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The impact of algal organic matter on coagulation of other impurities present in surface waters

Vliv organických látek produkovaných fytoplanktonem na koagulaci dalších znečišťujících příměsí přítomných v povrchových vodách

Ph.D. Thesis

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PROHLÁŠENÍ

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DECLARATION

I declare that I have prepared this thesis individually, using only the information sources and literature cited. This thesis or its substantial part has not been submitted for the award of the same or any other academic degree.

Prague, 15th September 2017

Magdalena Barešová

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PROHLÁŠENÍ O SPOLUPRÁCI NA ODBORNÝCH PUBLIKACÍCH

Jménem dalších spoluautorů prohlašuji, že se Mgr. Magdalena Barešová významně podílela na vzniku výše uvedených publikací. Rozsah jejího podílu je uveden u jednotlivých prací.

DECLARATION OF COOPERATION ON SCIENTIFIC PUBLICATIONS

On behalf of the other co-authors, I declare that Mgr. Magdalena Barešová contributed significantly to the preparation of the aforementioned publications. The extent of her participation in the individual papers is indicated in the List of publications.

Prague, 15th September 2017

doc. RNDr. Martin Pivokonský, Ph.D.

ABSTRACT

Drinking water treatment is facing an adverse impact of algae especially when they extensively decay and release cellular organic matter (COM). As the character and consequently the removal efficacy of COM depends on the individual species, the thesis compares algal organic matter (AOM) derived from four common phytoplankton species: green alga *Chlamydomonas geitleri*, diatom *Fragilaria crotonensis*, and cyanobacteria *Microcystis aeruginosa* and *Merismopedia tenuissima*. To evaluate the differences between extracellular and cellular organic matter, we monitored the growth of those organisms and compared AOM obtained at different growth phases. As COM is not only difficult to coagulate, but also hinders the coagulation of other substances, the thesis investigates the effect of simultaneous coagulation of COM with other impurities present in surface waters: kaolin, humic substances (HS) or cyanobacterial cells of *M. tenuissima*. Coagulation behaviour was studied by the jar tests performed with single components and their mixtures with and without a coagulant (ferric or aluminium sulphate). Special emphasis was paid to proteinaceous COM of *M. aeruginosa*; to enhance the removability of proteinaceous matter we implemented pre-oxidation.

Coagulation effectively removed turbidity (up to 99%) either of clay or algae origin at pH approx. 6-8 for ferric and 7-8.5 for aluminium coagulant. On the other hand, both humic and algal organic matter showed lower maximum removals (up to 65% for HS, 60-85% for proteinaceous COM of *M. aeruginosa*, and 43-53% for *M. tenuissima* COM, expressed as dissolved organic carbon (DOC)) at pH below neutral (approx. 4-6 for Fe and 5-6.5 for Al). In the case of mixtures, COM favoured coagulation although it modified the pH optimum for turbidity removal and hence the coagulation mechanisms. While both single cells and kaolin adsorbed preferably onto Al/Fe-oxide-hydroxides at about neutral pH, the COM-cell and proteinaceous COM-kaolin mixtures underwent charge neutralisation by Al/Fe-hydroxopolymers within the moderately acidic pH range more or less overlapping with that for single COM. Additionally, COM induced flocculation of those impurities even in the absence of a coagulant at acidic pH (approx. <4.5). It seems plausible that as a flocculant aid particularly high-molecular weights (MWs) (>10 kDa) involved in the adsorption processes and entailed inter-particle bridging of destabilised molecules and particles. In turn, high portions of low-MWs (<10 kDa) and predominantly hydrophilic nature most likely produced relatively low COM removals. Besides, at a narrow pH range about 6.2 for Fe and 6.8 for Al, coagulation was disrupted due to the formation of organo-metal complexes. However, this interference can be prevented by pH optimisation or by pre-oxidation with the benefit of heightened removals of proteinaceous COM by 5-12% compared to coagulation alone.

ABSTRAKT

Úprava pitné vody se potýká s výskytem fytoplanktonu zejména ve fázi jeho odumírání, kdy dochází k uvolnění značného množství celulárních organických látek (COM – Cellular Organic Matter). Vzhledem k druhové závislosti charakteru COM a tedy i účinnosti jejich odstranění, tato práce srovnává organické látky, tzv. Algal Organic Matter (AOM), produkované čtyřmi běžně se vyskytujícími druhy fytoplanktonu: zelenými řasami *Chlamydomonas geitleri*, rozsivkami *Fragilaria crotonensis* a sinicemi *Microcystis aeruginosa* a *Merismopedia tenuissima*. Odlišnost extracelulární a celulární frakce byla hodnocena monitoringem růstu a srovnáním AOM získaných v různých fázích rozvoje fytoplanktonu. Vzhledem k tomu, že COM jsou nejen obtížně odstranitelné, ale ztěžují i odstranitelnost dalších znečišťujících příměsí, práce zkoumá vliv souběžné koagulace COM a dalších látek přítomných v povrchových vodách: kaolinu, huminových látek (HL) a buněk *M. tenuissima*. Průběh koagulace byl studován pomocí sklenicových zkoušek s jednotlivými komponenty i jejich směsi za přítomnosti koagulantu (síran hlinitý či železitý) či bez něj. Důraz byl kladen na proteinovou složku COM *M. aeruginosa*. S cílem zvýšit odstranitelnost proteinových COM byla zařazena preoxidace.

