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Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms

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ABSTRACT

Algogenic organic matter (AOM) can interfere with drinking water treatment processes and comprehensive characterisation of AOM will be informative with respect to treatability. This paper characterises the AOM originating from four algae species (*Chlorella vulgaris*, *Microcystis aeruginosa*, *Asterionella formosa* and *Melosira* sp.) using techniques including dissolved organic carbon (DOC), specific UV absorbance (SUVA), zeta potential, charge density, hydrophobicity, protein and carbohydrate content, molecular weight and fluorescence. All AOM was predominantly hydrophilic with a low SUVA. AOM had negative zeta potential values in the range pH 2–10. The stationary phase charge density of AOM from *C. vulgaris* was greatest at 3.2 meq g^{-1} while that of *M. aeruginosa* and *Melosira* sp. was negligible. Lower charge density was related to higher hydrophobicity, while it was related in turn to increasing proteins >500 kDa:carbohydrate ratio. This demonstrates that AOM is of a very different character to natural organic matter (NOM).

1. Introduction

Algae are ubiquitous in rivers and reservoirs supplying drinking water treatment facilities. When these populations increase, treatment processes can be adversely affected. For example, coagulant demand is increased and floc formation is poor (Bernhardt, 1984) or membrane fouling is increased (Her et al., 2004). This is a result of not only increased cell concentration but also associated algogenic organic matter (AOM) which can form a substantial component of the algae system. AOM arises extracellularly via metabolic excretion, forming extracellular organic matter (EOM) or intracellularly due to autolysis of cells, forming intracellular organic matter (IOM), and is known to comprise proteins, neutral and charged polysaccharides, nucleic acids, lipids and small molecules (Fogg, 1983), of which polysaccharides can comprise up to 80–90% of the total release (Myklestad, 1995). The IOM proportion increases with increasing age of the algae system. AOM provides a significant contribution to the heterogeneous mixture of compounds that forms dissolved organic matter (DOM) in algal systems. Understanding the character of DOM, and therefore AOM, is essential in order to determine the level of process interference and treatability that may be anticipated.

The first major investigation into AOM character from a treatment perspective was undertaken by Bernhardt and team (Bernhardt et al., 1985; Hoyer et al., 1985; Lüsse et al., 1985). It was demonstrated that molecular weight (MW) and the concentration of dissolved organic carbon (DOC), carbohydrates and uronic acid was highly variable, depending on both species and culture age. MW is particularly important as high-MW AOM can act as a flocculant aid while low-MW AOM can increase the negative charge at the surface of particles (Bernhardt et al., 1985). A later study showed that alginate, which is frequently used as a model for AOM and comprises two uronic acids, has a similar capacity for aluminium ions as

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fulvic acid (Gregor et al., 1996). More recent investigations have demonstrated that proteins can interfere with coagulation (Tirado-Miranda et al., 2003), while both proteins and polysaccharides have been shown to foul membranes (Her et al., 2004).

In related fields involving treatment of organic systems, including that of natural organic matter (NOM) and wastewater biomass, additional parameters have been shown to be useful for linking organic matter character to treatment. For example, NOM is frequently characterised in terms of specific UV absorbance (SUVA), hydrophobicity, charge density and zeta potential (ZP) (Edzwald, 1993; Sharp et al., 2005). Furthermore, the character of soluble microbial products (SMP) and extracellular polymeric substances (EPS) in biomass, in terms of protein:carbohydrate ratios, charge density, hydrophobicity and MW distribution, has been linked to flocculation and membrane fouling potential (Brookes et al., 2003). Additionally, information on organic matter character has been gained from fluorescence excitation–emission matrices (EEMs) which can provide information specifically on protein and humic/fulvic-like substances in DOM and sewage effluent (Baker, 2002; Her et al., 2004; Nguyen et al., 2005).

The current paper aims to characterise AOM originating from algae that are commonly found in water sources using techniques that are commonly used by algae studies, as well as NOM and wastewater biomass studies. Specifically, the AOM character from four algae—the cyanobacteria, *Microcystis aeruginosa*; the green *Chlorella vulgaris*; and the diatoms, *Asterionella formosa* and *Melosira* sp.—is compared. Additionally, the overall character is compared to well-characterised and understood NOM and biomass systems. Consequently, the implications of the AOM character with respect to treatment are assessed.

2. Materials and methods

2.1. Algae cultivation procedure

The freshwater algae cultures of *C. vulgaris* (211/11B), *M. aeruginosa* (1450/3) and *A. formosa* (1005/9), were obtained from the Culture Collection of Algae and Protozoa, (Oban, Scotland), while *Melosira* sp. (JA386) was obtained from Sciento, Manchester, UK. Average cell surface areas for these species are 55, 95, 370 and 6000 μm^2 respectively. All algae were grown in aquarium tanks at 50 l volumes. The *C. vulgaris* and *M. aeruginosa* were grown at 20 °C using Jaworski Media, under 24 h radiation and mixed using a pump. *Melosira* sp. and *A. formosa* were grown using diatom media at 15 °C and a 14/10 h light/dark cycle, with daily mixing by hand. Sun-glo 30 W aquatic lights were used for lighting. Algae grew at various rates, reaching different cell concentrations. To illustrate, maximum concentrations of $1.2 \times 10^7 \pm 9.4 \times 10^5$, $1.5 \times 10^7 \pm 1.0 \times 10^6$, $2.9 \times 10^5 \pm 3 \times 10^4$ and $1.9 \times 10^4 \pm 1.9 \times 10^3$ cells ml^{-1} were achieved on Days 11, 32, 24 and 8 for *C. vulgaris*, *M. aeruginosa*, *A. formosa* and *Melosira* sp. respectively, marking the onset of the stationary phase of growth. AOM was extracted from all algae at this point and additionally during the exponential phase of growth for *C. vulgaris*, *M. aeruginosa* and *A. formosa*, corresponding to Days 8, 20 and 16. Daily checks were undertaken to ensure contamination had not occurred and to determine cell concentrations. Similar to previous observations during cultivation of algae on a comparable scale, if cultures were invaded by other organisms, this only occurred in the late stationary/decline phase (Lüsse et al., 1985). It is acknowledged that experiments are not based on sterile cultures; however, from a practical perspective this reflects natural environment algae blooms. Cell populations were measured by counting at least 100 cells in triplicate using a light microscope and hemocytometer or Sedgewick Rafter cells.

2.2. AOM extraction procedure

AOM was extracted by centrifuging the cell suspension at 10,000*g* for 15 min and subsequently filtering the supernatant (0.7 μm Whatman GF/F glass micro-fibre). When the DOC content of the filtered AOM solution was low, specifically for diatoms, concentration was undertaken using rotary evaporation at 70 mb and 40 °C followed by hardness ion removal using cation exchange resin (Dowex 50-WX-8, 200 mesh, Na^+ form) (Hoyer et al., 1985). This method extracts dissolved organic material as well as organics loosely bound to the cell surface, i.e., the EOM component. However, given that there may be IOM present as a result of autolysis the term AOM will be used for this material. No loss of DOC was observed on concentration and SUVA remained consistent. Furthermore, *C. vulgaris* AOM was used as a control as characterisation results from concentrated AOM were compared to those without concentration and no difference was observed. AOM extracted from *M. aeruginosa*, *C. vulgaris*, *A. formosa* and *Melosira* sp. will be denoted as MA-AOM, CV-AOM, AF-AOM and Msp-AOM respectively from henceforth.

