Novel methods for characterizing algogenic organic matter and associated nanofiltration membrane fouling

N. Her, G. Amy, J. Yoon and M. Song
Department of Civil and Environmental Engineering, University of Colorado, Boulder, CO 80309, USA
(E-mail: gamy@spot.colorado.edu)

Abstract Algogenic organic matter (AOM) has been extracted from blue-green algae (cyanobacteria) by various means and analyzed by UV absorbance scanning, HPSEC-UV-fluorescence-DOC, FTIR, and fluorescence excitation emission matrix (EEM). AOM extracted in water as a solvent showed a high hydrophilic fraction (57.3%) with a low SUVA (1.0 L/m-mg). The molecular weight (MW) distribution showed a significant heterogeneity (high value of polydispersivity) and high protein content (as indicated by specific fluorescence). A significant amount of proteinaceous components such as mycosporine-like amino acids (MAAs, UV-screening components) and phycobilins (light-harvesting pigment) was detected by UV/visible absorption. The confirmation of proteins was proven by FTIR (at 1,661 cm–1 and 1,552 cm–1) and EEM spectra (EX: 278–282 nm and EM: 304–353 nm). A bench-scale cross-flow unit, employing a flat-sheet membrane specimen, was used to examine nanofiltration (NF) membrane fouling and removal of natural organic matter (NOM) derived from different blends of Suwannee River humic acid (SRHA) and AOM. The flux decline and organic matter rejection as a function of delivered DOC showed significantly different results depending on the organic matter composition of samples even though the test conditions were the same (organic matter concentration, pH, temperature, inorganic salt composition and concentration, and recovery). A higher flux decline was observed with increasing proportions of AOM. Organic matter rejections also decreased with higher AOM contributions to the samples, indicating that lower MW AOM components were not well rejected by the NF 200 membrane having a 360 dalton molecular weight cutoff (MWCO). However, SRHA that shows a relatively high MW (5,000–1,000 daltons) and high SUVA (7.4 L/m-mg) was preferentially rejected through electrostatic repulsion/size exclusion by the NF 200 membrane, having a high negative charge (zeta potential: -15.6 mV), low MWCO, and relatively low hydrophobicity. Even though the DOC concentration of feed water is a decisive factor for membrane fouling along with membrane properties and operating conditions, the characteristics of organic matter are more influential in fouling potential. Protein-like and polysaccharide-like substances were found as major foulants by FTIR.

Keywords Algogenic organic matter; fluorescence excitation emission matrix; FTIR; HPSEC-UV-fluorescence-DOC; nanofiltration; Suwannee River humic acid

Introduction
Algae are abundant and diverse in drinking water supplies including lakes, reservoirs, rivers, and streams. In all habitats, they are important primary producers and an important food source for freshwater fish and crustaceans. Occasional algal blooms, comprised of blue-green algae (cyanobacteria) and/or green algae, cause significant challenges in drinking water treatment due to the release of organic compounds (algogenic organic matter, AOM) into water extracellularly and, upon cell lysis, intracellularly. The soluble AOM includes glycolic acid, carbohydrates, polysaccharides, amino acids, peptides, organic phosphates, enzymes, vitamins, hormonal substances, inhibitors, and toxins (Wetzel, 1975). The release rates of AOM are quite variable depending on the algal growth phase, type of algae, and physiological and environmental conditions. Blue-green (B-G) algae are enriched in proteins, generally 40–65% of dry weight (Chronakis, 2001).

In recent years, pressure-driven membrane processes have been considered as an economically viable alternative to conventional water treatment techniques for the removal
of natural organic matter (NOM) (Hong and Elimelech, 1997; Cho et al., 1999). In principle, membrane filtration also appears to be an attractive method of algal removal because its removal efficiency should be less influenced by certain water quality parameters that have an overriding influence on the performance of other conventional treatment processes (Chow et al., 1997). Algae and AOM were effectively removed by ultrafiltration membranes but membrane fouling was significant (Chow et al., 1997).

