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Characterisation of transparent exopolymer particles (TEP) produced during algal bloom: a membrane treatment perspective

L.O. Villacorte[a,b,c,*], Y. Ekowati[a], H. Winters[d], Gary L. Amy[a,c,d], J.C. Schippers[a], M.D. Kennedy[a,c]

[a]*UNESCO-IHE, Institute for Water Education, Westvest 7, 2611 AX, Delft, The Netherlands*
Email: l.villacorte@unesco-ihe.org
[b]*Wetsus, Center of Excellence for Sustainable Water Technology, Agora 1, 8934 CJ, Leeuwarden, The Netherlands*
[c]*Delft University of Technology, Stevinweg 1, 2628 CN, Delft, The Netherlands*
[d]*Water Desalination and Reuse Research Center, 4700 King Abdullah University of Science and Technology, Thuwal, Saudi Arabia*

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ABSTRACT

Algal blooms are currently a major concern of the membrane industry as it generates massive concentrations of organic matter (e.g. transparent exopolymer particles [TEP]), which can adversely affect the operation of membrane filtration systems. The goal of this study is to understand the production, composition and membrane rejection of these organic materials using different characterisation techniques. Two common species of bloom-forming freshwater and marine algae were cultivated in batch cultures for 30 days and the productions of TEP and other organic matter were monitored at different growth phases. TEP production of the marine diatom, *Chaetoceros affinis*, produced 6–9 times more TEP than the freshwater blue-green algae, *Microcystis*. The organic substances produced by both algal species were dominated by biopolymeric substances such as polysaccharides (45–64%) and proteins (2–17%) while the remaining fraction comprises of low molecular weight refractory (humic-like) and/or biogenic organic substances. MF/UF membranes mainly rejected the biopolymers but not the low molecular weight organic materials. MF membranes (0.1–0.4 μm) rejected 42–56% of biopolymers, while UF membranes (10–100 kDa) rejected 65–95% of these materials. Further analysis of rejected organic materials on the surface of the membranes revealed that polysaccharides and proteins are likely responsible for the fouling of MF/UF systems during an algal bloom situation.

Keywords: Algal bloom; Algal organic matter; Transparent exopolymer particles (TEP); MF/UF rejection; MF/UF fouling

1. Introduction

The presence of algae in water sources poses a major challenge for drinking and industrial water treatment [1–3]. These micro-organisms seasonally generate high concentrations of biomass (thousands to millions of cells/ml) and organic substances which can adversely affect the operation of several membrane-based water treatment plants, mainly because of

*Corresponding author.

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fouling problems. Recently, there have been some alarming cases of harmful algal blooms (HABs) in several desalination hotspots in the world, causing a major concern in the membrane industry [4,5].

Most algal blooms produce massive concentrations of transparent exopolymer particles (TEP). These biopolymeric substances are mainly made up of acidic polysaccharides released by algae [28]. TEP were recently identified as potential causes of biological fouling in reverse osmosis (RO) systems and organic fouling in ultrafiltration (UF) systems [6–13]. Because they are mainly invisible, they have been overlooked for many years by the membrane industry. Although the negative effects of TEP on membrane filtration have already been demonstrated in recent studies [10–13], the relative abundance of these materials in relation to the total organic matter produced by algae during algal bloom is still largely unknown. Moreover, TEP and their colloidal precursors are poorly studied in terms of their removal by MF/UF membranes. The aim of this study was to apply different characterisation techniques to further understand the production, composition and membrane rejection of TEP and other organic substances produced by common species of bloom-forming algae in marine and freshwater systems.

2. Materials and methods

Two common species of algae were selected for this study to simulate an algal bloom situation in fresh and saline surface water sources. Algal organic matter (AOM) produced by two species of algae was extracted from algal cultures and a series of analyses were performed to identify their composition and rejection by MF/UF membranes.

2.1. Algal culture

Two strains of algae were acquired from the Culture Collection of Algae and Protozoa (CCAP; Oban, Scotland): *Chaetoceros affinis* (1010/27) and *Microcystis* sp. (1450/13). *C. affinis* (CA) was inoculated in sterilized synthetic seawater containing nutrients and trace elements based on the $f/2+Si$ medium for diatoms. The synthetic seawater (SSW) was prepared to resemble the typical ion composition in seawater (TDS 34 g/L and pH 8 ± 0.2). *Microcystis* sp. (MSp) was grown in sterilised BG-11 medium for cyanobacteria. Both algal cultures were incubated at 20 ± 2°C room temperature under an artificial light source (fluorescent lamp) at 12/12 h light/dark regime and continuous slow mixing condition over a shaker. Light intensity was set at 40–50 and 10–15 μmol/m^2 s for CA and MSp cultures, respectively. The cell concentration was regularly measured using a counting chamber and a light microscope every 2–4 days to monitor their growth.

2.2. AOM extraction and membrane rejection

About 0.5 L of samples was collected during the exponential and stationary/death growth phases of the cultures for characterisation analyses. To separate AOM from the algae cells, the gently mixed samples were filtered through polycarbonate filters (Whatman Nuclepore PC membranes) by vacuum filtration at <0.2 bar. To collect as much AOM materials as possible, membrane with different pore sizes were used for each species depending on the minimal size of the algal cells: 5 μm PC for CA and 1 μm PC for MSp. For rejection experiments of AOM, membranes with different pore sizes were used, namely: 0.4 and 0.1 μm PC (Whatman) and 100 kDa polyethersulfone (PES, Pall) and 10 kDa regenerated cellulose (RC, Millipore). All membranes were soaked for 24 h and/or flushed with milli-Q water to remove any organic artefacts from the membrane before filtration. Permeate water samples were collected for further analyses.

2.3. Characterisation techniques

2.3.1. TEP staining and measurement

TEP were identified and measured by staining with a cationic dye Alcian Blue (AB) based on the method developed by Passow and Alldredge [14]. The stain specifically binds with anionic carboxylated and sulphated acidic polysaccharides retained on 0.4 μm PC filters. The staining solution was prepared from 0.025% (m/v) Alcian Blue 8GX (Standard Fluka) in acetic acid buffer solution (pH 2.5) and pre-filtered through 0.05 μm PC filter prior to TEP staining. Since AB coagulates when in contact with saline water, a rinsing procedure was added to the original method by filtering 1 ml of ultrapure water through the TEP gels prior to staining in order to replace the residual saline water adsorbed to it. No calibration with standard polysaccharides was performed. Hence, TEP concentrations were relatively presented in terms of abs/cm/L.