2.3. AOM characterisation procedures

All characterisation was undertaken within 4 days of extraction and conducted at pH 7 unless stated otherwise.

2.3.1. DOC and SUVA analysis

DOC was measured using a Shimadzu TOC-5000A analyser as the difference between total carbon and inorganic carbon. Each sample was analysed in triplicate with errors less than 2%. Furthermore, the DOC was measured for a minimum of duplicate AOM samples grown on separate occasions. UV_{254} absorbance was measured using a Jenway 6505 UV/Vis spectrophotometer and SUVA was calculated as UV_{254}/DOC.

2.3.2. Zeta potential

ZP measurements were obtained using a Malvern ZetaSizer 2000 (Malvern, UK) which measures the electrophoretic mobility and converts this to ZP based on the Smoluchowski Approximation which is valid when $\kappa a \gg 1$. The AOM solution ZP was measured for concentrated solutions (5 times), which correspondingly had 5 times the ionic strength. Hence, $\kappa a \gg 1$ for AOM greater than 4 nm. ZP was measured across a pH range of 1–10. All analyses were obtained in triplicate.

2.3.3. Charge density

A solution containing a known amount of AOM, 1 mM NaH_2PO_4/Na_2HPO_4 pH 7 buffer, excess 6.2 meq l^{-1} low-molecular-weight PolyDADMAC (Sigma, UK) and the indicator *ortho*-Toluidene blue was back-titrated against −1 meq l^{-1} poly(vinylsulphate) sodium salt (Sigma, UK) to measure charge density (Kam and Gregory, 2001). Solutions were standardised using +1 meq l^{-1} cationic cetyl-trimethylammonium bromide (Sigma, UK). The point of neutralisation was observed by measuring UV_{635} absorbance using a Jenway 6505 UV/Vis spectrophotometer (coinciding with a colour change from blue to purple). The measurement was repeated for three different volumes of AOM.

2.3.4. Carbohydrate and protein analysis

The carbohydrate content was determined using the phenol–sulphuric acid method (Zhang et al., 1999). The modified Lowry method was used for protein analysis (Frølund et al., 1995). Glucose and bovine serum albumin (BSA) were used for calibration respectively at UV_{480} and UV_{750} absorbance using a Jenway 6505 UV/Vis spectrophotometer. Carbohydrate and protein measurements were performed on triplicate samples.

2.3.5. XAD resin fractionation

An XAD-7HP/XAD-4 column pair was used to fractionate AOM into hydrophobic and hydrophilic components as described by Malcolm and MacCarthy (1992). A 2 l AOM sample of approximately 10 mg l^{-1}, acidified to pH 2, was passed consecutively through the XAD-7HP and XAD-4 resins (resin volume was 60 ml in each 15 mm column). The non-retained sample comprised the hydrophilic fraction (HPI). Each column was back-eluted with NaOH (0.1 M, 120 ml) such that the XAD-7HP and XAD-4 resin back-effluent comprised the hydrophobic fraction (HPO) and transphilic fraction (TPI), respectively. Each fractionation was completed in duplicate. The DOC and carbohydrate content of all fractions was measured as previously described.

2.3.6. Molecular weight fractionation

Nitrogen gas at a constant pressure of 1 bar was used to drive the AOM solution through Biomax 500, 100, 30 and 10 and Ultracell PL-3 and PL-1 ultrafiltration membranes (Millipore, Billerica, MA, USA) using the Amicon Stirred Cell (Model 8400) in series such that AOM was fractionated into portions of >500, 100–500, 30–100, 10–30, 3–10, 1–3 and <1 kDa. The stirred cell was operated at 75 RPM and 60% permeate, with the exception of initial filtration through the 500 kDa membrane for which only 40% permeate was obtained. This was due to a gelatinous layer developing on the membrane surface at a throughput >50% specifically for MA-AOM. Each MW fractionation was conducted only in the stationary phase and repeated in triplicate.

2.3.7. Fluorescence spectroscopy

EEMs were obtained using a Cary Eclipse Fluorescence Spectrophotometer (Varian, Surry, UK) and a 4 ml, 1 cm path length cuvette. Emission spectra were scanned from 300 to 500 nm at 0.5 nm increments and excitation spectra scanned from 250 to 400 nm with 5 nm increments (Baker, 2002). The slits for excitation and emission were 5 nm and the PMT voltage was set at 725 V. Deionised water blanks were run every 4 analyses and the intensity of the Raman line of water at 350 nm excitation wavelength measured to monitor instrument stability. However, EEMs have been used to provide a qualitative rather than quantitative insight to the investigation, as has previously been shown to be useful (Her et al., 2004).

3. Results

3.1. DOC

Stationary phase DOC averaged 27±9.7, 18.0±2.3, 7.5±2.3 and 3.6±1 mg l^{-1} as C for CV-, MA-, AF- and Msp-AOM, respectively. However, given the widely varying cell concentrations, results were primarily normalised for cell number. This resulted in AOM concentration varying in orders of magnitude with species (Fig. 1a). For example, in the stationary phase the DOC released per cell increased in the order MA-<CV-<AF-<Msp-AOM with values 0.00095<0.0029<0.019<0.65 ng $cell^{-1}$ as C respectively. The DOC results were reported additionally in terms of DOC per cell surface area (Fig. 1b). In this instance, DOC released per cell surface area at the stationary phase was 0.00002< 0.000051<0.000053<0.0001 ng μm^{-2} for MA-, AF-, CV- and Msp-AOM, respectively. Hence, although *Melosira* sp. had the lowest absolute concentration of DOC, on a per cell and per surface area basis it released more AOM than the other species. Additionally, DOC associated with the green algae, *C. vulgaris*, was consistently higher in concentration than that of the cyanobacteria, *M. aeruginosa*, despite having a smaller surface area, as was found in a previous study (Hoyer et al., 1985).

Stationary growth phase DOC concentrations per cell were consistently higher than those observed for the exponential phase. For example, DOC measured in MA- and CV-AOM increased from 0.00071±5×10^{-5} and 0.00088±0.00037 ng $cell^{-1}$ to 0.0015±0.0009 and 0.0029±0.0013 ng $cell^{-1}$, respectively, although DOC of AF-AOM only increased marginally from 0.018 to 0.019 ng $cell^{-1}$. Absolute DOC values for CV-AOM increased by 18 mg l^{-1} from exponential to stationary phase which is consistent with the literature where a 20 mg l^{-1} increase was observed (Hoyer et al., 1985). It has been postulated that while organic matter is actively excreted during the exponential growth phase, much of the material remains bound at the surface of the cell, to be worn away during the stationary phase (Konno, 1993).