Koagulace odstranila s až 99% účinností zákal jílového i organického původu přibližně při pH 6-8 v případě železitého a 7-8,5 u hlinitého činidla. Naopak u huminových látek a látek řasového původu bylo dosaženo nižší míry odstranění (do 68 % u HL, 60-85 % u proteinových COM *M. aeruginosa* a 43-53 % u COM *M. tenuissima*, vyjádřených jako koncentrace rozpuštěného organického uhlíku (DOC – Dissolved Organic Carbon)), a to v mírně kyselém pH (cca 4-6 pro Fe a 5-6,5 pro Al). V případě směsí COM koagulaci podpořily, přestože pozměnily optimální oblast pH a současně i koagulační mechanismy zákalotvorných částic. Zatímco samotné buňky i kaolin interagovaly spíše prostřednictvím adsorpce na hydratovaných oxidech Al/Fe v neutrální oblasti pH, směsi COM a buněk i proteinových COM a kaolinu podlehly nábojové neutralizaci Al/Fe-hydroxypolymery v mírně kyselém pH odpovídajícímu optimu samotných COM. COM rovněž vyvolaly flokulaci sledovaných příměsí přibližně při pH <4,5 i bez přídavku koagulantu. Jako pomocné flokulační činidlo se adsorpčních procesů účastnily pravděpodobně vysokomolekulární látky (>10 kDa), které umožnily agregaci destabilizovaných částic a molekul tvorbou mezičásticových můstků. Naopak vysoký podíl nízkomolekulární frakce (<10 kDa) a převážně hydrofilní povaha patrně odpovídají za relativně nízkou odstranitelnost COM. V úzkém rozsahu pH okolo 6,2 v případě Fe a 6,8 u Al byla koagulace rušena tvorbou organo-kovových komplexů. Tomuto narušení lze však předejít optimalizací reakčních podmínek či preoxidací, která navíc zvýšila míru odstranění proteinových COM v porovnání se samotnou koagulací o 5-12 %.

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LIST OF ABBREVIATIONS

AOM	Algal organic matter
BSA	Bovine serum albumin
COM	Cellular organic matter
DAF	Dissolved air flotation
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EOM	Extracellular organic matter
FA	Fulvic acids
HA	Humic acids
HS / HL	Humic substances / huminové látky
IOM	Intracellular organic matter
MCs (-LR)	Microcystins (-L = leucine, -R = arginine)
MW	Molecular weight
NOM	Natural organic matter
NTU	Nephelometric turbidity unit
PACl	Polyaluminium chloride
pH	Potential of hydrogen
pK_a	Acid dissociation constant
pzc	Point of zero charge
SOM	Surface-bonded/retained organic matter
SRFA	Suwannee River fulvic acids
SRHA	Suwannee River humic acids
SUVA	Specific UV absorbance
T&O	Taste and odour
TOC	Total organic carbon
WHO	World Health Organisation
WTP	Water treatment plant

1 INTRODUCTION

The quality of surface waters is significantly and permanently deteriorated by the presence of algae and cyanobacteria. They do not affect only aquatic ecosystems, but also limit the use of water resources for drinking purposes. In a temperate climate, water treatment faces the occurrence of algae mainly on the seasonal basis. However, due to a phytoplankton population increase associated with the ongoing eutrophication, the issue of water treatment is becoming topical worldwide. An outbreak of algal blooms may lead even to complete collapse of water treatment plants (WTPs) (Greene and Hayes, 1981; Henderson et al., 2008a; Zhang et al., 2010; Středa et al., 2015).

Algae and cyanobacteria have received increasing attention as they produce a variety of compounds denoted as algal organic matter (AOM), which can seasonally make up a bulk of natural organic matter (NOM) present in surface waters (Henderson et al., 2008b). As a complex mixture, AOM comprises 1) harmful cyanotoxins including cyclic peptide hepatotoxins (microcystins and nodularins) or alkaloid hepatotoxic cylindrospermopsin and neurotoxins (anatoxins and saxitoxins) (Carmichael, 1992; Wiegand and Pflugmacher, 2005; Bláhová et al., 2008; Dixon et al., 2010); 2) undesirable compounds affecting organoleptic properties of the source water such as 2-methylisoborneol and trans-1,10-dimethyl-trans-9-decalol (geosmin) producing musty earthy taste and odour (T&O) or various volatile organic sulphur compounds (Dixon et al., 2010; Zhang et al., 2010; Li et al., 2012; Zamyadi et al., 2015); and 3) precursors to hazardous disinfection by-products (DBPs) forming at water disinfection with chlorine and its compounds, specifically trihalomethanes (e.g. chloroform and dibromochloromethane) and halogenated acetic acids (e.g. dichloroacetic and trichloroacetic acid) (Nguyen et al., 2005; Fang et al., 2010; Li et al., 2012; Liao et al., 2015). Therefore, AOM poses a challenge for conventional water treatment processes (i.e. coagulation/flocculation – sedimentation – filtration).

The most pronounced disorder evoked by AOM seems to be its interference with coagulation/flocculation – the key broadly employed process among water treatment facilities whose efficiency predetermines the performance of all downstream processes (Ching et al., 1994; Vlaški et al., 1996; Kim and Kang, 1998; Cheng and Chi, 2002; Henderson et al., 2008a, 2010; Gonzalez-Torres et al., 2014). Beside other negative impacts on WTPs, not only does high organic content clog rapid sand filters with subsequent reduction of filter

runs, but it also limits the usage of advanced technologies, e.g. when it fouls ultra- and nano-filtration membranes or when it decreases the efficiency of activated carbon for adsorbing prevailingly low molecular organic matter or various micropollutants (Her et al., 2004; Dixon et al., 2010; Hnatukova et al., 2011; Huang et al., 2012; Kopecka et al., 2014). Hence, adverse effects of AOM result in increased residual coagulant concentrations, organic matter and turbidity of treated water (Greene and Hayes, 1981; Bernhardt et al., 1985, 1987; Takaara et al., 2007) and cause potential bacterial growth in distribution systems (Bernhardt et al., 1987, 1991; Widrig et al., 1996). To ensure satisfactory drinking water quality that would meet the collective legislation of the European Union under the Drinking Water Directive No. 98/83/EC (incorporated in the national regulation of the Ministry of Health of the Czech Republic under the decree No. 252/2004 Coll.), WTPs tentatively tackle this issue by increasing coagulant doses which, however, create subsidiary disorders including increased operating cost (Bernhardt et al., 1985, 1987; Sano et al., 2011; Ma et al., 2012a).

Significant participation of algae in the performance of drinking water treatment is given by their decay, which is followed by the release of a considerable amount of cellular algal organic matter (COM) (Zhang et al., 2010; Nicolau et al., 2015). However, despite relatively low treatability of AOM/COM by coagulation/flocculation (Widrig et al., 1996; Pivokonsky et al., 2009), owing to its widely variable features, it has been demonstrated to either hinder or in turn favour the removal of other impurities present in surface waters (Bernhardt et al., 1985, 1986, 1991; Bernhardt and Clasen, 1991; Dolejš, 1993; Ma et al., 2012a,b). This distinctive feature of algal organic matter motivates the objective of this thesis: to address the challenges posed by COM for water treatment. An emphasis was paid to its traits, potential of removal and impact on removability of other particles or molecules by means of coagulation/flocculation as the essential and simultaneously most vulnerable step in the overall solid-liquid separation scheme in drinking water treatment.