2.3.2. Total organic carbon

Total organic carbon (TOC) was measured by using a Shimadzu TOC-V_{CPN} TOC analyser. TOC was measured on selected days during the algal growth period. To keep the TOC samples free from algal cells,

water samples were first filtered through 5 μm and 1 μm PC membranes by mild vacuum filtration (−0.2 bar) for CA and MSp, respectively. The filter and filter holder were intensively flushed with ultrapure water to remove organic contaminants before sample filtration.

2.3.3. Liquid chromatography–organic carbon detection

Water samples extracted from algal cultures were analysed using liquid chromatography–organic carbon detection (LC–OCD) at DOC-Labor (Karlsruhe, Germany). The relative responses of organic carbon, ultraviolet and organic nitrogen at different retention times were measured with an online organic carbon detector (OCD), UV detector (UVD) and organic nitrogen detector (OND), respectively. Concentrations of biopolymers, humic substances, building blocks, low molecular weight (LMW) acids and neutrals were measured in terms of organic carbon based on size exclusion chromatography [15]. The chromatogram results were processed on the basis of area integration using a customised software program CHROMCalc (DOC-Labor, Karlsruhe). Since TEP are large macromolecules, LC–OCD analysis was performed without 0.45 μm pre-filtration. The theoretical maximum chromatographable size without sample pre-filtration is 2 μm based on the pore size of the sinter filters of the column (S. Huber, pers. com.).

Theoretically, biopolymers excreted by algae are mostly TEP. Hence, more attention was focused on this fraction. Biopolymers in surface waters are consisting mostly of polysaccharides and proteins. Protein concentration was estimated by assuming that all organic nitrogen which were detected by the organic nitrogen detector (OND) between 25 and 42 min retention time (biopolymer peak) were all bound to proteinic compounds. Most of the known protein compounds contain 14.5–17.5% of nitrogen and 49.7–55.3% of carbon [16]. Thus, the C:N ratio of proteinic biopolymers was assumed to be 3:1. Polysaccharide concentrations (C_{PS}) were calculated by subtracting the calculated protein concentration (C_{PR}) from the organic carbon concentration of biopolymers (C_{BP}).

$$C_{PS} = C_{BP} - C_{PR} \quad [\text{mg-C/L}]$$

$$C_{PR} = 3N_{BP} \quad [\text{mg-C/L}]$$

where N_{BP} is the organic nitrogen content of biopolymers (mg-N/L).

2.3.4. Fluorescence excitation-emission matrix

Fluorescence excitation-emission matrix (F-EEM) spectra were measured by using a FluoroMax-3 spectrofluorometer (HORIBA Jobin Yvon, Inc., USA) with a 150 W ozone-free xenon arc-lamp as a light source for excitation. The F-EEM measurements were performed at the excitation wavelength ranging from 240 to 450 nm with 10 nm increments and the emission wavelength ranging from 290 to 500 nm with 2 nm increments. The slit widths were set to 5 nm for excitation and emission. TOC concentration of the samples was diluted to 1 mg/L before the measurement. The three-dimensional spectrum data series from F-EEM were processed with MatLab R2007b to plot figures with contour lines. Background signals were minimised by subtracting the signals of the blank from the sample EEMs. The typical peaks which can be expected within the limits of the EEM spectra (Ex. 240–250 nm/Em. 290–500 nm) are given in Table 1.

For each samples, the fluorescence index (FI) was calculated based on the ratio of the fluorescence intensity at Em 500 nm/Ex 450 nm and Em 500 nm/Ex 370 nm [17]. The FI between 1.7 and 2.0 indicates that the fluorescent organic materials are autochthonous (microbial origin) while the FI between 1.3 and 1.4 indicates they are allochthonous (terrestrial origin).

Table 1
Typical EEM peaks of natural organic matter [22,27]

Code	Description	Fluorescence range	
		Excitation	Emission
H1	Humic-like primary peak	330–350	420–480
H2	Humic-like secondary peak	250–260	380–480
Hm	Marine humic-like	300–330	400–420
Pty	Protein-like (tyrosine) peak	270–280	300–320
Ptr	Protein-like (tryptophan) peak	270–280	320–350

2.3.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was used to identify the types of functional groups present on the surface of clean and fouled membranes. For this study, an attenuated total reflectance–FTIR Spectrum 100 instrument (PerkinElmer) was used to measure the infrared spectrum on the surface of flat sheet UF membranes before and after rejection experiments with extracted AOM from algal cultures.

3. Results and discussion

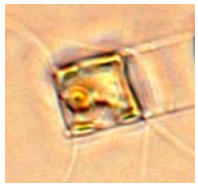
3.1. Algal growth and TEP production

The two species of bloom-forming algae, *C. affinis* (CA) and *Microcystis* Sp. (MSp), were selected for this study because they are ubiquitous in seawater and freshwater, respectively. Both micro-organisms belong to two major groups of phytoplankton (diatoms and cyanobacteria) responsible for several cases of HABs [18]. Typical characteristics of the two algae species are presented in Table 2.

CA is a marine diatom known to release massive concentrations of extracellular polysaccharides in the ocean including TEP [19]. In natural waters, this species usually thrive during the spring season. High concentration of this microalga can cause yellowish brown discolouration of the water. Algal cells resemble an oval cylinder geometric shape ranging from 7–30 μm in size. The cells tend to form chains where they are connected at the origin of the two setae located on both ends of the cell. In this study, a batch culture of this species was inoculated with a starting concentration of 200,000 cells/ml. The increase in cell concentration was very slow for the first seven days followed by very rapid growth until day 17 when the peak concentration of 875,000 cells/ml was reached (Fig. 1). Immediately after that, death growth phase commenced which is characterised by a rapid decrease of live cells down to <200,000 cells/ml in just seven days. The exponential growth phase apparently occurred between day 6 and day 13. Although there was no obvious indication of stationary growth phase, it was estimated to have occurred between day 13 and day 23, but for certainty, this was indicated as the stationary-death phase. The rapid decrease of algae after day 17 can be attributed to depletion of essential nutrients needed by diatoms to grow such as inorganic carbon, phosphate, nitrogen and silicon. The latter is an important limiting nutrient for diatoms as they are encased in frustules made up of silicates [20].