The aims of this study were to characterize AOM by various methods and to evaluate NF membrane fouling associated with AOM. For these experiments, the membrane employed was NF 200, having polypiperazine as an ultrathin top layer, a MWCO of 360 daltons determined by polyethylene glycol (PEG) rejection tests, a relatively low contact angle (22.5°), and a high negative surface charge (~16.5 mV at pH 7.0 and 300 µS/cm with KCl) (Her et al., 2000). The NF 200 membrane would be expected to cause less hydrophobic interactions that might lead to flux decline with hydrophobic NOM, and to reject well negatively charged NOM components (e.g. humic and fulvic acids) by electrostatic repulsion. Therefore, AOM that contains large amounts of proteins and polysaccharides may cause significant fouling of the NF 200 membrane due to its charge, hydrophilicity, and size of the AOM.

Materials and methods

AOM extraction and preparation for analyses

B-G algae were obtained from Klamath Blue Green Inc., CA. They were harvested from Klamath Lake located in the Cascade Mountains of Southern Oregon, whose morphological, biochemical, and immunological properties appeared to be mostly retained by the freeze-drying method. The composition of the B-G algae consists of 68% protein, 22% carbohydrate, 5% lipids, and 3% chlorophyll a (reported by the supplier). Extraction of AOM was performed using 0.45 µm cartridge (nylon filter) filtration. G-AOM corresponds to AOM extracted in water after physically grinding algal cells with a mortar and pestle while S-AOM corresponds to AOM extracted in water after ultrasonication of algal cells in water for 1 hour. AOM-Me is AOM extracted in 90% methanol over 24 hours. Chlorophyll a extracted from Anacystis nidulans algae was obtained from Sigma to help identify the AOM components by the UV/visible analyses. Chlorophyll a was dissolved in 90% methanol solvent.

The AOM dissolved in water after grinding the B-G algae was fractionated according to size using 0.45 µm (nylon) and 1.2 µm (GF/C, glass fiber) filters and solidified for functional group identification by FTIR.

Synthetic samples

Synthetic water samples were prepared with different blends of SRHA and S-AOM solutions to evaluate the effects of two different sources/types of organic matter on NF (NF 200) membrane fouling under the same conditions. The pH and conductivity were adjusted with H2SO4, NaOH, and Na2SO4 (pH 7 and 300 µS/cm) and the DOC concentration of synthetic waters was set to 10 mg C/L (SRHA 10 mg C/L, AOM 3 mg C/L + SRHA 7 mg C/L, AOM 7 mg C/L + SRHA 3 mg C/L, and AOM 10 mg C/L).

Sample analyses

Organic matter analysis. Dissolved organic carbon (DOC) and ultraviolet absorbance (UVA) at the wavelength of 254 nm were measured by a total organic carbon analyzer (TOC 800, Sievers) and a UV-visible spectrophotometer (UV-160A UV/Visible Spectrophotometer, Shimadzu) with a 1 cm quartz cell, respectively. NOM fractionation in feed waters was performed by XAD-8/-4 resin adsorption that defined the distribution of
mass fraction in terms of hydrophobic (HPO) vs. transphilic (TPI) vs. hydrophilic (HPI) DOC. UV absorbance spectra for AOM samples have been also obtained by scanning from 200 to 700 nm with a UV-visible spectrophotometer.

**ATR-FTIR**. To identify the functional groups of various solid samples and fouled membranes, spectra were collected with a Nicolet 752 Attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectrophotometer using a KBr pellet with solid samples or using a membrane itself. All spectra were collected between 4,000 and 400 cm⁻¹, averaging 32 scans at a nominal instrument resolution of 4.0 cm⁻¹. Some spectra were normalized after acquisition to a maximum absorbance of 1.0 for comparison.

**HPSEC-UV-fluorescence-DOC**. Separation by high performance size exclusion chromatography (HPSEC) was performed using a TSK-50S (Toyopearl HW 50S, 30 µm resin) column that had a length of 25 cm and an inner diameter of 2 cm. The HPLC (LC600 Shimadzu) was coupled with a UV detector (Shimadzu SPD-6A UV detector) operated at 254 nm, a fluorescence detector (Waters 470 Scanning Fluorescence detector) operated at excitation 278 nm and emission 353 nm, and a DOC detector (Modified Sievers Total Organic Carbon Analyzer 800 Turbo). Analysis was performed according to methods described in Her et al. (2002). The excitation and emission wavelengths for fluorescence were chosen to detect protein-like substances. Specific fluorescence indicates the DOC-normalized fluorescence obtained with fluorescence intensity divided by DOC concentration at each MW.