3.2. Zeta potential and charge density

The ZP of stationary phase AOM was demonstrated to decrease sharply between pH 1 and 3, reaching a plateau between pH 4 and 10. Isoelectric points (IEPs) for the AOM were 1.0, 1.8, 0.8 and 1.6 and the ZP stabilised at values of −21.5±0.94, −23.7±2.1, −33.3±3.5 and −15.9±1.7 mV for CV-, MA-, AF- and Msp-AOM, respectively. The AF-AOM and MA-AOM exponential growth phase ZP curves were similar to the stationary phase curves in that IEP values were 0.9 and 1.9, while average ZP values for pH 4–10 were −33.7±4.7 and −21.5±3.9 mV, respectively. However, the exponential phase ZP curve for CV-AOM was significantly different. From an IEP

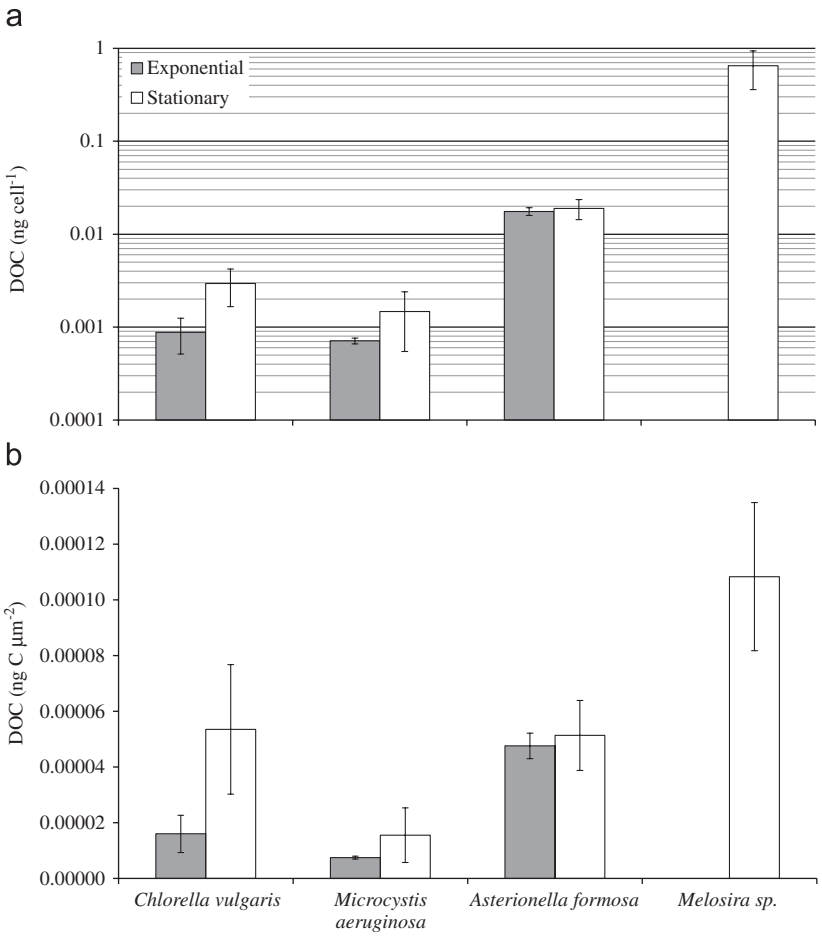

Fig. 1 – The DOC concentration for CV-AOM, MA-AOM, AF-AOM and Msp-AOM normalized: (a) by cell and (b) by surface area.

of 1.0, the ZP stabilised between pH 5–6 at −18.9 mV, before decreasing steeply from pH 6 to 10 to reach −35.3 mV. Overall, ZP values reported in the current study are comparable with the literature. For example, *Chlorella* had a relatively low ZP of between −17.4 and 19.8 mV (reported as −1.4 to −1.6 µmV s^{-1} cm^{-1}) independent of pH 4–10 (Edzwald and Wingler, 1990) and the ZP of the diatom *Nitzschia* was −28 mV in the stationary phase (Konno, 1993). The shape of the ZP curves relates to the ionisation of functional groups in the colloidal organics. In the case of the AOM reported here, the steep decrease observed between pH 1 and 4 can be attributed to the ionisation of carboxylic groups that are present in charged polysaccharides and proteins. Decreases at high pH values, such as that observed for the CV-AOM in the exponential growth phase, can similarly be attributed to the amino groups that have pK_a values of 9–11 (Lehninger, 1970).

During the exponential growth phase, the charge densities of CV-AOM and MA-AOM were 0.9 and 0.2 meq g^{-1} of C, whilst that of AF-AOM was negligible. An increase was observed for CV- and AF-AOM on transition to the stationary phase to 3.2 and 1.0 meq g^{-1} C, respectively. However, the charge density of MA-AOM decreased to 0.1 meq g^{-1} C (Fig. 2). Msp-AOM had a negligible charge density in the stationary phase. Charge density values obtained in the current study were comparable with literature values, for example, 3.1 and 2.3 meq g C^{-1} for AOM from *Dictyosphaerium* and *Pseudanabaena*, respectively (Bernhardt et al., 1985) and 0.5–1.8 meq g C^{-1} for *C. vulgaris*, *Scenedesmus quadricauda* and *Cyclotella* sp. (Paralkar and Edzwald, 1996). The charge density obtained for CV-AOM is comparable with those typically observed for NOM of 2.7–3.8 meq g^{-1} C (Sharp et al., 2005).

3.3. *Hydrophobicity—XAD resin fractionation and SUVA*

The AOM was largely hydrophilic (57% or more) for all species examined (Fig. 3), consistent with the presence of polysaccharides, sugars and hydroxy acids (Edzwald, 1993). Specifically, stationary phase MA- and Msp-AOM contained significantly more hydrophobic material at 30% and 32% in comparison to CV- and AF-AOM at 11% and 15%, respectively. On comparison of exponential and stationary growth phases, it was observed that the hydrophobic/hydrophilic proportions of MA- and AF-AOM remained relatively consistent, while the hydrophobic material of CV-AOM decreased from 24% to 11%, respectively. The transphilic proportion of the AOM varied from a minimum of 8% for Msp-AOM to a maximum of 17% for stationary phase CV-AOM. MA-AOM fractionation results compare well with a study investigating cyanobacteria AOM character (species not reported), where HPI and HPO results were 57% and 26%, respectively (Her et al., 2004).

The high hydrophilicity of the AOM as measured by the XAD-resin fractionation procedure was supported by SUVA

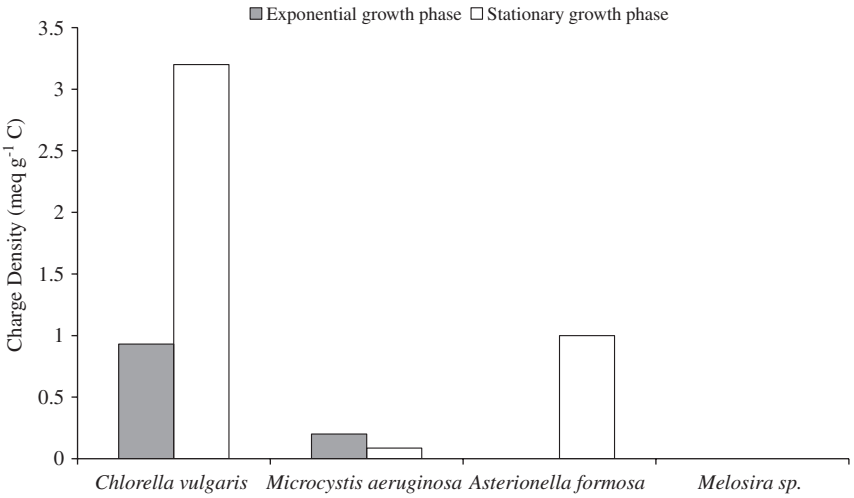

Fig. 2 – The charge density for each system in the stationary phase at pH 7.