1.1 PRINCIPLE OF AOM COAGULATION

In a simplified way, coagulation/flocculation process destabilises dissolved organic matter (DOM), colloids and particles, which collide with each other and combine into aggregated forms (Stumm and Morgan, 1996; Jiang and Graham, 1998; Bratby, 2006; Matilainen et al., 2010). What seems to be decisive for efficient coagulation is the charge distribution in the system that also governs running interaction mechanisms (Bernhardt et al., 1985, 1991; Henderson et al., 2010; Pivokonsky et al., 2012). The surface charge of individual impurities as well as the charge and character of coagulant species are controlled by the reaction pH reflecting dissimilar solubility and course of hydrolysis of coagulant metals (aluminium/iron) (Stumm and Morgan, 1996; Jefferson et al., 2004). Concisely, at acidic pH values (approximately pH <2 for iron and <4.5 for aluminium), Al^{3+}/Fe^{3+} ions prevail in an aqueous medium to about 99% as Al/Fe-hexaaquacomplex ($[Al(H_2O)_6]^{3+}$ or $[Fe(H_2O)_6]^{3+}$). As the pH rises, hydrolysis proceeds and forms cationic polynuclear Al/Fe-hydroxopolymers (e.g. $Al_7(OH)_{17}^{4+}$, $Al_{13}(OH)_{34}^{5+}$ or $Fe_3(OH)_4^{5+}$, $Fe_4(OH)_6^{6+}$) and subsequently Al/Fe-oxide-hydroxides (AlO(OH) or FeO(OH)). At alkaline pH (approximately above pH 8), Al/Fe largely occur as anionic hydroxocomplexes ($[Al(OH)_4]^-$ or $[Fe(OH)_4]^-$) (Bernhardt et al., 1985; Van Benschoten and Edzwald, 1990a; Stumm and Morgan, 1996).

Depending on which form of Al/Fe prevails, the coagulation is generally explained through several fundamental mechanisms. At slightly acidic pH and low coagulant doses (e.g. 0.01-1.0 mmol L^{-1} Fe and 0.002-0.1 mmol L^{-1} Al at pH 4-6 and pH 5-7, respectively, for inorganic colloids) mechanism of gradual charge neutralisation by mononuclear and medium polynuclear positively charged Al/Fe-hydroxopolymers plays the key role in the coagulation process (Johnson and Amirtharajah, 1983; Duan and Gregory, 2003; Bache and Gregory, 2007). Under conditions of charge neutralisation, optimum coagulant doses usually correspond to the zeta potential of closing to zero value (kept through a combination of pH adjustment and/or coagulant dose) in the coagulating system of either algal and clay colloid suspensions or humic solutions (Bernhardt et al., 1985; Bernhardt and Clasen, 1991; Kim and Kang, 1998; Briley and Knappe, 2002; Jefferson et al., 2004; Henderson et al., 2008a,c, 2010; Liu et al., 2009). The higher dosage could give consequential charge reversal and successive restabilisation of impurities (Bernhardt and Clasen, 1991, 1994; Wang et al., 2002; Duan and Gregory, 2003; Liu et al., 2009). Nevertheless, at sufficiently low coagulant doses,

coagulation at lower pH occurs also owing to the adsorption of Al/Fe-hydroxopolymers onto the surface of impurity particles forming electrostatic patches as postulated by Gregory (1973, 1996) for polymer adsorption. If the adjacent particles approach by patches of opposite charge, coulombic attraction may induce their aggregation (Ching et al., 1994; Bache and Gregory, 2007). However, such low coagulant concentrations are not likely to proceed to efficient charge neutralisation because of limited inter-particle collision rate. The collision rate is in turn enhanced at neutral to alkaline pH and higher coagulant doses or under an overdosing when a mechanism of sweep flocculation also known as enmeshment prevails and impurities become entrapped in the growing amorphous Al/Fe-hydroxide precipitate (Johnson and Amirtharajah, 1983; Van Benschoten and Edzwald, 1990a; Ching et al., 1994; Stumm and Morgan, 1996; Kim and Kang, 1998; Wang et al., 2002; Duan and Gregory, 2003; Bache and Gregory, 2007; Liu et al., 2009). At a transition between these two distinct mechanisms (charge neutralisation and sweep flocculation), adsorption onto the cationic surface of colloidal Al/Fe-oxide-hydroxides allowing inter-particle bridging tends to be influential depending on the concentration ratio of coagulant to impurities, otherwise it is not easily distinguishable (Cheng and Chi, 2002; Duan and Gregory, 2003; Kabsch-Korbutowicz, 2006; Liu et al., 2009). Then, for pH approaching the point of zero charge (pzc) of AlO(OH) or FeO(OH) (Stumm and Morgan, 1996), the coagulant species turn gradually negative and cause electrostatic repulsions in the interacting system (Duan and Gregory, 2003).

1.1.1 REMOVABILITY OF AOM

The complex AOM mixture consists of various compounds ranging from metabolic intermediates to storage macromolecules. AOM comprises volatile aldehydes and ketones, enzymes and vitamins, phosphorus compounds, amines, hydrocarbons, glycolic and other organic acids, lipids and fatty acids. Dominating components are saccharides (mono-, oligo- and polysaccharides) and nitrogenous substances (amino and nucleic acids, peptides and proteins) (Fogg, 1971; Hellebust et al., 1974; Maksimova et al., 2004; Pivokonsky et al., 2006; Nicolau et al., 2015). A specific composition of AOM not only differs with the microorganism's species and the growth phase, but also depends on physiological and growth conditions (Fogg, 1971; Hellebust et al., 1974; Hoyer et al., 1985; Brown et al., 1997;

Maksimova et al., 2004; Pivokonsky et al., 2006; Huang et al., 2012). What this chemical heterogeneity of AOM entails is that the removal efficiencies are dissimilar (Pivokonsky et al., 2009).

