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Characterisation of algal organic matter produced by bloom-forming marine and freshwater algae

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ABSTRACT

Algal blooms can seriously affect the operation of water treatment processes including low pressure (micro- and ultra-filtration) and high pressure (nanofiltration and reverse osmosis) membranes mainly due to accumulation of algal-derived organic matter (AOM). In this study, the different components of AOM extracted from three common species of bloom-forming algae (*Alexandrium tamarense*, *Chaetoceros affinis* and *Microcystis* sp.) were characterised employing various analytical techniques, such as liquid chromatography – organic carbon detection, fluorescence spectroscopy, fourier transform infrared spectroscopy, alcian blue staining and lectin staining coupled with laser scanning microscopy to indentify its composition and force measurement using atomic force microscopy to measure its stickiness. Batch culture monitoring of the three algal species illustrated varying characteristics in terms of growth pattern, cell concentration and AOM release. The AOM produced by the three algal species comprised mainly biopolymers (e.g., polysaccharides and proteins) but some refractory compounds (e.g., humic-like substances) and other low molecular weight acid and neutral compounds were also found. Biopolymers containing fucose and sulphated functional groups were found in all AOM samples while the presence of other functional groups varied between different species. A large majority (>80%) of the acidic polysaccharide components (in terms of transparent exopolymer particles) were found in the colloidal size range (<0.4 μm). The relative stickiness of AOM substantially varied between algal species and that the cohesion between AOM-coated surfaces was much stronger than the adhesion of AOM on AOM-free surfaces. Overall, the composition as well as the physico-chemical characteristics (e.g., stickiness) of AOM will likely dictate the severity of fouling in membrane systems during algal blooms.

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1. Introduction

The presence of algae in freshwater and marine surface water sources may cause serious challenges in drinking and industrial water production. Microscopic algae in surface water seasonally proliferate and generate varying concentrations of organic substances (Kirchman et al., 1991). These can cause water discolouration, anoxic conditions, odour and toxicity problems as well as operational problems in water treatment processes (Petry et al., 2007; Caron et al., 2010; Henderson et al., 2008a; Schurer et al., 2012; Villacorte et al., in pressa,b).

Organic substances produced by algae may comprise various forms and differing concentrations of polysaccharides, proteins, lipids, nucleic acids and other dissolved organic substances (Fogg, 1983; Bhaskar and Bhosle, 2005; Decho, 1990; Myklestad, 1995). Depending on the type or species of algae, AOM components are either produced through extracellular release in response to low nutrient stress or other stressful conditions (e.g., unfavourable light, pH or temperature), invasion by bacteria or viruses and/or through disruption and decay of algal cells (Fogg, 1983; Leppard, 1993; Myklestad, 1999). However, several species of algae may also release these organic materials under normal conditions (Fogg, 1983). Myklestad (1995) highlighted the significance of studying extracellular polysaccharides as they may comprise more than 80% of algal organic matter (AOM). In seawater, algal-derived acidic polysaccharides have been reported to be highly sticky and likely involved in the coagulation of colloidal/particulate materials in aquatic environments, which may result in the formation of mucilaginous aggregates such as marine snow and sea foam (Alldredge and Silver, 1988; Mopper et al., 1995). These sticky materials are often referred to as transparent exopolymer particles (TEP; see review by Passow, 2002).

Some AOM components, specifically TEP, were recently identified as potential causes of biological fouling in reverse osmosis (RO) membrane (Berman and Holenberg, 2005; Villacorte et al., 2009a,b; Bar-Zeev et al., 2012, 2015) and organic fouling in ultrafiltration (UF) membrane systems (Kennedy et al., 2009; Villacorte et al., 2010a,b; Berman et al., 2011; Schurer et al., 2013; Discart et al., 2014). However, their composition and characteristics which may be associated with membrane fouling are still not extensively investigated, especially for marine systems.

Over the years, different analytical techniques have been applied to characterise natural organic matter in aquatic systems, some of which have been adopted to specifically investigate the composition of algal-derived organic matter but mostly in freshwater (Fang et al., 2010; Qu et al., 2012; Pivokonsky et al., 2014; Qu et al., 2014; Her et al., 2004; Henderson et al., 2008b; Zhou et al., 2014; Chu et al., 2015; Lee et al., 2006; Mecozzi et al., 2001; Li et al., 2014). These techniques include conventional particulate/dissolved organic carbon, specific ultraviolet absorption (SUVA), protein and polysaccharide concentration measurements but also advanced techniques such as size exclusion chromatography (SEC), fluorescence spectroscopy and fourier transform infrared spectroscopy. However, latest staining techniques coupled with optical and advanced microscopy has not been (extensively) explored so far for AOM characterisation. For instance, staining with fluorochrome labelled lectins coupled with laser scanning microscopy to visualise and indentify carbohydrate components of extracellular polymeric substances has been extensively applied in microbial biofilms (Neu, 2000; Zippel and Neu, 2011). Lectins are proteins extracted from various organisms which can bind specifically to one or more carbohydrate functional groups. Furthermore, advancement in atomic force microscopy (AFM) offers possibility of characterising the stickiness and elasticity of high molecular weight AOM (e.g., biopolymers). Such application been applied to measure the adhesive strength of polysaccharides on polymeric membranes (Yamamura et al., 2008; Frank and Belfort, 2003) and the elastic character of diatom mucilages (Higgins et al., 2002).

Since naturally occurring algal blooms are often unpredictable, a better understanding of the physico-chemical characteristics of AOM produced by common species of bloom-forming algae in both fresh and saline waters is a significant step towards developing an effective strategy to minimise their adverse effects in membrane-based water treatment systems. The objective of this study is to investigate the composition and physico-chemical characteristics of AOM from three common species of bloom-forming algae in marine and freshwater sources by applying a wide range of characterisation techniques including liquid size exclusion chromatography, fluorescence spectroscopy, fourier transform infrared spectroscopy, staining techniques coupled with optical and laser scanning microscopy and force measurement using an atomic force microscope.

2. Materials and methods

2.1. Algal cultures

Three selected algal species were grown in batch cultures to represent an algal bloom situation in freshwater and seawater. Three strains were acquired from the Culture Collection of Algae and Protozoa (SAMS, Scotland), namely: *Alexandrium tamarense* (CCAP 1119/32), *Chaetoceros affinis* (CCAP 1010/27) and *Microcystis* sp. (CCAP 1450/13). *A. tamarense* and *C. affinis* were inoculated in sterilised synthetic seawater spiked with nutrients and trace elements based on the L1 and f/2 + Si medium, respectively. The artificial seawater (ASW) was prepared to resemble the typical ion composition of the North Sea (TDS 34 g/L, pH 8 ± 0.2), excluding the organic components. *Microcystis* was grown in sterilised BG-11 medium for freshwater algae. The composition of the prepared media and ASW is shown in the Supplementary data S1. All algal batch cultures were incubated at 20 ± 2 °C with an artificial light source (fluorescent lamps) at 12/12 h light/dark regime. Light intensities of the fluorescent lamps were adjusted to 40–50 μmol m^{-2} s^{-1} for *A. tamarense* and *C. affinis* cultures and 10–15 μmol m^{-2} s^{-1} for *Microcystis*. *C. affinis* and *Microcystis* cultures were continuously mixed on a shaker table while *A. tamarense* was mixed manually 1–2 times per day.

The average algal cell concentrations in the batch cultures were monitored by sampling every 2–4 days and counting the