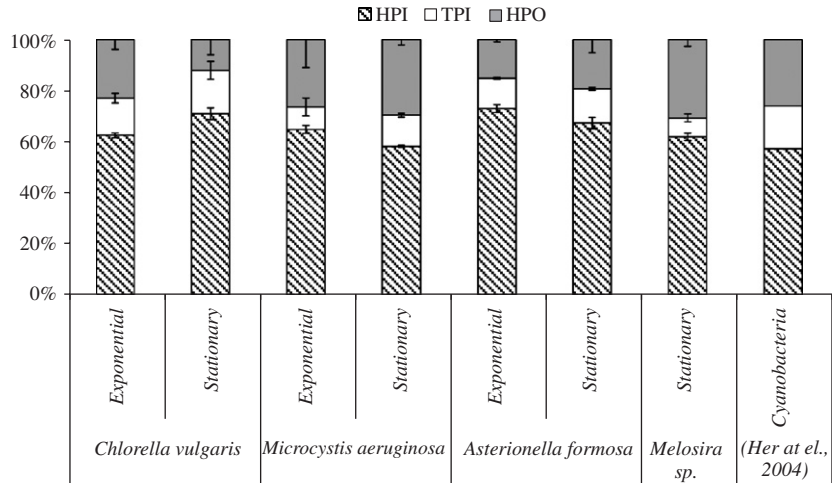

Fig. 3 – The proportion of AOM contained within hydrophilic (HPI), transphilic (TPI) and hydrophobic (HPO) fractions for *Chlorella vulgaris*, *Microcystis aeruginosa*, *Asterionella formosa* and *Melosira* sp. compared with that of cyanobacteria obtained using the same method in a separate study (Her et al., 2004).

results which similarly indicated the AOM was of a highly hydrophilic nature. SUVA values during the exponential growth phase were 1.29, 1.65 and 1.71 $l\,m^{-1}\,mg^{-1}$ for CV-, MA- and AF-AOM respectively while in stationary growth phases, it decreased to 0.54, 0.48 and 0.541 $l\,m^{-1}\,mg^{-1}$ for the same species and 0.581 $l\,m^{-1}\,mg^{-1}$ for Msp-AOM. Hence, regardless of the growth phase, the SUVA was consistent with a material of a very hydrophilic nature. The low SUVA is a result of the relatively low aromaticity associated with AOM such that it would not be expected to be highly absorbing (Hoyer et al., 1985). This correlates with larger-scale studies where increasing eutrophication and consequent DOC concentration coincided with a decrease in the SUVA (Cheng and Chi, 2003).

3.4. *Carbohydrates and proteins*

The stationary phase carbohydrate:DOC weight ratios were relatively similar at 1.1, 0.7, 1.0 and 0.8 $mg\,mg^{-1}$ as glucose:C for CV-, MA-, AF- and Msp-AOM, respectively; however, the protein:DOC ratio was more varied with values of 0.4, 0.64, 0.19 and 0.16 $mg\,mg^{-1}$ as BSA:C for the same systems (Fig. 4). Hence, it was protein concentration that was responsible for the variability observed in the protein:carbohydrate ratio of 0.4, 0.6, 0.2 and 0.2 $mg\,mg^{-1}$ as BSA:glucose for CV-, MA-, AF- and Msp-AOM. The exponential phase protein:carbohydrate ratios (not depicted here) were 0.58 and 0.31 $mg\,mg^{-1}$ for CV- and MA-AOM, respectively, demonstrating that while the amount of protein relative to carbohydrate decreased with age for CV-AOM, that of MA-AOM doubled. Protein:carbohydrate ratios for activated sludge were determined as 0.59 and 0.85 for a full-scale and lab process, respectively (Morgan et al., 1990), and are therefore similar to values obtained for MA-AOM in this study.

The proportion of total carbohydrates found in the HPO fraction was consistent irrespective of growth phase and species at between 9% and 17%, demonstrating that carbohydrates were predominantly hydrophilic or transphilic (Fig. 5). Proportions of carbohydrates found in the HPI fraction were

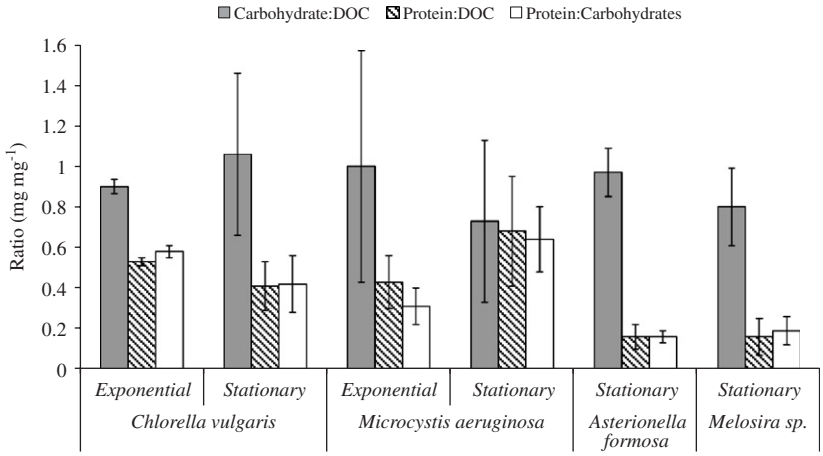

Fig. 4 – Carbohydrate:DOC, protein:DOC and protein:carbohydrate ratios for stationary phase CV-, MA-, AF-, and Msp-AOM.

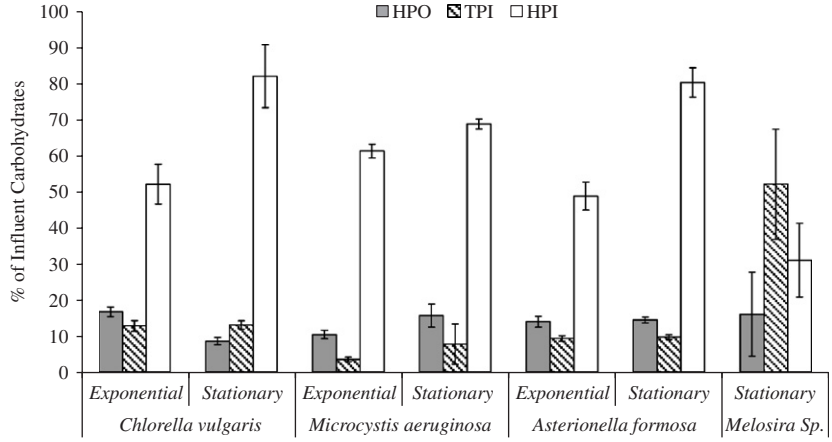

Fig. 5 – Percentage of total carbohydrates in the AOM present in the HPO, TPI and HPI fractions.