The potential of AOM to be coagulated depends mainly on the content of a range of functional groups, such as –OH, –COOH, $=NH_2^+$, $–CONH_2$, $–NH_3^+$, –SH etc. (Chang, 2005). AOM interactions are predominantly assigned to carboxyl groups that dissociate depending on their respective acid dissociation constants (pK_a) (e.g. proteinaceous aspartic acid with terminal α– and side β–COOH of pK_a=2.09 and 3.86 and glutamic acid with α– and γ–COOH of pK_a=2.19 and 4.25 (Chang, 2005) or saccharide alginic acid, composed of poly-D-mannuronic and L-guluronic acid with –COOH of pK_a=3.38 and 3.65, respectively (Haug, 1961)). Kopecka et al. (2014) recorded for proteinaceous part of cellular organic matter (COM) of the cyanobacterium *Microcystis aeruginosa* nearly 10 mmol of titratable carboxyl groups per 1 g of dissolved organic carbon (DOC) in the pH range 7.1-1.5. Similarly, alginate, a polysaccharide produced by some algae and bacteria, was also estimated to contain about 10 mmol of carboxyl groups per 1 g C (Gregor et al., 1996).

The pH optimum for AOM coagulation ranges within the moderately acidic pH, specifically between pH 4.5 to 6.5 for *M. aeruginosa* COM (Pivokonsky et al., 2009) and between 4.0 to 6.0 for a proteinaceous fraction of *M. aeruginosa* COM (Pivokonsky et al., 2012), both for ferric coagulant. Under certain pH conditions, deionized functional groups interact electrostatically with positively charged coagulant hydroxopolymers and hydrated oxides, which enable their coagulation by the most pronounced gradual charge neutralisation or adsorption mechanism. At about neutral pH (6-8 for Fe) adsorption may still take part in the coagulation. Nevertheless, it has been shown that the extent of DOM removal by the adsorption mechanism is usually less significant, since it is effective only if the ratio between DOC and a coagulant is relatively low (e.g. <0.33 for Fe). On the contrary, at a higher ratio, DOM tends to occupy the whole surface of hydrated oxide particles and causes their restabilisation (Bernhardt et al., 1985, 1991; Pivokonsky et al., 2009, 2012).

Despite the above listed prominent features, AOM generally shows relatively low treatability. For instance, Widrig et al. (1996) achieved through coagulation followed by sedimentation 18%, 25% and 49% DOC removal efficiencies for extracellular organic matter (EOM) of *M. aeruginosa*, the green algae *Scenedesmus quadricauda* and *Dictyosphaerium*

pulchellum, respectively (initial DOC 6 mg L^{-1}, a dose of 2.96 mmol L^{-1} Fe ($FeCl_3.6H_2O$), 0.5 mmol HCO_3^-, pH 5.0). Similarly, Pivokonsky et al. (2009) obtained under optimised conditions (pH 4.5-6.5) maximum DOC removal rates of 42% and 50% for COM of *M. aeruginosa* at initial DOC concentrations of 3 and 7 mg L^{-1} using 16 and 26 mg L^{-1} Fe ($Fe_2(SO_4)_3.9H_2O$), respectively. In the following study, they achieved more than 70% removal rates of the proteinaceous fraction of COM increasing with the initial DOC (1-8 mg L^{-1} DOC, 7 mg L^{-1} Fe, pH 4.0-6.0) (Pivokonsky et al., 2012). Such relatively low removals result from largely hydrophilic nature of AOM, high quantities of low molecular matter (depending on specific species) (Edzwald, 1993a; Pivokonsky et al., 2006, 2009, 2012; Henderson et al., 2008b, 2010; Ghernaout et al., 2010) and from the formation of organo-metal complex compounds (Bernhardt et al., 1985; Takaara et al., 2005, 2007; Pivokonsky et al., 2006, 2012; Ma et al., 2012a).

Lower coagulation efficiencies for a hydrophilic fraction in comparison to the hydrophobic matter have been widely reported (Edzwald, 1993a; Ghernaout et al., 2010; Henderson et al., 2010; Ma et al., 2012a). Sharp et al. (2006) concluded that initial concentrations of the hydrophilic matter correlate well with the residual DOC after coagulation among various water sources. High hydrophilicity (57-86%) of AOM was determined either by the DAX/XAD/IRA resin fractionation (Her et al., 2004; Henderson et al., 2008b; Huang et al., 2012; Li et al., 2012; Leloup et al., 2013) or by the analysis of specific UV absorbance (SUVA) with values as low as of about 0.5 L mg^{-1} m^{-1} as recorded for COM derived from *M. aeruginosa* (Fang et al., 2010; Liao et al., 2015). Moreover, it was documented that a degree of hydrophilicity rises and SUVA accordingly decreases as the phytoplankton culture grows (Henderson et al., 2008b; Fang et al., 2010; Huang et al., 2012; Li et al., 2012). For instance, the content of hydrophilic fraction increased from 63% at EOM fraction to 86% at COM (in this case titled as IOM – Intracellular Organic Matter) of *M. aeruginosa* in the study by Li et al. (2012), and correspondingly SUVA decreased from 1.38 for EOM to 0.58 L mg^{-1} m^{-1} for IOM of the same cyanobacterium in the study by Fang et al. (2010), and from 1.13 to 0.5 and from 0.87 to 0.47 L mg^{-1} m^{-1} for *M. aeruginosa* and the diatom *Cyclotella meneghiniana*, respectively, in the study by Liao et al. (2015).

Furthermore, particularly organics of mainly low molecular weights (MWs) (<10 kDa) were proved to be less prone to coagulation (Cheng and Chi, 2003; Pivokonsky et al., 2009,

2012; Ma et al., 2012a) if they are of either proteinaceous (Pivokonsky et al., 2012) or saccharide character (Bernhardt et al., 1985), and thus require employment of additional technologies such as adsorption onto activated carbon (Hnatukova et al., 2011; Kopecka et al., 2014) or membrane filtration (Teixeira and Rosa, 2005; Dixon et al., 2010). For instance, in the study by Henderson et al. (2010), highly hydrophilic (57% or more) EOM of the green alga *Chlorella vulgaris*, the cyanobacterium *M. aeruginosa* and the diatom *Asterionella formosa* with 30%, 38% and 81% portion <1 kDa showed 71%, 55% and 46% removal efficiencies (at initial concentrations of 5 mg L^{-1} as DOC) using optimised doses of 0.8, 1.2 and 1.5 mg Al mg^{-1} DOC, respectively ($Al_2(SO_4)_3.18H_2O$, pH 7).