MSp is a common species of freshwater cyanobacteria (blue–green algae) ubiquitous in lakes, rivers and reservoirs during the summer seasons. They are known to generate high concentrations of extracellular polymeric substances during a bloom situation [3,21]. In this study, *Microcystis* bloom was simulated in a batch culture by inoculating about 2,000,000 cells/ml in BG11 medium. Rapid growth of algae started immediately after inoculation, followed by slight decrease of growth rate as it nears the peak of the bloom (day 13;

Table 2
Characteristics of bloom-forming algae investigated in this work

	C. affinis	*Microcystis* Sp.
		
Strain	CCAP 1,010/27	CCAP 1,450/13
Type	Diatom	Blue–green algae/cyanobacteria
Geometric shape	Oval cylinder	Sphere
Typical dimensions	Diameter = 7–30 μm Height = 10–30 μm	Diameter = 3–6 μm
Cell surface area	~1,400 μm^2	~50 μm^2
Cell volume	~3,800 μm^3	~34 μm^3
Water discolouration	Yellowish brown	Bluish green
Natural habitat	Seawater	Fresh surface waters
Typical blooming period	Spring season	Summer season

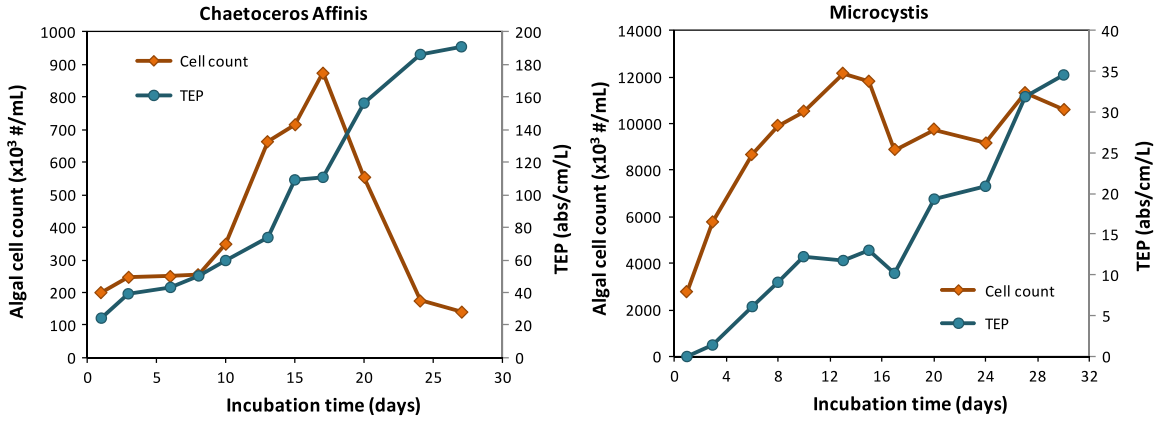


Fig. 1. Growth curve and TEP production of *C. affinis* and *Microcystis* sp.

~12,000,000 cells/ml). Afterwards, algal concentrations were slightly decreased and then slightly recovered. Based on the growth curve, exponential growth phase was between day 1 and day 8, while the stationary growth phase was after day 10. Unlike CA, the MSp batch culture did not manifest an apparent death phase within the 30 days of monitoring period. This is an indication that MSp was less sensitive than CA to nutrient limited conditions during the peak of the bloom.

Both CA and MSp produced significant concentrations of TEP (Fig. 2). The cumulative TEP produced by CA was about 6–9 times higher than MSp (Fig. 1). For CA, TEP increase coincided with the increase in cell concentration during the lag and exponential growth phases and continued to increase more rapidly during the stationary-death phase. TEP accumulation in MSp culture also increased with increasing cell concentration during the exponential growth phase, followed by very slow increase at the start of the stationary growth phase and then a rapid increase as more cells started to die. This means that in the latter phase intracellular TEP materials were also released. In general, TEP were produced by both actively growing (exponential growth phase) and nutrient limited cells (stationary/death phase). About 40% and 65% of total accumulated TEP were produced during the stationary/death phase of CA and MSp cultures, respectively. This may indicate that CA extracellularly produced TEP and not just released during deterioration of dead cells. On the contrary, actively growing and not nutrient limited MSp cells poorly produced TEP but release more of these materials when they were under nutrient limitation. Moreover, compromised or deteriorating cells during the stationary-death phase of MSp may have released more intracellular materials to the TEP pool.

3.2. Characterisation of organic matter generated during algal bloom

Although the organic materials released by the two species of algae were largely TEP, other forms of

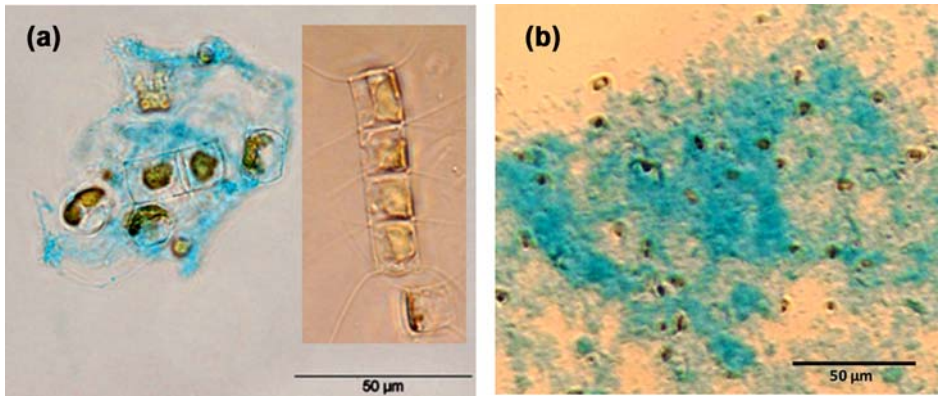


Fig. 2. Alcian blue stained TEP and algal suspension from batch cultures of CA (a) and MSp (b).

organic matter were also generated. These materials together with TEP are collectively known as AOM. AOM from two species of algae was investigated using different characterisation techniques to identify the main components that make up TEP and other organic materials that the algae produced.

