13}$C, and Fourier-transform infrared spectroscopy (FT-IR) in order to identify functional groups and inorganic species. Bulk water samples were directly used for liquid-chromatography analysis with continuous UV absorbance and organic carbon detection (LC-OCD).

Total and dissolved organic carbon were analyzed as non-purgeable dissolved organic carbon (NPDOC) by thermal combustion with infrared detection of CO$_2$ using a Shimadzu 5000 TOC-analyzer with an ASI-5000 autosampler (Standard Method 5310 B) (method detection limit (MQL) = 0.15 mg/L; lower level of detection (LLD) = 0.10 mg/L). UV-absorbance (UVA) was measured at a wavelength of 254 nm with a Hewlett Packard 8452A spectrophotometer (path length 1 cm) or a Safas spectrophotometer “Double energy system 190 DES” with 1 or 5 cm long quartz cells. The specific UV absorbance (SUVA) was calculated as ratio between UVA and DOC and is expressed in units of m$^{-1}$ L/mg C. The $^{13}$C-NMR spectra were performed on a Varian UnityPlus 400 spectrometer using a multinuclear Varian 5 mm CP/MAS probe operating at 100.58 MHz. Fourier transformation – infrared (FT-IR) spectroscopy was carried out on KBr pellets using a 2020 Galaxy Series FT-IR (Mattson Instruments) or a Nicolet 750 Magna-IR™. Each sample was scanned 16 times at a resolution of 2 or 4 cm$^{-1}$. A background spectra of CO$_2$-carbon was generated prior to each measurement and subtracted from each sample spectrum. For CHN element analysis a Perkin – Elmer 2400 Elemental Analyzer with acetanalide and apple leaves standard was employed. A certain number of samples were also analyzed at the CNRS-Solaize laboratory in France (Croue et al., 2000). $\delta^{13}$C analyses were conducted at the Laboratoire de Biogéochimie Isotopique of the University of Pierre et Marie Curie (Paris, France) according to the method published by Girardin and Mariotti (1991). Samples are combusted and converted to carbon dioxide, dinitrogen and water in an elemental analyzer (Carlo Erba Na 1500) coupled with an isotopic mass spectrometer (Sira X Micromass) and equipped with double inlet system and triple ionic collection. Samples for LC-OCD analysis were shipped to the DOC-Laboratory of Dr. Huber in Karlsruhe, Germany in ice-packed containers. Chromatographic characterization was performed using a high-pressure liquid chromatography-system (LC-OCD) equipped with a conventional GAT PHD-500 UV-Vis detector in association with a Grätzel thin film photochemical reactor combined with an IR-Ultramat 3 from Siemens. The method is described in Huber and Frimmel (1992).

Results and discussion

Size-exclusion chromatography using NOM and EfOM samples

Drinking water and hydraulically corresponding wastewater samples prior to and after groundwater recharge were sampled and subsequently used for high-pressure liquid chromatography with online DOC and UVA detection (LC-OCD). This analytical technique uses bulk water samples and allows to differentiate organic carbon by integrating the chromatograms into five different fractions: (1) humics (HS), (2) humic substances (HS) hydrolysates, (3) low molecular mass acids, (4) low molecular mass neutrals and amphiphilics, and (5) polysaccharides. The LC-OCD chromatograms of a NOM and EfOM sample after simulated groundwater recharge in a laboratory soil-column set-up exhibited a similar molecular weight distribution (Figure 1).

Although the EfOM sample prior to groundwater recharge exhibited increased concentrations of all five chromatographic fractions (Table 1), organic carbon chromatograms of
Houston drinking water and reclaimed water after simulated soil-aquifer treatment revealed the presence of significant less humics, less molecular weight acids and only slightly higher low molecular weight neutrals. Although, the organic carbon concentration of humics in the EfOM sample after soil-aquifer treatment is lower as compared to humics in drinking water, the specific UV absorbance (SUVA) of this fraction is slightly higher and points to a slightly higher aromatic carbon content in the EfOM sample. The sources of the additional aromaticity are probably either of anthropogenic origin or soluble microbial products (SMPs) formed during the activated sludge process. Rostad et al. (2000) pointed out that aromatic carbons in EfOM samples are primarily sulfonated anionic surfactant metabolites. However, Drewes et al. (2001) generated SMPs by feeding a pilot activated sludge system (capacity 100 L/d) with low molecular weight carbon only (glucose and glutamic acid in distilled water) and the SUVA increased from essentially zero in the influent to 0.7 to 1.2 mg–1 L m–1 in the effluent of the pilot plant indicating an increase of aromaticity by generated SMPs.