The HPSEC mobile phase was prepared with a phosphate buffer (0.0024 M NaH₂PO₄ + 0.0016 M Na₂HPO₄, pH 6.8) and 0.025 M Na₂SO₄, producing an ionic strength of 0.1 M. The flow rates were 1 mL/min and sample injection volume was 2 mL. The intensity of the chromatograms for UVA at 254 nm and DOC were both measured in volts and calibrated to general units such as m⁻¹ unit for UVA and mg/L unit for DOC by direct input of potassium hydrogen phthalate (PHP) standard solutions (0.05, 0.1, 0.5, 1, 3, and 6 mg/L) to HPLC. PHP standards were also used for calibration to quantify UV and DOC peak heights of chromatograms. Polystyrene sulfonate (PSS) standards have been shown to be more representative of NOM than PEG in SEC characteristics (hydrodynamic radii, viscosity, etc.) (Perminova et al., 1998). However, this system was calibrated using polyethylene glycol (PEG) standards ranging from 200 to 10,000 daltons because PSS displayed more undesirable interactions than did the PEG with the Toyopearl HW resin within the column. A semi-log calibration curve ($r^2>0.99$) was used to calculate the MW. The ionic strength of samples was adjusted with a concentrated eluent solution to match the ionic strength (0.1 M) of the HP-SEC mobile phase. AOM-Me, extracted in 90% methanol, was evaporated to remove methanol at 50°C using a Rotavapor and diluted in eluent for the SEC analysis.

**Fluorescence EEM**. Fluorescence EEM spectra were measured by a JY-Horiba/Spex Fluoromax-2 fluorometer with a xenon lamp as the excitation source. EEM spectra are a collection of a series of emission spectra over a range of excitation wavelengths. In these determinations, EEM spectra were collected with subsequent scanning emission spectra at 2 nm increments by varying the excitation wavelength at 10 nm increments. The intensity of EEM is represented by contour lines. Spectral subtraction was performed to remove blank spectra mainly caused by Raman scattering.

**Membrane test unit**
A Millipore Mini-Tan system (a bench-scale cross-flow unit employing a flat-sheet membrane specimen) was used to perform bench-scale flux decline and rejection experiments.
with the NF 200 membrane. The Mini-Tan system consisted of a 4 litre reservoir from which a small, variable speed gear pump provided water to the test cell. The total membrane surface area in the test cell is approximately 56.27 cm$^2$ (5.8 cm × 9.7 cm) and the total cross flow area into the test cell is 0.41 cm$^2$ (5.8 cm × 0.07 cm).

All permeates and retentates were recycled to the 4 L feed tank due to limited sample volume. The pure water permeability (PWP) of the NF200 membrane was 1–3 L/m$^2$·day·psi. The recovery ($Q_p/Q_f$) was set at approximately 10% and the operating pressure was maintained at a constant 90 psi (constant pressure declining flux mode of operation). The system recovery ratio was managed through the use of by-pass and back-pressure controllers. The flow rate of retentate was monitored by a flow-meter and permeate flow volumes were measured in a graduated cylinder over time. The pressures of the feed and retentate were assumed to be equal and were measured by a pressure sensor. Feed water temperature is an important factor in obtaining constant specific flux, and it was maintained using a temperature controller.

Initial permeate flux was established using Millipore Milli-Q (MQ) water, with MQ water first filtered through a flat sheet membrane for two days until an approximately constant flux was observed. Permeate flux and quality (UVA$_{254}$ and DOC rejections) were monitored at a constant pressure (90 psi) as a function of delivered DOC (mg/cm$^2$).