Another aspect of limited removals of AOM follows from its ability to form polynuclear complexes with coagulant species. Coagulant metals are bound in a form of stable soluble or microcolloid organo-metal complexes that consequently consume the coagulant, prevent its hydrolysis, and thus hinder the formation of positively charged Al/Fe-polyhydroxocomplexes required for destabilisation of both dissolved and particulate impurities. This leads to decreased coagulant efficiencies, disproportional increase in effective coagulant dosages and also increased residual concentrations of both coagulant and organic matter in treated water (Bernhardt et al., 1985, 1986, 1987, 1991; Takaara et al., 2005, 2007; Pivokonsky et al., 2006, 2012; Sano et al., 2011; Ma et al., 2012a,b).

The affinity of metals to organic matter can be explained by the model of surface complexes. This model describes the formation of complex compounds by coordinative electrostatic bonds between particularly the deprotonated carboxyl functional groups, especially those on the biopolymer side chains, and the positively charged surface groups of metal hydroxypolymers and hydrated oxides. In the case of polysaccharides, metals are bound to carboxyl in uronic acids (Bernhardt et al., 1985, 1987, 1991). As the mechanisms of complex formation are largely of electrostatic character, the level of this interference also relates closely to the reaction pH that controls both the hydrolysis of metal species and dissociation of carboxyl groups. In the case of proteinaceous COM of *M. aeruginosa*, the maximum binding capacity was found at about pH 6 for iron and was quantified to be of about 1.38 mmol Fe g^{-1} DOC (Pivokonsky et al., 2012). Similarly, Gregor et al. (1996) determined binding capacity of about 1.4 mmol Al g^{-1} C at pH 6 for alginate. As complex forming compounds mainly low-MWs were isolated, specifically those of apparent MW of 1,

2.8, 6, 8, 8.5, 10, 52 and 60 kDa (Pivokonsky et al., 2006, 2012) and between 43 and 67 kDa (Takaara et al., 2005) in the case of peptides/proteins derived from *M. aeruginosa*. Nevertheless, it was shown that the removability of organic matter can be controlled by pre-oxidation as will be discussed later.

1.2 INTERFERENCE OF AOM WITH OTHER IMPURITIES

Although algal and cyanobacterial cells and related algal organic matter (AOM) can be present in surface waters together with other impurities, most attention has been paid to the removal of individual components. Besides the generally low treatability by coagulation/flocculation (Widrig et al., 1996; Pivokonsky et al., 2009), AOM has been found to affect also the removal of another natural matter including inorganic colloids represented by quartz and kaolin particles (Bernhardt et al., 1985, 1986, 1991; Dolejš, 1993; Takaara et al., 2007, 2010) or algal cells themselves (Bernhardt and Clasen, 1991; Henderson et al., 2010; Ma et al., 2012a,b). Owing to its widely variable features, such as overall amount, hydrophobicity, content of proteinaceous matter, molecular weight (MW) distribution and surface charge, AOM may exhibit both favourable and adverse effects on the coagulation (Bernhardt et al., 1985, 1986, 1991; Bernhardt and Clasen, 1991; Dolejš, 1993; Ma et al., 2012a,b). Already Bernhardt and co-authors, who carried out the first major investigation into AOM from the perspective of drinking water treatment (Bernhardt et al., 1985, 1986, 1991), suggested that under certain conditions AOM of particularly high MWs may enhance the coagulation. It has been found that AOM may act akin to natural biopolymer polyelectrolytes (e.g. as chitosan, sodium alginate, starches, guar gums, tannins or seeds of the *Moringa olejfera* tree) applied in water treatment as a flocculant aid (Bernhardt et al., 1985; Bratby, 2006; Ma et al., 2012a; Pranowo et al., 2013) or, moreover, may even induce coagulation/flocculation of other particles or molecules (Paralkar and Edzwald, 1996; Plummer and Edzwald, 2002).

1.2.1 TREATMENT OF TURBID WATERS

Responsible for turbidity in surface waters are ubiquitous inorganic colloids. Stability of particles such as quartz or kaolin is given mainly by an electrical double layer that governs their surface charge (Bernhardt et al., 1985, 1986, 1991; Bratby, 2006; Bache and Gregory,

2007). In natural conditions, inorganic particles usually prevail as negatively charged (Duan and Gregory, 2003). For example, the point of zero charge (pzc) of kaolinite fluctuates around pH 3, approximately at pH 2.6 in the study by Coles and Yong (2002) or in the pH range of 3.5-4.5 in the study by Dohnalová et al. (2008) depending on the electrolyte solution, specific composition of kaolin samples or employed analytical techniques. Above pzc, kaolin and such colloids bear a negative surface charge measured either as electrophoretic mobility or zeta potential (Ching et al., 1994; Kim and Kang, 1998).

The optimum for turbidity removal ranges typically about neutral pH. Kim and Kang (1998) observed efficient coagulation of kaolinite between pH 6.7 and 8.2 by ferric nitrate obtaining removal rates that increased with a coagulant dose to more than 95% (initial particle concentration of 25 mg L^{-1} ~ 24 NTU (Nephelometric turbidity unit) and average size of 1.8 µm, 5-20 mg L^{-1} $Fe(NO_3)_3.9H_2O$, 5 mmol $NaNO_3$). In the study by Ching et al. (1994), optimum for kaolin was enlarging from pH 6.4-7.2 to 6.4-8.0 with the coagulant doses of 3 and 36 µmol Fe ($FeCl_3.6H_2O$) and with the residual turbidity levels of about 20 NTU or below 5 NTU, respectively (initial solid concentration of 50 mg L^{-1} ~ 40 NTU, 1 mmol $NaHCO_3$). Such pH values close to the pzc of Al/Fe indicate that kaolinite/kaolin particles are preferably destabilised due to absorption onto Al/Fe-oxide-hydroxides or through enmeshment by Al/Fe-hydroxide precipitate (Ching et al., 1994; Kim and Kang, 1998; Duan and Gregory, 2003).