**DOC distribution (hydrophobicity/hydrophilicity) of NOM and EfOM**

Specific UV absorbance of bulk water samples is primarily associated with hydrophobic acids and neutrals (HPO) which adsorb onto XAD-8 resins. However, transphilic and

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**Figure 1** LC-OCD chromatogram of Houston NOM and corresponding tertiary effluent (EfOM) after simulated short-term soil-aquifer treatment

<table>
<thead>
<tr>
<th></th>
<th>NOM</th>
<th>EfOM prior to soil-aquifer treatment</th>
<th>EfOM after soil-aquifer treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DOC (mg/L)</td>
<td>4.62</td>
<td>6.65</td>
<td>4.21</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>0.08</td>
<td>0.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Low molecular weight neutrals</td>
<td>0.69</td>
<td>1.46</td>
<td>0.85</td>
</tr>
<tr>
<td>Low molecular weight humics</td>
<td>0.31</td>
<td>0.096</td>
<td>0.064</td>
</tr>
<tr>
<td>HS hydrolysates</td>
<td>0.91</td>
<td>1.35</td>
<td>0.96</td>
</tr>
<tr>
<td>Humics (HS)</td>
<td>2.63</td>
<td>3.45</td>
<td>2.26</td>
</tr>
<tr>
<td>Total UVA (m–1)</td>
<td>10.05</td>
<td>14.53</td>
<td>11.15</td>
</tr>
<tr>
<td>Low molecular weight neutrals</td>
<td>1.11</td>
<td>0.8</td>
<td>1.26</td>
</tr>
<tr>
<td>HS-hydrolysates</td>
<td>1.81</td>
<td>4.43</td>
<td>2.56</td>
</tr>
<tr>
<td>Humics (HS)</td>
<td>7.12</td>
<td>9.3</td>
<td>7.34</td>
</tr>
<tr>
<td>Total SUVA (L mg–1 m–1)</td>
<td>2.18</td>
<td>2.18</td>
<td>2.65</td>
</tr>
<tr>
<td>Humics (HS)</td>
<td>2.71</td>
<td>2.70</td>
<td>3.25</td>
</tr>
</tbody>
</table>
hydrophilic carbon can also contain significant amounts of specific UV absorbance. Considering organic matter samples from various locations, the percentage of NOM adsorbed onto XAD-8 varied between 28 and 79 percent, and increased with increasing SUVA (Figure 2). SUVA of NOM samples investigated varied between 0.6 and 5 mg\(^{-1}\) L m\(^{-1}\). The proportion of HPO of EfOM samples varied between 28 and 48 percent with SUVA varying between 1 and 3 mg\(^{-1}\) L m\(^{-1}\), and a correlation between carbon adsorbed onto XAD-8 resin and SUVA was not obvious. SUVA of TPI fractions of NOM and EfOM did not show a clear difference or distinct trend. However, the hydrophilic carbon (i.e. fraction of NOM that do not adsorb on both XAD resins) decreased with increasing SUVA both for NOM and EfOM showing an overall linear regression coefficient \(R^2\) of 0.79.

As a general trend the proportion of hydrophilic NOM appeared to be significantly higher in the EfOM. Whereas NOM usually represents well degraded and altered organic matter, EfOM samples can still contain significant proportions of non-aromatic, easily degradable carbon which might also contribute to overall lower SUVA values.

### Aromaticity of NOM and EfOM

The percentage of aromatic carbon content of HPO and TPI isolates of NOM and EfOM samples was determined using \(^{13}\)C-NMR spectroscopy. Results are presented in Figure 3. Both, NOM and EfOM HPO and TPI isolates exhibit positive correlations indicating that increasing SUVA values are reflected in increasing aromaticity. However, for the same SUVA value EfOM HPO and TPI isolates exhibited higher aromatic carbon contents as compared to NOM isolates. This structural difference is clearly reflected in the \(^{13}\)C-NMR spectra (Figure 4).