**Results and discussion**

**Characteristics of AOM**

Water quality parameters of feed waters are summarized in Table 1. The specific UVA (SUVA, representing an index of NOM aromaticity) values of SRHA and S-AOM were 7.4 L/m-mg and 1.0 L/m-mg. AOM exhibits a much lower aromaticity compared to SRHA. Table 2 shows the distribution of DOC fractions for SRHA and AOM. The HPI fraction was high for AOM (57.3%) while SRHA, as expected, showed a high HPO fraction (93.5%). The HPO fraction can potentially exhibit high hydrophobicity associated with a high aromatic structure (high SUVA).

UV/visible absorption spectra are shown in Figure 1 for G-AOM, S-AOM, AOM-Me, and chlorophyll $a$. UV/visible spectra shows that the extraction of organic matter does not highly depend on the physical method (i.e. grinding or sonicating) but on solvents (i.e. water or methanol). The spectra were similar for G-AOM and S-AOM, extracted in water as a solvent. However, AOM-Me showed additional absorption peaks around 400 nm and at 664 nm. The absorption around 400 nm may be from scytonemin that is phenolic and an indolic derivative produced in the sheaths of B-G algae (Cockell and Knowland, 1999).

**Table 1** Water quality of feed waters

<table>
<thead>
<tr>
<th>Feed waters</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>UVA$_{254}$ (cm$^{-1}$)</th>
<th>DOC (mg/L)</th>
<th>SUVA (L/mg-m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRHA 10 mg C/L</td>
<td>6.80</td>
<td>300</td>
<td>0.74</td>
<td>10</td>
<td>7.4</td>
</tr>
<tr>
<td>(S-AOM 3 mg + SRHA 7 mg) C/L</td>
<td>6.80</td>
<td>300</td>
<td>0.58</td>
<td>10</td>
<td>5.8</td>
</tr>
<tr>
<td>(S-AOM 7 mg + SRHA 3 mg) C/L</td>
<td>6.80</td>
<td>300</td>
<td>0.42</td>
<td>10</td>
<td>4.2</td>
</tr>
<tr>
<td>S-AOM 10 mg C/L</td>
<td>6.80</td>
<td>300</td>
<td>0.10</td>
<td>10</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table 2** DOC fractionation of feed waters by XAD-8/4 resins

<table>
<thead>
<tr>
<th></th>
<th>HPO (%)</th>
<th>TPI (%)</th>
<th>HPI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRHA</td>
<td>93.5 (0.96)</td>
<td>5.2 (0.49)</td>
<td>1.4 (0.49)</td>
</tr>
<tr>
<td>S-AOM</td>
<td>25.9 (1.41)</td>
<td>16.8 (2.69)</td>
<td>57.3 (4.1)</td>
</tr>
</tbody>
</table>

( ): Standard deviation
and/or from the chlorophyll $a$. Chlorophyll $a$, exhibiting a green color, also shows strong absorption of red light at 664 nm. Chlorophyll $a$ appears not to be dissolved well in water as a solvent.

All AOM materials showed a high UV/visible absorption by mycosporine-like amino acids (MAAs) at 329 nm. MAAs, which consist of a cyclohexenone (UV-B, 280–315 nm, screening core structure) or cyclohexeneimine (UV-A, 315–400 nm, screening core structure) substituted with amino compounds, are water- and methanol-soluble and have a high molar absorptivity (Sommaruga and Garcia-Pichel, 1999; Cockell and Knowland, 1999). The concentrations of MAAs are directly related to the intensity of the UV radiation (maximum at 320 nm) received by the cells (Garcia-Pichel et al., 1993). The B-G algae used in this research were cultured above an elevation of 1,200 m that may receive more UV-B radiation. UV irradiances increase due to the natural increase of UV radiation with elevation (20% increase per 1,000 m of elevation for UV-B radiation) (Sommaruga and Garcia-Pichel, 1999). The high concentration of MAAs may be produced for UV-screening to reduce the inhibition of heterosyst formation in B-G algae (Sinha et al., 1996). The peaks at 609 nm (for AOM-Me) and 621 nm (for G-AOM and S-AOM) may be from proteinaceous phycobilin that appears as a blue color. Phycobilin is used for photosynthesis as a light-harvesting pigment in addition to the chlorophyll $a$ in B-G algae. The absorbance peak around 260 nm in the UV-C (200–280 nm) region resulted from intense short-wavelength $\pi$ to $\pi^*$ transitions found in most conjugated molecules (Cockell and Knowland, 1999).