Although clay particles and AOM differ in both optimal reaction pH and prevailing coagulation mechanisms, only handful studies have dealt with the potential effect of AOM on turbidity removal (Bernhardt et al., 1985, 1986, 1991; Takaara et al., 2007, 2010; Sano et al., 2011). Plausible interactions between microbial products and clay colloids in an aqueous medium arise from the evidence of natural organics (of either proteinaceous or saccharide character) adsorbing onto clay minerals in soil environments (e.g. Goring and Bartholomew, 1952; Greaves and Wilson, 1969; Parfit and Greenland, 1970). The adsorption efficacy depends highly on the type and the concentration ratio of both organics and clay particles, when especially concentrated solutions proved to cause an increased adsorption. Moreover, adsorption efficacy could be enhanced by the addition of electrolyte (Goring and Bartholomew, 1952; Greaves and Wilson, 1969). Nevertheless, pH value occupies a crucial role. In the case of pH above 5, only a weak adsorption was observed for nucleic acids onto

montmorillonite, whereas pH decreasing from 5 to 3.5 significantly increased the adsorption efficacies (Greaves and Wilson, 1969). The ability of model polysaccharides to induce flocculation of mineral colloids at about neutral pH and in the presence of $CaCl_2$ was investigated by several other studies (Labille et al., 2005; Devrimci et al., 2012). Devrimci et al. (2012) recorded effective clay coagulation using sodium alginate at pH 7.3 (predominantly smectite of initial 150 and 80 NTU) similarly to Labille et al. (2005), who evaluated flocculation of montmorillonite suspension using several polysaccharides of bacterial origin at pH 7 (50 mg L^{-1} of Na-montmorillonite, polysaccharide concentration of 0.5 g L^{-1}, 1.25 mmol $CaCl_2$,). At about neutral pH, both clay particles and polysaccharides prevail negatively charged, hence their contact was most likely mediated by divalent calcium cations. Calcium in these studies probably compressed the electrical double layer of particles, reduced the distance and repulsive forces and allowed them to approach close enough to be bridged by adsorbing macromolecules (Labille et al., 2005; Devrimci et al., 2012). The ability to bridge particles emerges from polysaccharide macromolecular configuration and their acidity (amount, location and thus the accessibility of the acidic sites) (Labille et al., 2005). Indeed, the highest flocculent ability among polysaccharides used by Labille et al. (2005) was proved by succinoglycan with two acidic moieties per unit appropriately arranged on the side chain.

Considering the composition of AOM, some attempts assumed similar behaviour to those polymers to be observed even between AOM and inorganic colloids. The enhancing effect of AOM on the particles removal was reported already by Bernhardt and co-authors (e.g. Bernhardt et al., 1985, 1987). They demonstrated that extracellular (EOM) polysaccharides extracted from the green alga *Dictyosphaerium pulchellum* and model alginic acids may at low levels (<1-2 mg L^{-1} as C) serve as anionic and non-ionic polyelectrolytes and contribute to the coagulation and agglomeration of quartz particles. These polysaccharides behave analogously to polymer flocculants. They adhere on the surface of impurity particles through hydrogen and covalent bonds, create so called inter-particle polymer bridges, and consequently decrease consumption of a conventional hydrolysing coagulant (Bernhardt et al., 1985, 1987, 1991). By contrast, increased EOM levels (>2 mg L^{-1} C), in dependence on pH, cause restabilisation of particles, prevent them from aggregation, and thus increase residual organic content requiring elevated coagulant doses (Bernhardt et al., 1985, 1987, 1991). Nevertheless, the key factor is the ratio of

coagulant to organic matter. Bernhardt et al. (1991) reported the appropriate ratio to be 2-3 mg Fe per mg TOC (Total Organic Carbon). Furthermore, the effect and the crucial ratio are species specific, and even the growth phase of the culture plays a role (Bernhardt et al., 1987; Paralkar and Edzwald, 1996). For example, coagulation of quartz (initial 25 mg L^{-1} of sikron particles with grain size 2-12 µm, pH 6.5, 5 mg L^{-1} Fe) was favoured by EOM of the cyanobacterium *Pseudonabaena* at concentrations up to about 1.5 mg L^{-1} DOC, but by only up to 0.5 mg L^{-1} DOC in the case of EOM derived from the diatom *Melosira* sp. (Bernhardt et al., 1987). Another study by Paralkar and Edzwald (1996), who in contrast to Bernhardt et al. (1985, 1986, 1987, 1991) focused on the ability of EOM to stand as the only coagulant, demonstrated a contributing effect of EOM and model alginic acids onto coagulation of latex particles (initial concentration of 5×10^5 particles mL^{-1} of mean diameter 3.98 µm). EOM of the diatom *Cyclotella* sp., which contained, in comparison to other species used in that study, the highest portion of high-MW (90% of EOM >30 kDa), assisted coagulation at concentrations up to 0.01 mg L^{-1} as C when adsorbed onto the positive surface of latex particles and causing their charge neutralisation. Other species – the green algae *Scenedesmus quadricauda* (65% of EOM >30 kDa) and *Chlorella vulgaris* (more than 40% of EOM >30 kDa) as well as model alginic acids of 100 and 250 kDa proved to be of benefit at about up to 0.1 mg L^{-1} as C. Similarly to the studies by Bernhardt et al., with increasing DOC of all the species, EOM presence induced gradual charge reversal and restabilisation of the latex particles as supported by the measurements of electrophoretic mobility (Paralkar and Edzwald; 1996).

Another study concerning the impact of AOM on turbidity removal (Takaara et al., 2007) compared the effect of EOM and cellular organic matter (COM) (in this study titled as IOM) of *Microcystis aeruginosa* on kaolin coagulation. They reported much stronger coagulation inhibition in the case of COM compared to EOM. EOM brought just weak inhibition even in 10 times higher DOC concentration than those of COM (EOM of DOC 112.5 and 214.9 mg L^{-1} reduced kaolin removal rate from 97.7% gained at single kaolin tests to 91.5% and 81.5%, respectively), while COM at initial DOC of 7.7 and 20.6 mg L^{-1} caused the decrease to 79.8% and completely prevented coagulation, respectively (initial kaolin concentration of 20 mg L^{-1}, a dose of 10 mg L^{-1} polyaluminium chloride – PACl, pH 7). In another study, Takaara et al. (2010) focused on the hydrophilic surface-bonded/retained

organic matter (SOM) including lipopolysaccharides extracted in several ways from *M. aeruginosa* and proved their potential to inhibit kaolin coagulation as well.