Figure 4 represents \(^{13}\)C-NMR spectra of HPO and TPI isolates of NOM and hydraulically corresponding EfOM samples prior to and after simulated SAT. The difference between NOM HPO and EfOM HPO is obvious in an increased carbohydrate peak (70 ppm), the occurrence of anomeric carbon (90–110 ppm), generally stronger aromatic peaks (110–160 ppm), and most commonly a distinct phenolic peak at 140 ppm in the EfOM HPO which is according to Rostad \textit{et al.} (2000) attributed to sulfonated compounds.
derived from laundry detergents. Simulated subsequent SAT obliterate these distinctive
differences between NOM and EfOM HPO with the exception of aromatic carbon which is
diminishing only slightly. The comparison of TPI fraction revealed no significant differ-
ence between the different spectra and only a slight difference for aromatic carbon I
(110–140 ppm) between NOM and EfOM samples which also tends to persist during subse-
quent simulated short-term SAT. Results of field studies at groundwater recharge sites
revealed that aromatic carbon of EfOM HPO and TPI fractions decreased further during
longer retention times of more than 6 months in the subsurface (Drewes and Fox, 1999).

Elemental composition of NOM and EfOM
Elemental analysis of HPO and TPI fractions can provide further insight into the structural
differences between NOM and EfOM. Elemental analysis results are presented in Table 2.

Whereas no difference seems to exist between the H/C ratio of NOM and EfOM isolates,
the N/C ratios of EfOM isolates resembled river water NOM isolate samples, ranging from
0.05 to 0.11 and 0.04 to 0.11, respectively. In contrast, humic acid isolate originated from
the peat bog showed a H/C ratio of 0.22 and a N/C ratio of 0.021, respectively, indicating

Table 2 Elemental analysis of hydrophobic and transphilic carbon isolated from NOM and EfOM samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Elemental distribution</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (%)</td>
<td>H (%)</td>
</tr>
<tr>
<td>NOM Tempe HPO</td>
<td>52</td>
<td>6.4</td>
</tr>
<tr>
<td>NOM Tempe TPI</td>
<td>36</td>
<td>5.0</td>
</tr>
<tr>
<td>NOM Seine HPO</td>
<td>51.29</td>
<td>5.37</td>
</tr>
<tr>
<td>NOM Seine TPI</td>
<td>43.89</td>
<td>5.2</td>
</tr>
<tr>
<td>NOM Vienne HPO</td>
<td>49.03</td>
<td>5.51</td>
</tr>
<tr>
<td>NOM Vienne TPI</td>
<td>45.21</td>
<td>5.63</td>
</tr>
<tr>
<td>EfOM Mesa HPO</td>
<td>42.2</td>
<td>4.9</td>
</tr>
<tr>
<td>EfOM Mesa TPI</td>
<td>41.3</td>
<td>5.2</td>
</tr>
<tr>
<td>EfOM Saint Julien HPO</td>
<td>49.3</td>
<td>5.2</td>
</tr>
<tr>
<td>EfOM Saint Julien TPI</td>
<td>48.6</td>
<td>5.6</td>
</tr>
<tr>
<td>EfOM Model Wastewater HPO</td>
<td>41.1</td>
<td>4.87</td>
</tr>
<tr>
<td>EfOM Model Wastewater TPI</td>
<td>39.0</td>
<td>5.12</td>
</tr>
</tbody>
</table>
relatively less nitrogen and hydrogen in the peat bog isolate (Peschel et al., 1988). HPO and TPI fractions isolated from a model wastewater representing SMPs exhibited the highest N/C ratios of 0.13 and 0.17, respectively. High N/C ratios might be caused by proteins and aminosugars (i.e. peptidoglycan structure) from microbiological activity. Hydrophobic NOM (i.e. fulvic acids) isolated from Antarctic lakes that were known to be derived exclusively from microbial sources was found to be characterized by a high N/C ratio and low aromatic character as compared to organic materials isolated from lignin-rich sources (Aiken and Cotsaris, 1995). Although particularly nitrogen rich compounds are discharged into wastewater by consumers, pyrolysis GC-MS results revealed a clear dominance of fragments derived from the biodegradation of proteins (peptone, egg albumen etc.) and carbohydrates (methylfuran, furfural etc.) in spectra of river and effluent isolates but not in the spectrum of allochthonous organic matter (Peschel et al., 1988; Dignac et al., 2000).