Figure 2 shows HPSEC-UV-fluorescence-DOC chromatograms for S-AOM and SRHA. The results of MW estimations in Table 3 for SRHA and S-AOM were obtained using the UV and DOC spectra portrayed in Figure 2. S-AOM has a high weight-averaged MW ($M_w$) and low number-averaged MW ($M_n$) for both detectors, resulting in a high value of polydispersivity ($\rho$, $M_w/M_n$). S-AOM shows multiple peaks (greater heterogeneity) with a lower SUVA (maximum: 0.73 L/m-mg), compared to SRHA. On the contrary, SRHA shows a single peak ($\rho = 1.7–2.0$) around 3,400 daltons with a highest SUVA value (8.58 L/m-mg) and a low specific fluorescence intensity (0.01 height unit). The specific fluorescence is much higher for S-AOM than for SRHA, indicating a higher protein content in AOM.

Figure 3(a) shows the FTIR spectra of B-G algae, AOM materials, and SRHA. All samples displayed strong absorption bands at 3,430 cm$^{-1}$, characteristic of hydrogen bonded OH. Relatively high aliphatic CH$_2$ absorption bands were seen at 2,926 cm$^{-1}$ (asymmetric stretching) and 2,853 cm$^{-1}$ (symmetric stretching) for AOM-Me. The 1,720 cm$^{-1}$
absorption bands, mainly associated with C=O of C(OH), were stronger for SRHA than for all AOM materials. The absorption intensity of C(OH) increased in the order: B-G algae, G-AOM, S-AOM, and AOM-Me. The absorption bands at 1,613–1,622 cm⁻¹ may be associated with aromatic C=C and ionized carboxylic acids. B-G algae showed distinctive protein peaks at 1,661 cm⁻¹ and 1,552 cm⁻¹. Proteins, like polypeptides in general, consist of chains of amino acid residues joined end-to-end by secondary amide bonds. The absorption at 1,661 cm⁻¹ is the stretching vibration bands associated primarily with the peptide carbonyls (C=O, amide I band). The amide II bands are seen at 1,552 cm⁻¹ resulted from the interaction between the N-H bending and the C-N stretching of the C-N-H group (Tadesse et al., 1991).

Figure 3(b) shows the FTIR spectra of B-G algae on the basis of size after fractionation in water solvent following the grinding of the B-G algae. The absorption bands by hydrogen bonded NH were much stronger at 3,308 cm⁻¹ for the algal components above 0.45 µm and the original B-G algae, while dissolved components less than 0.45 µm (G-AOM) showed a stronger absorption by hydrogen bonded OH at 3,430 cm⁻¹. The absorption by amide I and II bands also occurs significantly at 1,661 cm⁻¹ and 1,552 cm⁻¹ for the algal components above 0.45 µm. This suggests that dissolved AOM contains lower nitrogen-containing components compared to colloidal/particulate algal components. However, the content of polysaccharide-like substances observed at 1,040–1,150 cm⁻¹ are relatively higher for dissolved AOM than larger size algal components.

Figure 4 shows the EEM spectra of S-AOM (5–10 mg C/L) and SRHA (around 2 mg C/L). The EEMs show protein-like substances (at EX: 279–282 nm and EM:...
(304–353 nm) and humic substances (at EX: 352 nm and EM: 441 nm) (Her et al., 2001). The EEM peak maxima of SRHA (EX: 341 nm and EM: 453 nm) shows a red shift (longer excitation and emission wavelengths) compared to humic substances in AOM (EX: 352–360 nm and EM: 441 nm), indicating a more oxidized form of SRHA and more electron-donating substituents in AOM.