3.2.1. Total organic carbon

The bulk organic matter in terms of TOC produced by the two species of bloom-forming algae was monitored during the different phases of algal growth. Since TOC was measured in algal cell free samples (see Section 1.3.2), the organic matter measured was mostly extracellular organic matter and not from cell wall or intracellular materials. The release of intracellular organic matter through rupture or autolysis of cells during filtration was assumed to be minimal because algae cells were removed by mild vacuum filtration [14] and not by centrifugation [3].

AOM productions of actively growing algae during the exponential growth phase were estimated based on the rate of increase in TOC per day. The rate of AOM release was normalised in terms of cell concentration by dividing the average increase in TOC per day with the average increase of cell concentration. In addition, the rate of release was also estimated per cell surface area and cell volume for better comparison of the two species. The estimated rate of release per cell of CA was 3.89 pgC/cell/day, which was about 100 times higher than MSp (0.04 pgC/cell/day). However in terms of cell surface area, the rate of release of CA was only about 4 times higher than MSp while this was relatively similar if the rate was calculated per cell volume (Fig. 3). Although the rate of release of CA and MSp was relatively similar in terms of biovolume, the absolute increase in AOM was still significantly higher for CA because it has much higher (~6 times) maximum biovolume than MSp. This was largely in agreement with the TEP production of the two species, indicating that TEP are major component of the AOM. It was not possible to estimate the rate of AOM release during the stationary growth phase because the growth rate of the algae was either irregular or negative.

3.2.2. Liquid chromatography–organic carbon detection

LC–OCD has been considered as a reliable tool for characterising natural organic matter in fresh and marine waters [15]. In this study, this technique was used to determine the types of organic matter that made up the AOM from CA and MSp cultures. Organic carbon detector chromatograms of selected samples of AOM extracts at different growth phases and the culture media (blank) are shown in Fig. 4.

For CA, two major peaks were observed, namely: biopolymers (25–40 min) and low molecular weight (LMW) acids (45–55 min). Some minor peaks were also observed for building blocks and LMW neutrals. The blank culture medium on which the alga was inoculated showed a similar acid peak which was quite stable at different growth phases. This signal was found to have originated from the chelating agent (EDTA) which was added to the medium to minimise precipitation of metals. Discounting the latter would indicate that CA produced AOM that were mainly biopolymers (e.g. polysaccharides and proteins) with low concentrations of building blocks (LMW humics) and LMW organic neutrals (weakly and uncharged organic compounds). The concentration of these AOM fractions has increased significantly between the exponential and stationary-death growth phases by about 95, 50 and 40% for biopolymers, building blocks and LMW neutrals.

The OCD chromatograms of MSp samples showed four peak signals: biopolymers (28–42 min), building blocks (42–51 min) and LMW acids and humic sub-

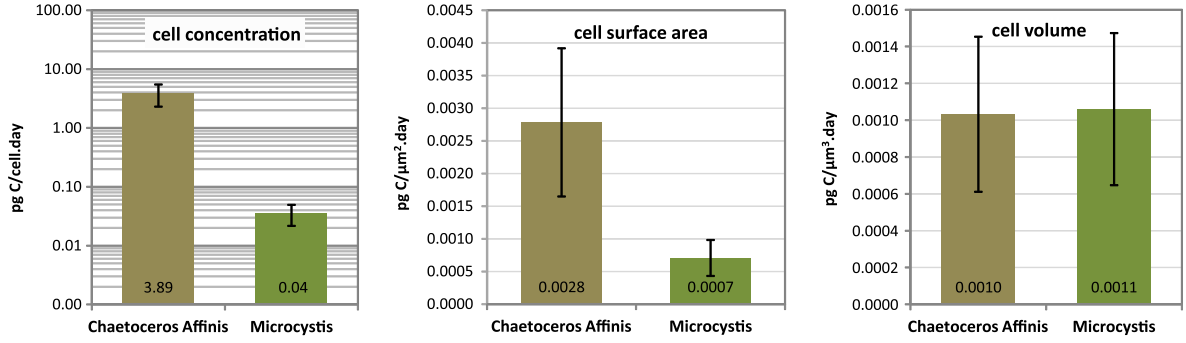


Fig. 3. Rate of extracellular release of organic carbon in CA and MSp cultures during the exponential growth phase.

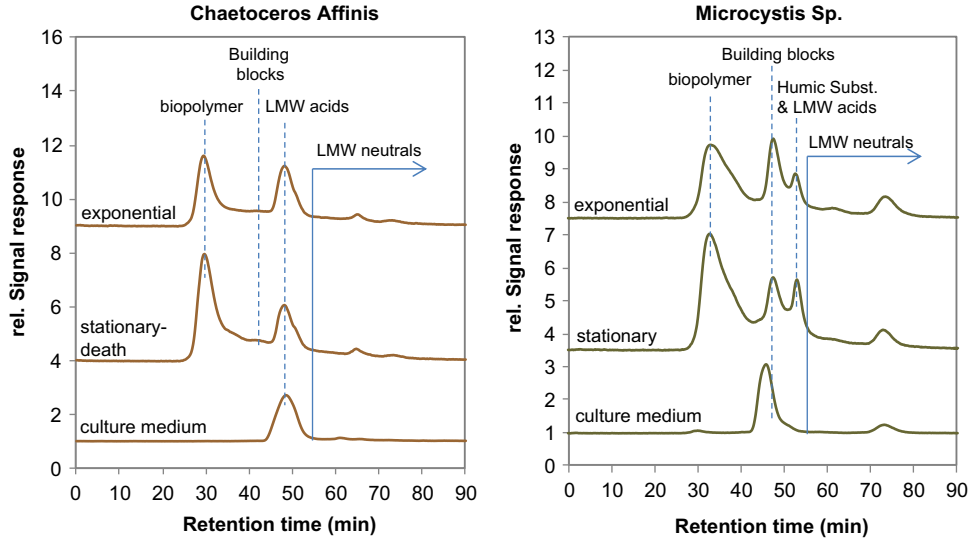


Fig. 4. LC-OCD chromatograms of blank culture media and AOM from CA and MSp at different growth phases.

stances (51–55 min). Contrary to CA, the LMW acid peak for MSp was mainly comprised of lower molecular weight humic substances based on the UV signal peak that was observed at similar retention time. The medium OCD chromatogram showed a peak (42–53 min) near the building block peak of MSp. This peak was attributed to the presence of citric acid (a metal solubilising agent) in the culture medium which may have been transformed to building blocks presumably by algal activities. Assuming that the building block peak originated entirely from the medium, MSp excreted mainly biopolymers and low concentrations of humic substances, LMW organic acids and LMW organic neutrals. The relative increase of each fraction between the exponential and stationary growth phases was about 150, 180, 220 and 240% for biopolymers, humic substances, LMW neutrals and LMW acids, respectively. These clearly indicate that majority of the accumulated AOM from MSp were produced during the stationary phase. The significant increase of low molecular weight organics is also indication of autolysis or deteriorating algal cells contributed significantly to the AOM pool during this phase.