52%, 61%, and 49% in the exponential growth phase and 82%, 69% and 80% in the stationary phase for CV-, MA- and AF-AOM, respectively. The recovery of carbohydrates across the fractionation procedure varied from 72% to 82% in the exponential phase and 93–104% in the stationary phase, which suggests that some carbohydrate material in the exponential growth phase becomes irreversibly associated with the resin. This accounts for the difference observed between exponential and stationary phase carbohydrates. Hydrophilic compounds are described as neutral polysaccharides, low-MW mono- and di-carboxylic carboxylic acids and acidic sugars (Edzwald, 1993), thus supporting observations in the current study.

3.5. *Fluorescence EEM*

Fluorescence in the current study has been designated according to Coble (1996) as follows: Peaka T_1 and T_2 are tryptophan-like (protein-like); Peak A is humic-like; Peak B is tyrosine-like (protein-like); and Peak C is also humic-like. Tryptophan-like rather than humic/fulvic acid-like fluorescence dominated in all EEMs, with the exception of exponential phase CV-AOM (Fig. 6). This is common for microorganisms and has previously been observed for algae, such as the diatom *Nitzschia* (Determann et al., 1998), the green algae, *S. quadricauda* (Nguyen et al., 2005) and planktonic bacteria including *Pseudomonas aeruginosa* at culture ages of 168 h (Elliott et al., 2006). MA- and AF-AOM had comparable EEMs (Fig. 6). For example, in the exponential phase both had Peaks T_1 and T_2 maxima at $\lambda_{emission} = 340$ nm and $\lambda_{exitation} = 305$ nm (T_1) and 240 nm (T_2). On transition to the stationary phase the excitation wavelengths of Peaks T_1 and T_2 maxima decreased to 285 and 225 nm for MA-AOM and similarly to 280 and 230 nm for AF-AOM. Given that the solution environment remained stable in terms of composition, temperature and pH, this is attributable to a change in protein structure. Furthermore, in both cases the ratio of relative peak intensities decreased with age by six (T_1) and two (T_2) times for MA-AOM and 87 (T_1) and 55 (T_2) times for AF-AOM. This indicates that tryptophan-like substances were present in lower quantities during the stationary phase. The EEM of MA-AOM was similar to that obtained for cyanobacteria in previous studies as tryptophan-like fluorescence was detected; however, additional fluorescence was also detected in locations attributable to humic/fulvic-like substances (Her et al., 2003; Nguyen et al., 2005). Similar to AF-AOM, there was only very low intensity fluorescence at $\lambda_{emission} = 335$ nm and $\lambda_{exitation} = 225$ (T_2) for Msp-AOM,

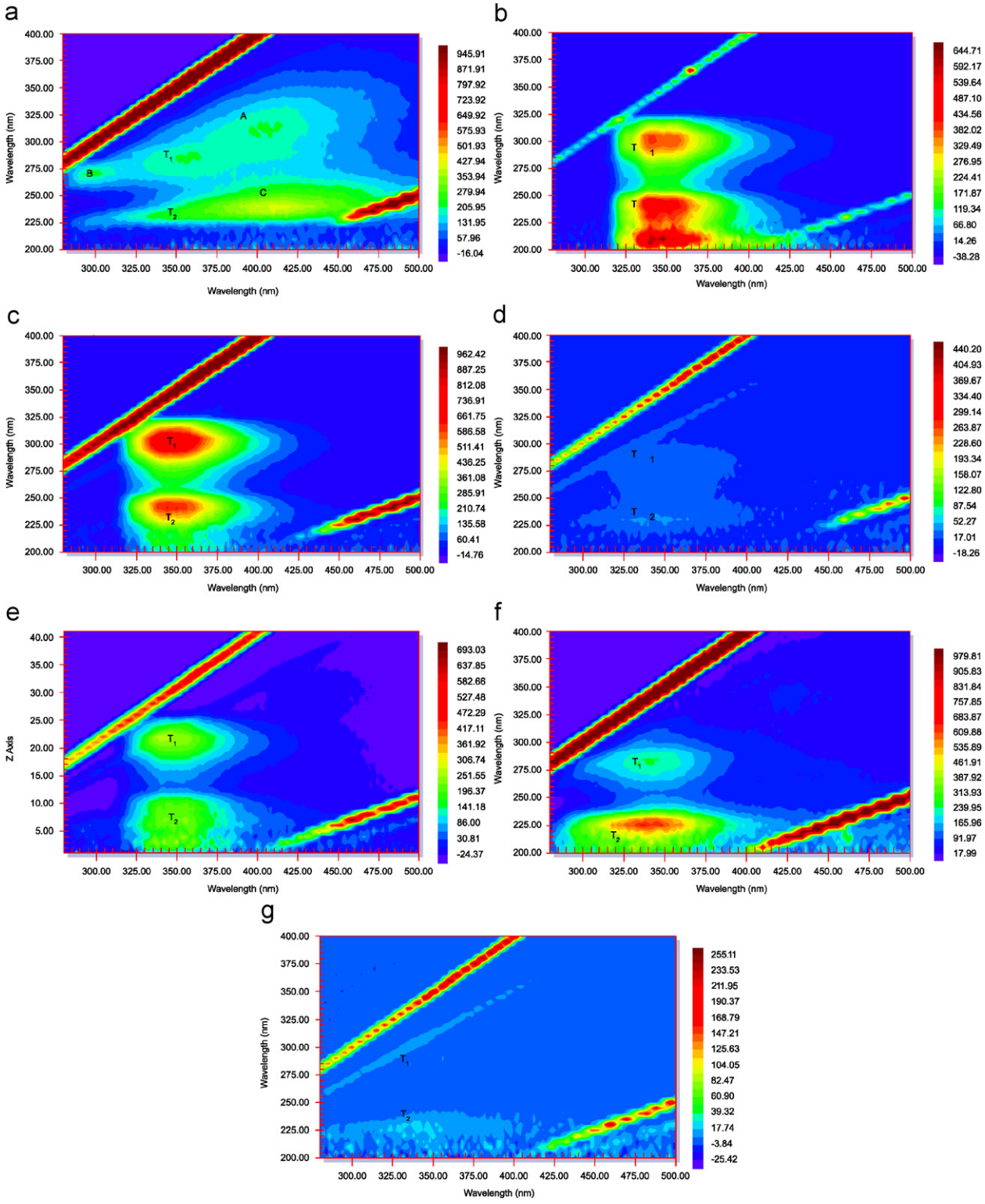

Fig. 6 – Examples of fluorescence excitation–emission matrix (EEM) spectra for (a) exponential CV-EOM; (b) stationary CV-EOM; (c) exponential AF-EOM; (d) stationary AF-EOM; (e) exponential MA-EOM; (f) stationary MA-EOM; and (g) stationary Msp-AOM. Z-axis = excitation (nm); X-axis = emission (nm); and Y-axis = intensity.

demonstrating that only low levels of tryptophan-like fluorescence was detected (Fig. 6g).