Results of membrane tests
Flux decline and organic matter rejection were studied for each of the four blend samples of SRHA and S-AOM. Figure 5 shows the results of flux decline and organic matter rejection as a function of delivered DOC (based on summation of DOC flux in feed water) at 20°C. Even though the test conditions were the same (NOM concentration, pH, temperature, inorganic salt concentration, and recovery), significantly different results were obtained depending on the organic matter composition of the feed waters. When 12 mg of DOC was delivered per cm² of membrane, flux decline was 4.4% for the membrane fed with 10 mg C/L of SRHA only and increased up to 20.0% for the membrane fed with 10 mg C/L of AOM only (Figure 5(a)). A higher flux decline was observed with increasing AOM proportion. However, organic matter rejection measured by DOC (Figure 5(b)) decreased with an increasing AOM contribution to the samples, indicating that the lower MW AOM components were not well rejected by the NF 200 having a 360 dalton MWCO.
Figure 5 Flux decline and organic matter rejection (based on DOC) as a function of delivered DOC for SRHA, AOM, and blend samples

Figure 6 Permeate quality as a function of delivered DOC: (a) DOC and (b) SUVA
The DOC and SUVA values of membrane permeate are shown in Figure 6 as a function of delivered DOC. The decrease in DOC of permeate over time may be due to the additional separation by an increase in fouling. The DOC concentrations of permeate at 12 mg/cm² delivered DOC increased from 0.45 mg/L with SRHA only to 1.34 mg/L with AOM only. Permeate SUVA values were highly dependent on those of the corresponding feed water: 3.01 L/mg-m with SRHA only and 1.04 L/mg-m with AOM only, respectively.

The FTIR spectra of clean and fouled membranes were compared in Figure 7. A C=O stretching peak from carboxylic acid was seen at 1,740 cm⁻¹ from the membrane fouled with SRHA 10 mg C/L. However, with the increase of AOM proportion, this peak was not clearly seen probably due to a higher intensity peak at 1,650 cm⁻¹ corresponding to a stretching vibration of C=O connected to amides from AOM. The C=O stretching vibration is coupled with the adjacent N-H bending vibration peak appearing at 1,550 cm⁻¹ (N-H stretching: 3,300 cm⁻¹). The peak near 1,000–1,120 cm⁻¹ is associated with alcoholic C-O absorption. Alcoholic C-O bonds may originate from polysaccharide-like substances. Both protein and polysaccharide like substances were found as major foulants whose FTIR peaks were more significant for fouled membranes associated with AOM.

Conclusions
AOM showed a high HPI fraction (57.3%) and a low SUVA (1.0 L/m-mg). A significant amount of proteinaceous components such as MAAs (at 329 nm by UV/visible absorption) and phycobilin was detected by UV/visible absorption. MW distribution showed a greater heterogeneity (high value of polydispersivity) and higher protein content for 21,500 dalton components (by specific fluorescence). The existence of protein was proven by FTIR (at 1,661 cm⁻¹ and 1,552 cm⁻¹) and EEM (EX: 278–282 nm and EM: 304–353 nm). However, SRHA showed a high HPO fraction (93.5%) with high SUVA (7.4 L/m-mg). A large amount of C(=O)OH functional groups (at 1,720 cm⁻¹) and aromatic rings (at 1,622 cm⁻¹) was found by FTIR in SRHA.

Our observations in membrane tests suggest that (negatively charged) humic substances of relatively high MW (5,000–1,000 daltons) and high SUVA were preferentially rejected through electrostatic repulsion/size exclusion by the NF 200 membrane, having a high negative charge (zeta potential: −15.6 mV), low MWCO (360 daltons), and relatively low
hydrophobicity. However, in the case of AOM that reflects a wide MW range of components with less aromatic and more hydrophilic fractions, the NF 200 membrane showed high fouling and poor rejection (the smaller constituents passed through the membrane). Even though the DOC concentration of feed water is a decisive factor for membrane fouling in addition to membrane properties and operating conditions, the characteristics of organic matter are more likely associated with fouling potential. Protein-like and polysaccharide-like substances were found as major foulants by FTIR.

Acknowledgements
This work was funded by the U.S. National Science Foundation (NSF) Center for Membrane Applied Science and Technology (MAST, Project 01-2) at the University of Colorado.

References