In terms of relative abundance, biopolymers (62–70%) were the main constituents of the AOM produced by CA and MSp at any of the growth phases (Fig. 5). This AOM fraction was further divided into polysaccharides and proteins. For CA, protein concentration was estimated to be around 14% of the total AOM while polysaccharides ranged from 50 to 57%. For MSp, protein substances were 2–17% of AOM while polysaccharides were between 45 and 64%. For

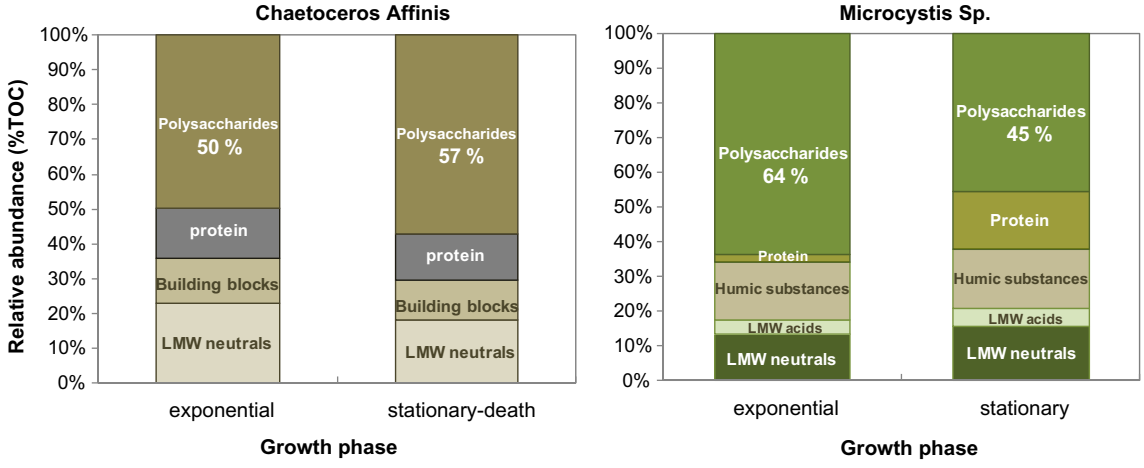


Fig. 5. The relative abundance of different fractions of the AOM released by CA and MSp at different growth phases.

CA, the low molecular weight organic fractions (building blocks and organic neutrals) were about 36% of the AOM during the exponential phase while it decreased to 29% during the stationary-death phase. This fractional decrease was attributed to higher production of biopolymers during the stationary-death phase when the algae were under nutrient limitation. Although MSp also produced more biopolymers during the stationary growth phase, the relative abundance of the low molecular weight fraction in the AOM pool has increased from 34% during the exponential phase up to 38% during the stationary phase. This was due to the higher production of humic substances, LMW acids and LMW neutrals during the latter phase. The protein fraction recorded the most significant increase for MSp during the stationary phase (from 2 to 17%) possibly as a result of the reaction of algae to nutrient limitation or the release of proteins bound to the cell wall and interior of dying and deteriorating algal cells.

3.2.3. Fluorescence excitation-emission matrix

The EEM spectra of the AOM released by the two species of algae were analysed to verify their composition. The analyses were performed with and without pre-filtration of samples to assess if AOM materials which were loosely and/or tightly bound to algal cells have different compositions than the one that were free (unbound). All previous studies include pre-filtration of samples through 0.45 μm filters to remove particulate materials that may interfere in the analysis (e.g. [3,22]). However, large biopolymers like TEP are mostly in the particulate size range, so they were excluded in these studies. The EEM spectra of filtered and unfiltered samples (Fig. 6) showed similarities in terms of the identified peak signals of the different AOM fractions, but with remarkable difference in fluorescence response. The fluorescence from the chlorophyll-a (Ex. 425 nm/Em. 680 nm) and chlorophyll-b (Ex.470 nm/Em.650 nm) of algal cell walls were not expected to influence the results because their