In contrast, the EEMs of CV-AOM were significantly different. Tryptophan-like, tyrosine-like and humic/fulvic acid-like fluorescence was detected in the exponential growth phase (Fig. 6a), where fluorescence centres were observed at $\lambda_{emission} = 350\,nm$ and $\lambda_{excitation} = 230\,nm$ (T_2) and 285 nm (T_1) in addition to humic/fulvic acid-like fluorescence at $\lambda_{emission} = 410\,nm$ and $\lambda_{excitation} = 240\,nm$ (C) and 320 nm (A). This was comparable to EEMs obtained in previous algae

characterisation studies (Her et al., 2003; Nguyen et al., 2005). However, only in the current study was tyrosine-like fluorescence observed (B). By the stationary phase, the fluorescence EEM had changed significantly (Fig. 6b). Specifically, there were no fulvic-acid-like peaks by this stage. However, the fluorescence observed was not consistent with that usually observed for tryptophan-like material given the detection of three peaks; although emission was still observed at 350 nm and excitation wavelengths of T_1 and T_2 were comparable to those obtained for exponential phase MA- and AF-AOM. Further work is required to elucidate current observations.

3.6. *Molecular weight fractionation*

The MW distribution of AOM was dependent on the species of algae. MA- and CV-AOM had bimodal distributions with 55% and 62% greater than 30 kDa and 38% and 30% less than 1 kDa, respectively (Fig. 7). Additionally, it was observed that within the high-MW portion (between 30 kDa and 0.7 μm in size—note that 20,000 kDa approximates to 0.1 μm), 46% of the MA-AOM was between 500 kDa and 0.7 μm compared to 5% of CV-AOM, demonstrating that MA-AOM was overall much larger than that of CV-AOM. The diatomic AF- and Msp-AOM were of smaller MW and not bimodal with 9% and 28% greater than 30 kDa, 16% and 22% between 1 and 30 kDa, and 81% and 53% less than 1 kDa, respectively. These observations are in agreement with Lüsse et al. (1985), who demonstrated bimodal distributions for stationary phase green algae including *Chlorella* sp., *Scenedesmus obliquus* and *Dictyosphaerium* sp. and the cyanobacteria, *Pseudanabaena catenata*, using 0.2 μm and <1.1 nm (~2 kDa) membranes. Similarly, the same study showed that AOM of MW <1.1 nm dominated for the diatom *Melosira granulata*.

Carbohydrate and protein analysis of the membrane permeate demonstrated that 78%, 77%, 81% and 62% of the total carbohydrates and 72%, 62%, 60.3% and 90.3% of the total proteins were in the range 30 kDa to 0.7 μm for CV-, MA-, AF- and Msp-AOM (Fig. 7). This demonstrates that much of the high-MW AOM is carbohydrates and proteins, suggesting that lower-MW material was dominated by smaller molecules not measured by either the carbohydrate or the protein methods.

4. Discussion

4.1. *Comparisons of AOM from different species and with NOM and EPS/SMP*

Irrespective of growth phase or species, AOM comprised more than 57% hydrophilic compounds, SUVA values of less than 2.0 l m^{-1} mg^{-1}, tryptophan-like fluorescence and similar carbohydrate-to-DOC ratios (Table 1) indicating that all AOM was dominated by compounds with low absorbance at 254 nm, including both hydrophobic proteins and hydrophilic polysaccharides (Edzwald, 1993). Interestingly, aromatic tryptophan-like proteins were not detected by SUVA. Hence, SUVA is only indicative of humic/fulvic type aromaticity. All AOM was negatively charged for pH 2–10 and with the exception of exponential phase CV-AOM, shared a similar ZP profile across the range pH 1–10 that was typical of systems dominated by carboxylic acid functional groups.

Comparisons of the different AOM revealed the major differences in character to be associated with charge density, hydrophobicity, protein:carbohydrate ratios and MW fractionation. Analysis across stationary phase AOM samples showed that increased charge density was related to lower hydrophobicity (Table 1). For example, CV-AOM had a charge density of 3.2 meq g^{-1} with a hydrophobicity of 11% whereas MA-AOM had a charge density of 0.1 meq g^{-1} with a hydrophobicity of 30%. This observation is inconsistent with previous knowledge concerning NOM where highly charged material has been associated with the hydrophobic humic acid fraction and fulvic acid fraction (Sharp et al., 2005). To illustrate, 8.8 and 1.0 meq g^{-1} were associated with the hydrophobic and hydrophilic components of NOM, respectively. However, the lack of humic/fulvic acid-like fluorescence (Table 1), with the exception of exponential phase CV-AOM, demonstrates that these compounds were not present in the AOM. In fact, the AOM was dominated by hydrophobic proteins and hydrophilic polysaccharides. The charge density of AOM occurs as a result of hydrophilic, charged polysaccharides including acetylamino sugars, sulphated sugars and carboxylated sugars (uronic acids) (Leppard, 1995) where the latter has been directly related to metal complexation capacity (Kaplan et al., 1988).

The lack of humic/fulvic material implies that proteins govern hydrophobicity in AOM systems. Indeed, one study determined that the HPO of EPS comprised predominantly protein and not carbohydrate components, as a result of amino acids with hydrophobic side groups (Jorand et al. (1998). Furthermore, it has been proposed that as the protein:carbohydrate ratio of EPS/SMP in biomass solutions increases, the charge density decreases (Morgan et al., 1990) and hydrophobicity increases (Jorand et al., 1998). In this study, MA-AOM and Msp-AOM had high hydrophobicity and low charge; however, their protein:carbohydrate ratios were very high and low, respectively. Hence, MA-AOM adheres to the correlation observed for EPS/SMP, while that of Msp-AOM does not, suggesting that the relationship is only valid for proteins excreted by bacteria, including cyanobacteria such as *M. aeruginosa*. In fact, increases in AOM hydrophobicity were more closely related to an increase in the ratio of high-MW proteins (>500 kDa) to carbohydrates, rather than the bulk protein content (Table 1). This observation is likely a result of more hydrophobic proteins tending to associate to form larger "gel-like" substances as a result of their hydrophobicity. Furthermore, proteins have been implicated as charge neutralisers as amino groups in some proteins carry positively charged groups that neutralise anionic functional groups, e.g., carboxylic acids, hence decreasing the net surface charge (Liao et al., 2001).