**FT-IR spectra of NOM and EfOM**

FT-IR-spectroscopy of HPO and TPI isolates displayed highly similar spectra for all samples investigated, however, functional groups containing nitrogen were more abundant in EfOM as compared to river water NOM which is consistent with observed increased N/C ratios in EfOM isolates and previous studies (Peschel et al., 1988). Figure 5 and 6 present examples of HPO and TPI isolates from NOM and EfOM samples, respectively. The hydrophobic and transphilic fractions of the NOM sample (Seine River) are both typical for aquatic humic-type material represented by a dominant carboxyl peak at 1,720 cm⁻¹. Additionally, TPI exhibits more hydrophilic alcohol groups near 1,045 cm⁻¹ than the HPO NOM fraction and shoulders at 1,660 cm⁻¹ and 1,550 cm⁻¹ that are typical of the presence of proteins (amide 1 and 2 bands) (Leenheer et al., 2000). HPO and TPI of the EfOM sample (Saint Julien) also exhibit very prominent peaks at about 1720 cm⁻¹. The major difference between EfOM and NOM isolates is the series of distinct peaks at 1,170 cm⁻¹, 1,125 cm⁻¹, 1,038 cm⁻¹, and 1,010 cm⁻¹ frequently detected in HPO and TPI isolates of EfOM samples and also observed in some NOM isolates which according to Field et al. (1992) are derived from anionic surfactant degradation products including sulfophenyl carboxylates and dialkyltetralin sulfonates. Efforts were made during this study to neglect the impact of anthropogenic sources and NOM and EfOM which consists of SMPs derived from model wastewater were examined (Drewes et al., 2001). The FT-IR spectra of the HPO and TPI isolated from these SMPs are presented in Figure 7. Beside a peak at about 1,720 cm⁻¹ indicating carboxyl carbon and the peaks at 1,660 cm⁻¹ and 1,550 cm⁻¹ that are strong indicators of the presence of proteinaceous material (i.e. intense microbial activity), the two distinct peaks at 1,172 cm⁻¹ and 1,038 cm⁻¹ are also obvious but the use of surfactants during these experiments can be excluded. This result points to a biological origin of compounds causing these peaks and further research is necessary to determine the structure of these compounds.
δ13C analysis results of NOM and EfOM isolates

δ13C analyses show strong differences between EfOM and NOM isolates. If we consider that aromatic carbon is an indicator of terrestrial origin (C3 plant) we can see logically that the higher the aromatic carbon the lower the δ13C. The more the hydrophilic the NOM fractions, the higher the incorporation of materials due to algae and microbial activities (i.e. carbohydrates, proteins) into the NOM structure which correspond to higher δ13C. The use of carbonates as a carbon source during intense bacterial activity could possibly generate organics with higher δ13C. An other explanation could be that higher oxidized material (i.e. biologically or chemically) lead to higher δ13C content organic structures considering that 12C will be easier oxidized than 13C. Nevertheless, for the same aromatic carbon content EfOM show a significantly higher δ13C content, for both HPO and TPI fractions which indicate as stated earlier a higher contribution of microbial products in EfOM as compared to NOM. So again, the aromatic moieties present in the EfOM matrix are probably of different origin and structure (i.e. degree of substitution and nature of the substituents) showing different molar absorptivity than the aromatic moieties of the NOM as indicated by the relationship we have established between SUVA and the aromatic carbon content.

Conclusions

By applying a variety of analytical tools, a structural similarity between EfOM and river water NOM samples was demonstrated. Findings of this study reveal that a combination of categorization protocols with state-of-the-art characterization techniques can highlight potential differences and similarities between NOM and EfOM. The physical, chemical, and biological processes that generate and modify organic compounds in natural and engineered systems share many similarities. As a result, the chemical characteristics of effluent derived and naturally derived organic compounds overlap extensively. Based on findings of this study, wastewater organics shifted during subsequent groundwater recharge toward a distribution characteristic of natural systems. However, their was evidence that the aromatic moieties of EfOM are probably of different origin than the moieties of NOM.
contribution of sulfonated compounds to the aromatic character of EfOM as reported previously could not be confirmed.

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