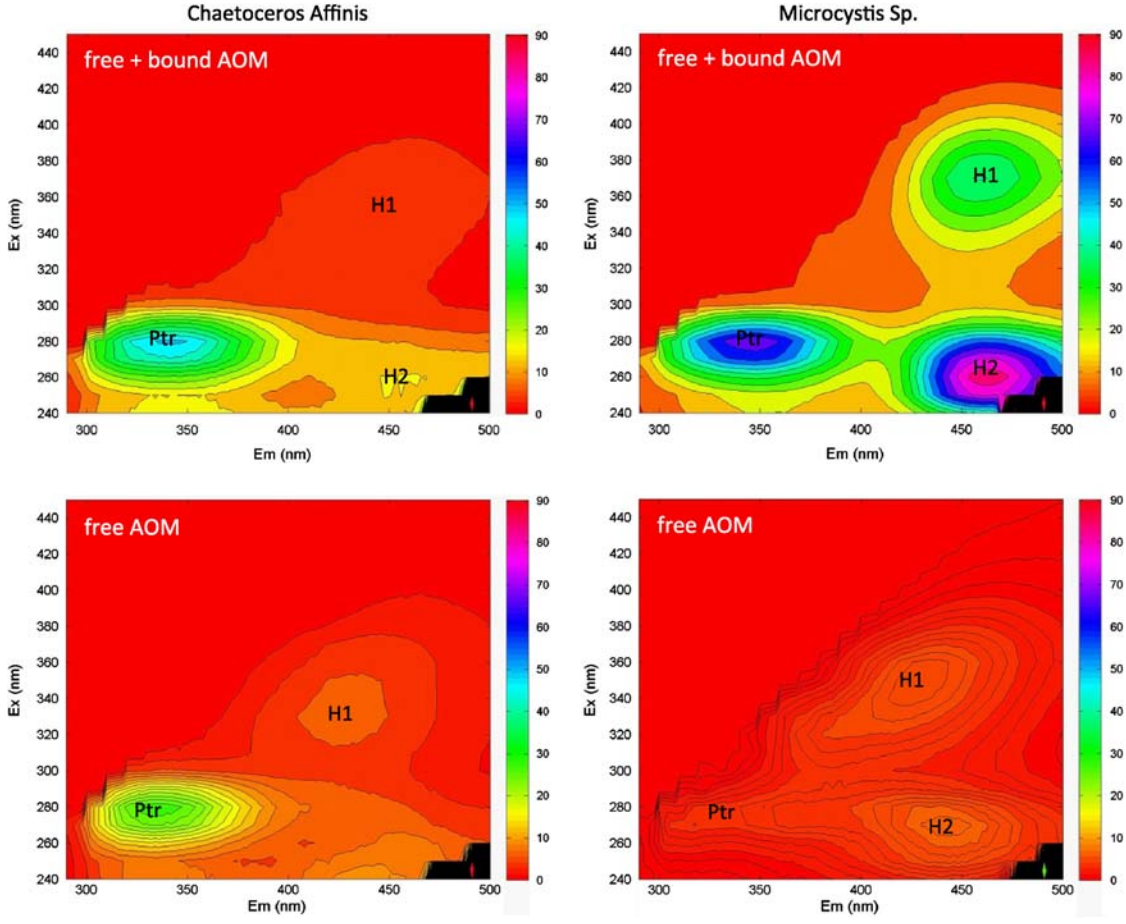


Fig. 6. Typical EEM spectra of unfiltered (total AOM) and filtered (free AOM) samples from CA and MSp.

EEM spectra are outside the range investigated in this study. Thus, the presence of algal cells in unfiltered water samples was assumed to have minimal interference in the analysis as the EEM spectra still showed the typical spectra of the target AOM fractions similar to pre-filtered samples. The peak responses for unfiltered samples were referred to as the relative response of total AOM (free and bound to cells), while the peak signals for the filtered samples were referred to as the free AOM. Since polysaccharides are non-fluorescent organic compounds, the focus of this analysis was to check the presence of protein-like and humic-like materials in the AOM pool.

The AOM fractions identified in the EEM spectra for CA and MSp were protein-like (Ptr) and humic-like substances (H1 and H2). For CA, majority of the fluorescence responses originated from protein-like (tryptophan) materials which were about 3–7 times more than the humic-like materials (Table 3). Around 40–55% and 35–80% of the fluorescence response were attributed to free protein-like and free humic-like materials, respectively. This means that around half of the AOM materials were either loosely or tightly bound to the algal cell or have formed aggregates larger than 5 μm. The latter is a likely scenario considering that TEP aggregates of 100s of micrometers in size were reported during diatom blooms [23]. In all the identified peaks, a significant increase of fluorescence response was observed between the exponential and stationary-death phases for both total and free AOM. The FI of the AOM was between 1.7 and 2.0, confirming their autochthonous origin.

For MSp, humic-like responses were comparable to that of protein-like (tryptophan) materials for total AOM while the fluorescence response was dominated mainly by humic-like materials for the free AOM. The free AOM spectra were similar to what was reported in *hypereutrophic* waters [24] and *Microcystis aeruginosa* cultures [21,25]. Between 5 and 15% of the fluorescence response of the total AOM from MSp were attributed to free AOM. This may indicate that a huge majority of the protein-like and humic-like materials were loosely or tightly bound to or aggregated with the algal cells. The latter may also include free AOM but were entrapped by these aggregates during the pre-filtration process. Both free and total AOM consistently increased between exponential and stationary phases (Table 3). This was contrary to a previous study of MSp where a decrease of fluorescence response for the tryptophan peak was observed [3]. The possible reason for the discrepancy is that the previous study analysed the samples after centrifugation and filtration through 0.7 μm pore size filters, while the current study analysed the samples with (through 1 μm PC) and without pre-filtration. As more AOM where produced during the stationary phase, more aggregates were formed and less free AOM can be extracted through a 0.7 μm pore size filter. This might be a testament of how important it is to consider the particulate fraction of AOM, specifically the TEP-like materials.

3.3. Rejection of AOM by MF/UF membranes

The removal of the different AOM components, including TEP and their precursors, by MF/UF membranes was investigated using the different analytical techniques applied in the characterisation studies. Moreover, FTIR spectroscopy was also performed to analyse the rejected AOM on the surface of the membrane. AOM solutions from CA and MSp extracted during the stationary-death phase were filtered through membranes with various pore sizes and residual AOM concentrations of the permeate water were measured to calculate membrane rejection.

Rejections of AOM in terms of TOC varied widely with membrane pore sizes and algal species (Fig. 7). Almost half of AOM (44%) from CA were rejected by

Table 3
EEM peak signals (Raman units) of the AOM fractions released by CA and MSp at different growth phases

EEM characteristic	AOM fraction	Peak location	CA		MSp	
		Excitation/emission	Exponential	Stationary	Exponential	Stationary
Protein-like	Total	280/336–348	41.9	51.3	43.9	70.7
	Free	270–280/318–334	16.5	29.0	1.8	3.5
Humic-like I	Total	360–370/444–460	6.0	8.3	25.7	42.4
	Free	330–360/430–442	3.2	6.8	3.9	5.6
Humic-like II	Total	250–270/448–472	12.4	17.3	51.9	88.2
	Free	250–270/448–473	4.5	9.5	5.1	6.5
Fluorescence index (FI)	Total	–	1.8	1.7	1.5	1.7
	Free	–	2.0	2.0	2.6	2.1

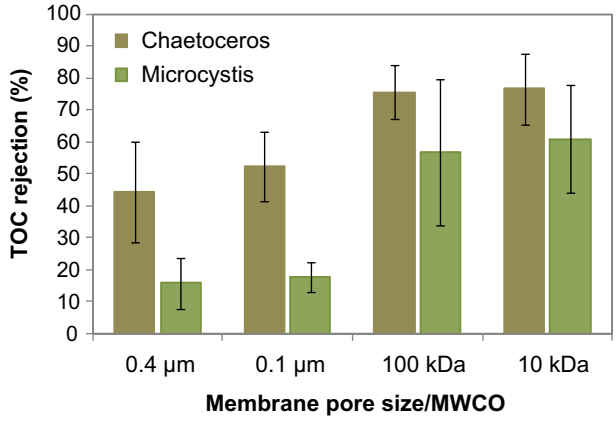


Fig. 7. AOM rejection in terms of TOC by MF/UF membranes.

