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The impact of interactions between algal organic matter and humic substances on coagulation

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ABSTRACT

This study focuses on the effects of molecular interactions between two natural organic matter (NOM) fractions, peptides/proteins derived from cyanobacterium *Microcystis aeruginosa* (MA proteins) and peat humic substances (HS), on their removal by coagulation. Coagulation behaviour was studied by the jar tests with MA protein/HS mixtures and with single compounds (MA proteins or HS). Aluminium sulphate was used as a coagulant. Besides MA proteins, bovine serum albumin (BSA) was used as a model protein. For the MA protein/HS mixture, the removal rates were higher (80% versus 65%) and the dose of coagulant substantially lower (2.8 versus 5.5 mg L^{-1} Al) than for coagulation of single HS, indicating the positive effect of protein-HS interactions on the coagulation process. The optimum coagulation pH was 5.2–6.7 for MA proteins and 5.5–6 for HS by alum. The optimum pH for the removal of MA protein/HS mixture ranged between pH 5.5–6.2, where the charge neutralization of negatively charged acidic functional groups of organic molecules by positively charged coagulant hydroxopolymers lead to coagulation. MA proteins interacted with HS, probably through hydrophobic, dipole–dipole and electrostatic interactions, even in the absence the coagulant. These interactions are likely to occur within a wide pH range, but they result in coagulation only at low pH values (pH < 4). At this pH, the negative charge of both MA proteins and HS was suppressed due to the protonation of acidic functional groups and thus the molecules could approach and combine forming aggregates. Virtually the same trends were observed in the experiments with HS and BSA, indicating that BSA is a suitable model for MA proteins under experimental conditions used in this study. The study showed that increases in organic content in source water due to the release of algae products may not necessarily entail deterioration of the coagulation process and a rise in coagulant demand.

1. Introduction

Natural organic matter (NOM) which occurs in water reservoirs is mainly present in two forms: (i) allochthonous NOM of terrestrial origin dominated by humic substances (HS, mostly humic and fulvic acids) and (ii) autochthonous NOM, including mainly compounds derived from algae and cyanobacteria, i.e. algal organic matter (AOM). As well as HS, AOM has received increasing attention as it can cause either aesthetic concerns (i.e., colour, taste and odour) or undesirable health effects associated with disinfection by-products (DBPs) (Lui et al., 2011; Li et al., 2012), cyanobacterial toxins (Harada, 2004) and other toxic compounds contained in NOM (Ghernaut et al., 2011). Moreover, AOM may instigate serious problems in drinking water treatment processes, especially during the decline phase of an algal bloom, when high concentrations of cellular organic matter, consisting mainly of saccharide-like and protein-like substances, are released into the source water (Zhang et al., 2010; Nicolau et al., 2015). Though the two forms of organic matter, HS and AOM, can be present together in surface water supplies (Knauer and Buffle, 2001), most studies have focused on either HS or AOM removal (Bernhardt et al., 1985; Ghernaut et al., 2010; Henderson et al., 2010; Matilainen et al., 2010; Pivokonsky

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et al., 2012), while little attention has been paid to their simultaneous removal.

Jiang et al. (1993) investigated the coagulation of algal cells and algae-derived organic matter by four different inorganic coagulants (polyferric sulphate, ferric sulphate, aluminium sulphate, polyaluminium chloride) in the presence of HS. They found that the addition of HS to the solutions of diatom *Asterionella formosa* lead to a reduction of coagulation performance with increasing concentration of HS. This was the case for both dissolved AOM and algal cell removal. Higher doses of coagulants were required to achieve overall charge neutralization with greater HS concentration. Several studies have demonstrated that humic and fulvic acids adsorb onto the surfaces of freshwater phytoplankton cells and that the HS-cell interaction is strongly dependent on pH value (Campbell et al., 1997; Knauer and Buffle, 2001; Vigneault et al., 2000). Moreover, some investigations have indicated that HS can interact with microbial products (polysaccharides and proteins) during membrane filtration, thus influencing the rate of flux decline, the reversibility of fouling and species rejection (Jermann et al., 2007; Katsoufidou et al., 2010; Myat et al., 2014). Myat et al. (2014) found that humic acid (HA) interacts with alginate (used as a model compound for algal polysaccharides) via Ca^{2+}-mediated interactions. Moreover, HA was demonstrated to interact with bovine serum albumin (BSA, as a representative protein) even in the absence of Ca^{2+}. It can be, therefore, assumed that proteins contained in AOM may, in a similar manner to the BSA model protein, interact with HS and these interactions may influence the water treatment process.

This paper investigates the molecular interactions between AOM proteins and humic substances and the impact of these interactions on the coagulation process. The study focuses on understanding the interaction mechanisms between AOM, HS and coagulant. Furthermore, the comparison between coagulation behaviour of AOM proteins and BSA protein is made in order to assess the suitability of BSA as a model compound for AOM proteins.