1.2.2 TREATMENT OF HUMIC WATERS

Except for seasonally elevated AOM concentrations, most of natural organic matter (NOM) in surface waters is usually created by aquatic humic substances (HS) comprising humic and fulvic acids (HA and FA) (Thurman, 1985). HS are of rather unclear structure with an aromatic framework including aliphatic moieties, both giving hydrophobic nature, and various ionisable hydrophilic carboxyl and phenolic functional groups, which dissociate in relation to their respective pK_a values (Thurman, 1985; Collins et al., 1986; Newcombe, 1994; Duan and Gregory, 2003; Sharp et al., 2006). The predominant contribution to the net negative charge of humic matter probably comes from the deprotonation of carboxyl groups with an effective pK_a of around 4 (Campbell et al., 1997; Vigneault et al., 2000; Kam and Gregory, 2001; Duan and Gregory, 2003). HA and FA contain 3.5-4.5 mmol g^{-1} and 5.0-6.0 mmol g^{-1} of carboxyl groups (Thurman, 1985), on average. Newcombe (1994) determined even 31 mmol titratable groups per 1 g DOC of humic material. For Suwannee River humic and fulvic acid fractions (SRHA and SRFA), Thorn (1995) reported a content of 4.9 mmol g^{-1} and 6.1 mmol g^{-1} of carboxyl groups, respectively. Humic acids, which have a lower content of carboxyl groups and larger molecular size resulting in their lower aqueous solubility compared to fulvic acids (Thurman, 1985), show favoured treatability, since similarly to AOM, coagulation effectively removes HS with MW above 500 or 1000 Da and that of lower carboxyl acidity and hence, lower anionic charge densities (Collins et al., 1986; Van Benschoten and Edzwald, 1990b; Jiang and Graham, 1998; Wang et al., 2002; Matilainen et al., 2006; Ghernaout et al., 2009).

Typically, HS are effectively coagulated by alum in the pH range of 5-7 depending on a coagulant type and both HS and coagulant concentrations (Van Benschoten and Edzwald, 1990b; Huang and Shiu, 1996; Wang et al., 2002; Duan and Gregory, 2003; Pivokonska et al., 2008; Liu et al., 2009). In the case of HA, Cheng and Chi (2002) observed effective coagulation within pH 4-5 enlarging to 4-7.5 as a coagulant dose increased from 2 to 10 mg L^{-1} Fe (polyferric sulphate, commercial HA – 2-50 kDa, Aldrich Chemical Co., No. H1,675-2). Non-depending of the used coagulant dose, they observed 90% maximum removal rates as analysed by TOC and fluorescence intensity. Markedly lower removals

(about 40-50% TOC) of HS (isolated from lake sediment in Xi'an, China) were attained by Wang et al. (2002) at pH 5 and 7. They gained the highest HS removal at pH 5 at a dose of 0.17 mg Al mg^{-1} TOC ($Al_2(SO_4)_3.18H_2O$) whereas, for the maximum removal at pH 7, about 5 times higher alum dose was required. Similarly, Van Benschoten and Edzwald (1990b) observed also slightly better removals (by 10% on average determined as UV absorbance at 254 nm) of FA (extracted from the Provencial Brook in Princeton, Massachusetts, USA) at pH 7 than at pH 5.5, but again with a double dose of PACl (0.5 vs 1 mg Al mg^{-1} DOC approx.). The coagulant demand increasing as pH rises could be attributed to a higher portion of deprotonated HA/FA functional groups resulting in a higher charge density and accordingly higher consumption of the coagulant, which shows a decreasing charge neutralizing capacity at the same time (Kam and Gregory, 2001; Cheng and Chi, 2002). Since HS occur in dissociated form over a wide pH range, when being coagulated, they interact with positively charged either Al-hydroxopolymers or Al/Fe-oxide-hydroxides. Charge neutralisation, sweep flocculation and adsorption are thereby commonly referred as removal mechanisms of HS (Van Benschoten and Edzwald, 1990b; Huang and Shiu, 1996; Cheng and Chi, 2002; Wang et al., 2002; Duan and Gregory, 2003; Ghernaout et al., 2009).

Although both HS and AOM may occur in surface waters at the same time, simultaneous coagulation of AOM/cells and HS has not been sufficiently clarified. According to Jiang et al. (1993), the presence of HS (extracted from upland raw water) disrupted coagulation of the diatom *Asterionella formosa*. They investigated the removal of cells and associated AOM released into the culture medium at a concentration of 2.5 mg L^{-1} DOC by four different coagulants: polyferric sulphate, ferric sulphate, aluminium sulphate and PACl. They found that the increasing HS concentrations (0-5 mg L^{-1} DOC) systematically reduced coagulation performance for both cells and DOC and increased anticipated coagulant consumption. For example, HS at 2 mg L^{-1} reduced cell removals from 96 to 86%, from 80 to 61%, from 72 to 55%, from 90 to 70% and DOC removals from 61 to 45%, from 33 to 24%, from 30 to 25%, from 52 to 32% for polyferric sulphate, ferric sulphate, PACl and aluminium sulphate, respectively, under the same reaction conditions (about $5x10^4$ cells mL^{-1}, 0.2 mmol L^{-1} Al/Fe, pH 7.5). On the other hand, potentially beneficial interactions between AOM and HS follow from the evidence of accumulation of HS on the surface of algal cells (Campbell et al., 1997; Vigneault et al., 2000; Knauer and Buffle, 2001). These studies concurred on the favoured adsorption of organic matter at biological surfaces at acidic pH.