0.4 µm membrane. Rejection increased to 52, 76 and 77% when filtered through 0.1 µm, 100 kDa and 10 kDa membranes, respectively. In contrast, rejection of AOM from MSp was rather low for 0.4 µm (16%) and 0.1 µm (18%) while higher rejection was observed for 100 kDa (57%) and 10 kDa (61%) membranes. Based on rejection with different pore size membranes, the apparent size of AOM from MSp was smaller than the AOM from CA. This is an indication that UF membranes are likely to be more fouled by AOM from CA than MSp but at the same time, this also mean that UF is less effective pre-treatment when the source of AOM in the water is MSp.

Since UF is not expected to remove the dissolved fraction of AOM, TOC analysis is not a comprehensive technique to understand AOM membrane rejection. Hence, further specific rejection analysis was performed using LC–OCD. OCD chromatograms showed significant removal of AOM biopolymers by MF/UF membranes, while low molecular weight organics (e.g. building blocks, humics and organic acids) were not or poorly rejected in the filtration processes (Fig. 8). One remarkable difference between the biopolymer peak of CA–AOM over MSp–AOM is the retention time. The biopolymers from CA (30 min) eluted ahead of biopolymers from MSp (34 min) which confirms their larger apparent size.

A comparison of the rejection of biopolymers by various MF/UF membranes is shown in Fig. 9. Rejections of biopolymers from CA were 47, 56, 83 and 95% by 0.4 µm, 0.1 µm, 100 kDa and 10 kDa membranes, respectively. For biopolymers from MSp, membrane rejections were 42, 52, 65 and 83% by 0.4 µm, 0.1 µm, 100 kDa and 10 kDa membranes, respectively. Since majority of biopolymers produced by both species during the stationary-death phase were polysaccharides (70–85%), rejected biopolymers were dominated by these macromolecules. Rejection of polysaccharides from CA increased with decreasing pore size of the membranes while protein removals were relatively similar. For MSp, membrane rejection of both polysaccharides and proteins increased with decreasing membrane pore size.

Although biopolymers are usually much larger than 20 kDa [15], about 5% of biopolymers from CA and 17% of biopolymers from MSp were not rejected by 10 kDa membranes. This may be due to the wide pore size distribution of the RC membranes. However, biopolymers like TEP mostly originate from nanogels as small as 3 kDa [26] which means that biopolymers from algae could be much smaller than 10 kDa. The actual pore size of the membrane was expected to decrease as more rejected biopolymer materials accumulate on its pores. As the cake/gel layer build-up and compressed on the surface of the membrane, it

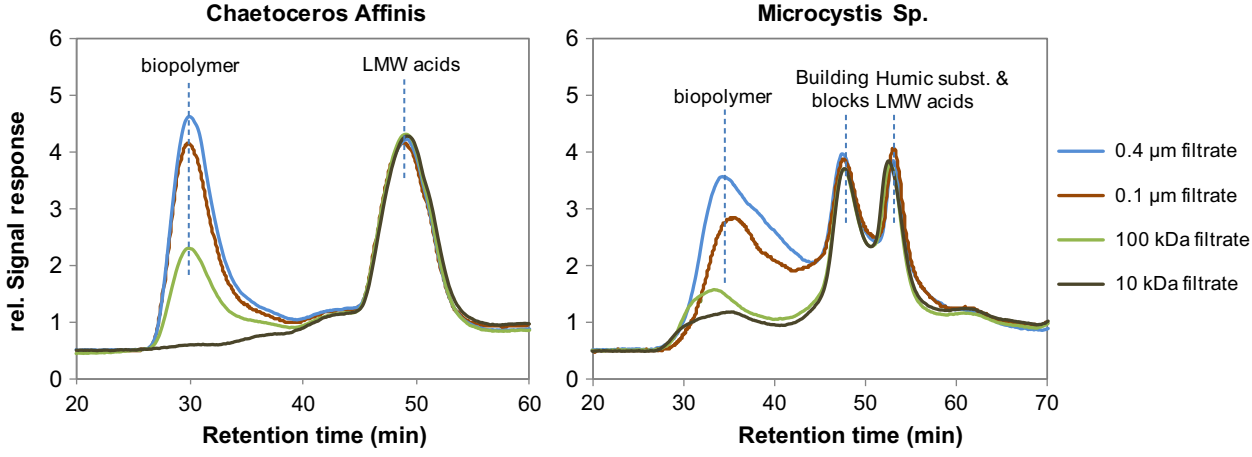


Fig. 8. OCD chromatograms of filtrates of various MF/UF membranes feed with AOM from CA and MSp.

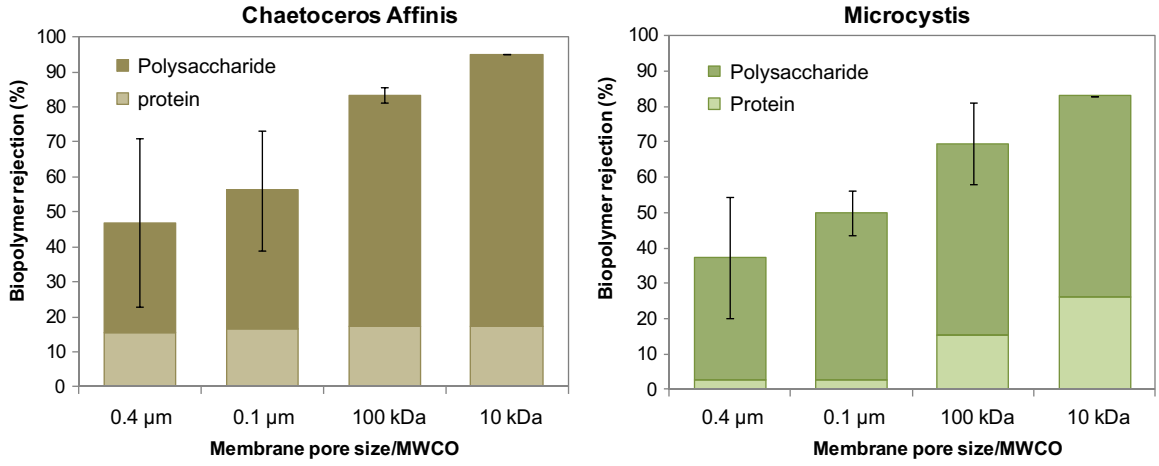


Fig. 9. Rejection of biopolymers from CA and MSp by various MF/UF membranes.