4.2. *Implications for water treatment*

Water that is characterised by a large hydrophilic portion and low SUVA (less than 3), such as the AOM in the current study, is generally assumed to have a low coagulant demand due to its low charge density and relatively low DOC removals can be anticipated on coagulation (Edzwald, 1993). One study

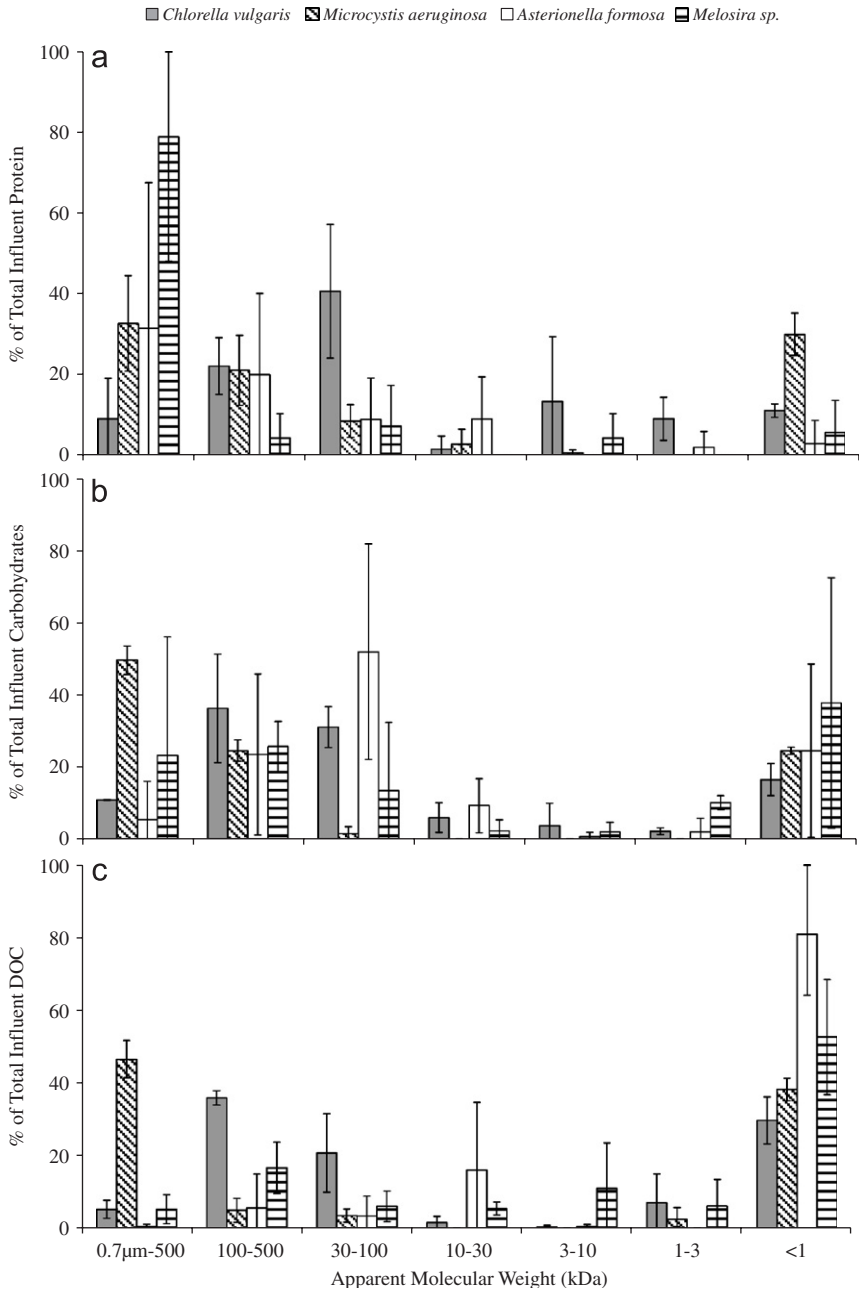

Fig. 7 – Molecular weight UF membrane fractionation results for (a) protein, (b) carbohydrate, and (c) DOC. Results are presented as the percentage of the total influent respective parameter.

demonstrated that the concentration of the hydrophilic component for NOM-dominated water could be used as a good indicator for the proportion of DOC that was untreatable (Sharp et al., 2005). However, as shown AOM and NOM exhibit very different properties. Crucially, the hydrophilic material in NOM tends to be uncharged whilst that of AOM can carry a significant proportion of the total charged load. The importance of such differences is that previously found predictive relationships are unlikely to hold for systems that contain AOM.

AOM levels from all algal species tested exceeded 2 mg L^{-1} at the cell concentrations commonly reported during blooms (Henderson et al., 2007) suggesting that AOM should have a significant influence on the coagulation process. CV- and MA-AOM was predominately of a large MW with concentrations of high-MW (>100 kDa) protein that equated to a coverage of 9–20 mg m^{-2}. Such levels are significantly higher than the 2.5 mg L^{-1} reported to provide high coverage of BSA on latex and thus inhibited coagulation (Tirado-Miranda et al., 2003). In contrast, the Msp- and AF-AOM was predominately below 10 kDa which is unlikely to extend beyond the double layer and so should mainly influence charge density and hence coagulant demand but not effect removal potential (Bernhardt et al., 1985).

Comparison with previous studies on algae-related treatment indicate that MA- and CV-AOM should produce the

Table 1 – Summary table of characterisation for each species in the exponential growth phase (EG) and stationary growth phase (SG)

	CV-AOM		MA-AOM		AF-AOM		Msp-AOM
	EG	SG	EG	SG	EG	SG	SG
SUVA ($l\,m^{-1}\,mg^{-1}$)	1.29	0.54	1.65	0.48	1.7	0.54	0.58
Isoelectric point	1.0	1.0	1.9	1.8	0.9	0.8	1.6
Hydrophilicity (%)	60	71	59	57	73	70	64
Hydrophobicity (%)	22	11	24	30	15	20	32
Charge density ($meq\,g^{-1}$)	0.9	3.2	0.2	0.1	Neg.	1.0	Neg.
Fluorescence EEMs peaks	T_1, T_2, A, B, C	T_1, T_2	T_1, T_2	T_1, T_2	T_1, T_2	T_1, T_2	T_1, T_2
Carbohydrate:DOC ($mg\,mg^{-1}$)	0.9	1.1	1.0	0.7	–	1.0	0.8
Trans-/hydrophilic carbohydrates (%)	65	95	64	77	58	90	83
Protein:DOC ($mg\,mg^{-1}$)	0.53	0.40	0.40	0.64	–	0.19	0.16
Protein:carbohydrate ($mg\,mg^{-1}$)	0.58	0.4	0.30	0.6	–	0.2	0.2
>500 kDa proteins:carbohydrate ($mg\,mg^{-1}$)	–	0.037	–	0.2	–	0.05	0.15
>500 kDa proteins:carbs/hydrophobicity	–	0.0034	–	0.0067	–	0.025	0.0047
AOM > 30 kDa (%)	–	62	–	55	–	9	30
AOM < 1 kDa (%)	–	30	–	38	–	81	53

Neg. = negligible.

higher THM levels up on chlorination due to there higher protein contents (Scully et al., 1988). Similar studies on nanofiltraton of algae have demonstrated higher-MW AOM to be the predominant foulant and as such greater operational problems could be expected than when treating the diatoms. Importantly, comparing the different treatment stages indicates that the varying character of AOM from the different algae means that each species will cause a unique set of challenges restricting the ability to generalise about the treatment of algae.