2. Material and methods

2.1. Material

Bovine serum albumin (BSA, Sigma Aldrich) was used as a model protein in this study, because its structure and properties are well characterised, it is easily accessible and has been already used as a model protein for microbial proteins (Myat et al., 2014). It has a molecular weight of 66 kDa and an isoelectric point of 4.7. When characterised by high performance size exclusion chromatography (HPSEC), two peaks are usually detected, dimeric and monomeric with a stronger UV absorbance signal (Myat et al., 2014; see Fig. S4 in Supplemetary Data). On its side chains, BSA bears 99 acidic, 98 basic and 116 non-charged polar functional groups that are fairly uniformly distributed over the whole molecule. This makes BSA relatively hydrophilic and very soluble in aqueous media (Carter and Ho, 1994), especially at pH values close to the isoelectric point, where BSA molecule adopts the compact form.

HS were purchased as a water extract from mountain fibre peat (Aqua exotic, Slovakia). They possess a variety of largely oxygen-containing functional groups dominated by carboxylic and phenolic groups. HS were characterized in terms of apparent molecular weight (MW) distribution, determined by HPSEC, and acidity via the potentiometric titration method (see sections S1–S3 in Supplementary Data).

Cyanobacterium *Microcystis aeruginosa* was cultivated and its cellular peptides and proteins were isolated as described in the studies of Safarikova et al. (2013) and Pivokonsky et al. (2014). The isoelectric points of these peptides and proteins are 4.8, 5.1, 5.3, 5.5, 5.6, 5.8, 6.1, 6.3, 6.5, 6.6, 7.0, 7.4, 7.8, 7.9 and 8.1 (Safarikova et al., 2013). The peptide/protein MWs were determined by HPSEC (see Fig. S3 in Supplementary Data).

2.2. Methods

2.2.1. MW fractionation

MW distributions by HPSEC analyses were performed with a diode array detector (DAD). Wavelengths that are commonly used for the detection of protein-like substances (280 nm) and humic-like substances (254 nm) were applied (Aitken and Learmonth, 2002; Matilainen et al., 2011). Agilent Bio SEC-5100 Å and 300 Å columns (7.8 × 300 mm, 5 μm) connected in series (separation range 100–1,250,000 Da) were used at the temperature of 23 °C with a 0.15 M phosphate buffer (pH 7.0) as the mobile phase. The columns were operated with a flow-rate of 1 mL/min and a 50 μL injection volume. Peptide and protein SEC standards (Sigma–Aldrich, USA) of MW range from 244 Da to 900 kDa were used to calibrate the system.

2.2.2. Jar testing

In order to describe the effect of BSA and peptides/proteins of *M. aeruginosa* (MA proteins) on the coagulation of HS, jar tests with single compounds (HS or BSA or MA proteins, initial dissolved organic carbon (DOC) concentration of 5 mg L^{-1}) and BSA/HS or MA proteins/HS mixtures (DOC = 5 + 5 mg L^{-1}) were performed. These concentrations represent the typical HS and peptide/protein content in natural surface water (Knauer and Buffle, 2001; Pivokonska et al., 2008; Matilainen et al., 2011). Aluminium sulphate ($Al_2(SO_4)_3 \cdot 18H_2O$; Sigma–Aldrich, USA) was used as coagulant. Firstly, coagulant doses were optimized for HS, BSA and MA proteins and also for BSA/HS or MA proteins/HS mixtures with Al doses ranging from 0.2 to 10 mg L^{-1} Al (0.007–0.370 mmol L^{-1} Al). Secondly, jar tests with the optimized coagulant doses were performed in the pH range 3–8.5. The target pH was reached by adding predetermined amounts of 0.1 M $NaHCO_3$, 0.1 M NaOH or 0.1 M HCl prior to the addition of the coagulant. The experiments were carried out using the variable speed eight position paddle stirrer (LMK 8-03, IH ASCR, Czech Republic) and 2 L jars. The samples were rapidly mixed at shear rate of 200 s^{-1} for 1 min, followed by a slow stir phase at shear rate of 50 s^{-1} for 15 min and 60 min settling period. The supernatants were analysed for pH value, DOC, UV absorbance at 254 nm (UV_{254}) and residual Al. DOC, referring to the concentration of dissolved residual organics, was measured using a total organic carbon analyser (TOC-V_{CPH}) (Shimadzu, Japan). Residual DOC of HS was determined from UV absorbances at 254 nm, measured using a UV-VIS 8452A spectrophotometer (Agilent Technologies, USA) with 1 cm quartz cuvette. When compared to HS, the absorbances at 254 nm were very low for BSA or MA proteins and thus were considered to be negligible. Residual DOC value of BSA or MA proteins was calculated as the difference between total DOC and DOC of HS. Moreover, residual organics remaining after jar tests under optimum pH conditions were analysed via HPSEC. The samples were concentrated in a rotary evaporator (Laborota 4002, Germany), operated at 23 °C and pressure 19 mbar, prior to the HPSEC analyses to reach concentrations of 100 mg L^{-1} DOC. The possible aggregation of the organic molecules during pre-concentration was tested by measuring turbidity of samples and DOC of samples filtered through a 0.22 μm membrane filter (Millipore, USA). It was found that pre-concentration to 100 mg L^{-1} DOC did not lead to aggregation.

To investigate the possible interactions between BSA/MA proteins and HS, jar tests without coagulant addition were also carried out in pH range 2.5–5.5. Three different protein/HS ratios (initial