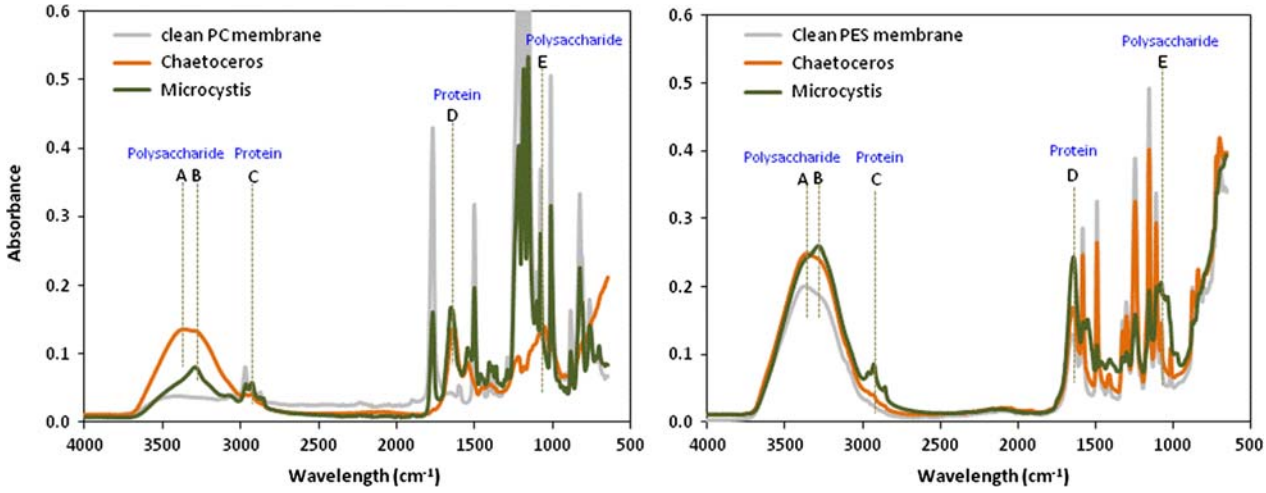


Fig. 10. FTIR spectra of AOM from CA and MSp rejected by 0.4 µm PC and 100 kDa PES membranes.

becomes an additional barrier and may reject more biopolymers than the membrane itself. On the other hand, TEP and other biopolymers are fibrillar and flexible in nature that it might squeeze through the membrane pores smaller than their apparent size [26]. Hence, the rejections of biopolymers by MF/UF membranes are governed by several factors which include concentration of biopolymers in the bulk solution as well as the characteristics of both the membrane and biopolymer themselves.

The rejected AOM on the surface of MF/UF membranes were verified by analysing the FTIR spectra of fouled membranes used in rejection studies in comparison with clean membranes (Fig. 10). The spectra of fouled and clean membranes were notably different indicating that the surface chemistry of the membrane has changed with increasing accumulation of AOM. Three distinct IR peaks ($A = 3{,}365\,cm^{-1}$, $B = 3{,}290\,cm^{-1}$ and $C = 2{,}930\,cm^{-1}$) and two similar IR peaks ($D = 1{,}655\,cm^{-1}$ and $E = 1{,}080\,cm^{-1}$) were indentified from the rejected AOM of two species that were not associated with the surface chemistry of the membranes. Peaks *A* and *B* correspond to the O–H stretching and peak *E* corresponds to the C–O stretching of alcohol components associated with polysaccharides. Peak *C* was related to the O–H stretching of dimeric carboxylic acids likely associated with protein-like substances. Peak *D* was related to NH_3^+ bending of amino acids from proteins.

The FTIR spectra showed that both polysaccharides (*A*, *B*, *E*) and proteins (*C*, *D*) from CA and MSp were rejected by 0.4 µm PC and 100 kDa PES membranes. The FTIR results were in agreement with a previous study by Lee et al. [24] of colloidal organic

matters in surface waters largely impacted by bloom-forming algae. Hence, the AOM investigated in this study may represent the characteristics of AOM in natural waters, their treatability by MF/UF membranes as well as their potential role in the fouling of these membranes.

4. Conclusions

This study investigated the production, composition and MF/UF rejection of organic matter from marine and freshwater bloom-forming algae using different analytical techniques. The following are the main findings:

(1) Simulated blooms of laboratory cultured algae showed that *C. affinis* (marine diatom) released about six times more TEP than *Microcystis* Sp. (freshwater blue-green algae). The former produced more TEP during the exponential growth phase while the latter released most of the TEP during the stationary phase.
(2) The rate of organic matter release of non-nutrient limited algae was higher for CA than MSp in terms of TOC per cell (~100 times) and per cell surface area (~4 times). However, the release rate was relatively similar in terms of TOC per biovolume because of the differing cell sizes of the two species.
(3) The AOM from CA and MSp species comprised mostly of biopolymers (62–70%) and some low molecular weight organic substances (e.g. humics, organic acids and neutrals). It was estimated that >70% of the biopolymers were polysaccharides, while the rest were identified as proteinic substances. Significant concentrations of refractory compounds (e.g. humic substances) were also identified in the AOM of MSp, most of which were tightly or loosely bound to algae cells.
(4) Rejections of AOM by MF/UF membranes (10 kDa-0.4 μm) vary with membrane pore sizes and algal species. AOM from CA was better rejected (44–77%) by MF/UF membranes than those from MSp (16–77%) mainly because of their larger size. The membrane rejection of AOM was mainly dependent on the removal of polysaccharides (20–97%) and proteins (22–95%) considering that the low molecular weight fractions of AOM were poorly rejected by MF/UF membranes. FTIR spectroscopy analyses verified the accumulation of rejected polysaccharides and proteins on the surface of the membranes.

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