5. Conclusions

1. The following similarities were observed for all samples: (a) AOM was dominated by hydrophilic polysaccharides and hydrophobic proteins; (b) low SUVA values were exhibited for all species at both growth phases, signifying a lack of UV_{254} absorbing compounds, and indicating that aromatic tryptophan-like proteins were not detected by SUVA; and (c) all AOM had a negative ZP of between −15 and −35 mV for pH 4–10 with, i.e., p values of 1–2.
2. Two relationships were observed for AOM: (a) charge density was observed to decrease as hydrophobicity increased; and (b) increasing hydrophobicity was related to increasing proteins >500 kDa:carbohydrate ratio. The first was inconsistent with previous knowledge for NOM; while the second was similar to that reported for EPS/SMP systems. These observations are explained as follows: (a) charge density of AOM is attributable to hydrophilic, acidic carbohydrates and not hydrophobic, fulvic and humic acids as it is for NOM; (b) proteins of MW greater than 500 kDa govern hydrophobicity in the absence of humic/fulvic acids; and (c) hydrophobic proteins may neutralise some of the acidic groups thus reducing the charge density.

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REFERENCES

Baker, A., 2002. Fluorescence properties of some farm wastes: implications for water quality monitoring. Water Res. 36, 189–195.

Bernhardt, H., 1984. Treatment disturbances with water out of eutrophic reservoirs as a consequence of extensive algal development. Water Supply 2, SS4-7–SS4-15.

Bernhardt, H., Hoyer, O., Schell, H., Lusse, B., 1985. Reaction mechanisms involved in the influence of algogenic organic matter on flocculation. Z. Wasser. Abwass. For. 18 (1), 18–30.

Brookes, A., Judd, S., Reid, B., Germain, E., Smith, S., Alvarez, H., Le-Clech, P., Stephenson, T., Turra, E., Jefferson, B., 2003. Characterisation and impact of biomass foulants in membrane bioreactors. In: Proceedings of the Fifth International Membrane Science and Technology Conference, Sydney, 10–14 November 2003.

Cheng, W.P., Chi, F., 2003. Influence of eutrophication on the coagulation efficiency in reservoir water. Chemosphere 53, 773–778.

Coble, P.G., 1996. Characterisation of marine and terrestrial DOM in seawater using excitation–emission matrix spectroscopy. Mar. Chem. 51, 325–346.

Determann, S., Lobbes, J.M., Reuter, R., Rullkötter, J., 1998. Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. Mar. Chem. 62, 137–156.

Edzwald, J.K., 1993. Coagulation in drinking water treatment: particles, organics and coagulants. Water Sci. Technol. 27 (11), 21–33.

Edzwald, J.K., Wingler, B.J., 1990. Chemical and physical aspects of dissolved air-flotation for the removal of algae. Aqua 39, 24–35.

Elliott, S., Lead, J.R., Baker, A., 2006. Characterisation of the fluorescence from freshwater, planktonix bacteria. Water Res. 40, 2075–2083.

Fogg, G.E., 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. Bot. Mar. 26, 3–14.

Frølund, B., Griebe, T., Nielsen, P.H., 1995. Enzymatic activity in the activated-sludge floc matrix. Appl. Microbiol. Biotechnol. 43, 755–761.

Gregor, J.E., Fenton, E., Brokenshire, G., van den Brink, P., O'Sullivan, B., 1996. Interactions of calcium and aluminium ions with alginate. Water Res. 30 (6), 1319–1324.

Henderson, R.K., Chips, M., Cornwell, N., Hitchins, P., Holden, B., Hurley, S., Parsons, S.A., Wetherill, A., Jefferson, B., 2007. Experiences of algae in UK waters: a treatment perspective. Water Environ. J., in press, doi:10.1111/j.1747-6593.2007.00100X.

Her, N., Amy, G., McKnight, D., Sohn, J., Yoon, Y., 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. Water Res. 37 (17), 4295–4303.

Her, N., Amy, G., Park, H.-R., Song, M., 2004. Characterizing algogenic organic matter (AOM) and evaluating associated NF membrane fouling. Water Res. 38 (6), 1427–1438.

Hoyer, O., Lusse, B., Bernhardt, H., 1985. Isolation and characterisation of extracellular organic matter (EOM) from algae. Z. Wasser. Abwass. For. 18, 76–90.

Jorand, F., Boué-Bigne, F., Block, J.C., Urbain, V., 1998. Hydrophobic/hydrophilic properties of activated sludge exopolymeric substances. Water Sci. Technol. 37 (4–5), 307–315.

Kam, S.-K., Gregory, J., 2001. The interaction of humic substances with cationic polyelectrolytes. Water Res. 35 (15), 3557–3566.

Kaplan, D., Christiaen, D., Arad, S., 1988. Binding of heavy metals by algal polysaccharides. In: Stadler, T., Karamanas, Y., Mollion, J., Morvan, H., Mollion, J., Christiaen, D. (Eds.), Algal Biotechnology. Elsevier, London, pp. 179–187.

Konno, H., 1993. Settling and coagulation of slender type diatoms. Water Sci. Technol. 27 (11), 231–240.

Lehninger, A.L., 1970. Biochemistry: The Molecular Basis of Cell Structure and Function. The John Hopkins University, Worth Publishers, Inc., New York, pp. 67–75.

Leppard, G.G., 1995. The characterisation of algal and microbial mucilages and their aggregates and their aggregates in aquatic ecosystems. Sci. Tot. Environ. 165, 103–131.

Liao, B.Q., Allen, D.G., Droppo, I.G., Leppard, G.G., Liss, S.N., 2001. Surface properties of sludge and their role in bioflocculation and settleability. Water Res. 35 (2), 339–350.

Lüsse, B., Hoyer, O., Soeder, C.J., 1985. Mass cultivation of planktonic freshwater algae for the production of extracellular matter (EOM). Z. Wasser. Abwass. For. 18, 67–75.

Malcolm, R.L., MacCarthy, P., 1992. Quantitative evaluation of XAD-8 and XAD-4 resins used in tandem for removing organic solutes from water. Environ. Int. 18, 597–607.

Morgan, J.W., Forster, C.F., Evison, L., 1990. A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. Water Res. 24 (6), 743–750.

Myklestad, S.M., 1995. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. Sci. Tot. Environ. 165, 155–164.

Nguyen, M.-L., Westerhoff, P.E., Baker, L., Hu, Q., Esparza-Soto, M., Sommerfield, M., 2005. Characteristics and reactivity of algae-produced dissolved organic carbon. J. Environ. Eng. 131 (11), 1574–1582.

Paralkar, A., Edzwald, J.K., 1996. Effect of ozone on EOM and coagulation. J. Am. Water Works Assoc. 88 (4), 143–154.

Scully, F.E., Howell, G.D., Kravitz, R., Jewell, J., 1988. Proteins in natural waters and their relation to the formation of chlorinated organics during water disinfection. Environ. Sci. Technol. 22 (5), 537–542.

Sharp, E.L., Parsons, S.A., Jefferson, B., 2005. Coagulation of NOM: linking character to treatment. Water Sci. Technol. 53 (7), 67–76.

Tirado-Miranda, M., Schmitt, A., Callejas-Fernández, J., Fernández-Barbero, A., 2003. The aggregation behaviour of protein-coated particles: a light scattering study. Eur. Biophys. J. 32 (2), 128–136.

Zhang, X., Bishop, P.L., Kinkle, B.K., 1999. Comparison of extraction methods for quantifying extracellular polymers in biofilms. Water Sci. Technol. 39 (7), 211–218.