Campbell et al. (1997) recorded that the amount of FA associated with the surface of cells of the cyanobacterium *Synechococcus leopoliensis* and the green alga *Chlorella pyrenoidosa* significantly increased as the pH dropped from 7 to 4 and as FA concentrations rose from 0 to 20 mg L^{-1} FA. This observation corresponds to the findings of Vigneault et al. (2000), who observed higher accumulation of SRHA/SRFA at the cell surface of the green alga *Selenastrum capricornutum* at pH 5 than at pH 7 (0.8 vs 0.1 mg C m^{-2} for SRHA and 0.6 vs 0.0 mg C m^{-2} for SRFA of initial concentrations 5 mg C L^{-1}). Similarly, Knauer and Buffle (2001), who dealt with SRFA and three green algae: *Chlorella* sp., *Scenedesmus subspicatus* and *Chlamydomonas reinhardtii*, observed specifically no adsorption onto algal surfaces at pH 7 and 6 compared with up to 4 and even 31 mg SRFA m^{-2} at pH 5 and 4, respectively, for *C. reinhardtii*. In addition, accumulation of HS at cell surface appears to be also species-specific. In comparison to *C. reinhardtii*, the adsorbed amount was 6 times lower for *Chlorella* sp. and even 28 times lower for *S. subspicatus* under the same conditions (10 mg L^{-1} SRFA at pH 4). On the contrary, Campbell et al. (1997) observed in case of *S. leopoliensis* and *C. pyrenoidosa* almost equal exposed cell surface area of 9.7 and 10×10^9 μm^2 mg^{-1} FA, respectively, corresponding to maximum surface adsorption of about 20 mg FA m^{-2} at pH 4, although, initial concentrations of FA in the experiments with those two species differed (10 vs 14 mg L^{-1} FA). Favoured accumulation at the cell surface as the pH is lowered from circumneutral to pH 4 might be ascribed to the character of HS regarded as natural anionic polyelectrolytes (Thurman, 1985; Duan and Gregory, 2003; Ghernaout et al., 2009). When pH drops close to or below dissociation constants of carboxyl groups comprised in both HS and cell surface, their ionization suppresses and they become protonated (Campbell et al., 1997). Due to a decrease in the negative net cell surface charge potential as monitored by the reduced absolute values of electrophoretic mobility (Campbell et al., 1997; Knauer and Buffle, 2001), HS may attach to the cell surface. In this way, especially macromolecular structures might be thus able to aggregate cells through the formation of bridges similar to inorganic colloids (Ghernaout et al., 2009). On contrary, as pH increases, adsorbed layers of HS, together with deprotonated functional groups of other surface-retained organics increase the negative surface charge of algal cells and enhance their colloid stability (Campbell et al., 1997; Kam and Gregory, 2001; Knauer and Buffle, 2001). It is likely that HS interact with AOM and the cell surface through 1) electrostatic interactions, 2) hydrogen bonding involving polar hydrophilic ionisable functional groups present either in HS or on the

external cell surface, and 3) hydrophobic bonds forming between the hydrophobic regions of organics adsorbed on the cell surface, where they interact with the HS hydrophobic domain of either aliphatic or aromatic moieties (Campbell et al., 1997; Myat et al., 2014).

1.2.3 TREATMENT OF ALGAE-LADEN WATERS

The most challenging for water treatment plants (WTPs) is the collapse of algal blooms when both algal cells and consequently high levels of cellular organic matter (COM) are present in water. This could lead even to a breakdown of WTPs (Greene and Hayes, 1981; Henderson et al., 2008a; Zhang et al., 2010; Středa et al., 2015). Therefore, it is well preferred to remove intact cells without any damage that would cause the additional release of organic matter and related adverse effects (Chow et al., 1999; Teixeira and Rosa, 2006, 2007; Fan et al., 2013). Cell stability is generally ascribed to two factors: firstly, repulsive interactions that result from the electronegative surface charge forming diffuse electrical double layers, and secondly to the hydrophilic or steric effects caused by adsorbed water or macromolecules and extracellular organic matter (EOM) on cell surfaces (Bernhardt and Clasen, 1991, 1994; Edzwald, 1993b; Henderson et al., 2008a,c, 2010; Ma et al., 2012a; Vandamme et al., 2012). This explains the easy combination of algal cells with the commonly applied coagulants bearing a positive charge as proved by highly effective removal rates of algal or cyanobacterial cells achieved in various studies (Henderson et al., 2008a,c, 2010; Vandamme et al., 2012; Garzon-Sanabria et al., 2013; Gonzalez-Torres et al., 2014).

Algal cells are usually effectively coagulated through either charge neutralisation or sweep flocculation depending on the applied pH value, type and dose of a coagulant, generally above pH 5 (Bernhardt and Clasen, 1991, 1994; Edzwald, 1993b; Chow et al., 1999; Briley and Knappe, 2002; Vandamme et al., 2012; Wu et al., 2012; Garzon-Sanabria et al., 2013; Gonzalez-Torres et al., 2014). For instance, Gonzalez-Torres et al. (2014) reached 95.9-97.6% and 98.6-99.6% removals of *M. aeruginosa* using aluminium and ferric coagulant ($Al_2(SO_4)_3$ and $FeCl_3$), respectively, at concentrations of up to 10 mg L^{-1} Al/Fe (initial $(8\pm4)\times10^5$ cells mL^{-1}, at pH 6 and 7).

In spite of these highly effective removals, algal cells may not be removable by the conventional treatment processes, i.e. coagulation/flocculation – sedimentation – followed by filtration. This is given by their diverse morphologies and physiologies, especially their

physical dimensions, low specific density, tendency to float, negative charge of surface and high motility, which prevents flocs from settling and allows for their breakthrough rapid sand filters into treated water (Bernhardt and Clasen, 1991, 1994; Briley and Knappe, 2002; Teixeira and Rosa, 2006, 2007; Henderson et al., 2008a,c; Ghernaout et al., 2010). To enhance cell flocs separation efficacy, the dissolved air flotation (DAF) tends to be employed to replace the considerably problematic conventional clarification by sedimentation that usually precedes filtration in a two-phase separation of suspension. Taking advantage of algal buoyancy and low density (<1.2 g cm^3), DAF is less influential by the aggregation efficiency compared to settling and separates even poorly aggregated suspension, which appears to contain smaller and low dense flocs including those of algal or cyanobacterial cells (Edzwald, 1993b; Vlaški et al., 1996; Teixeira and Rosa, 2006, 2007; Ghernaout et al., 2009; Henderson et al., 2010). Nevertheless, as DAF performance was proved to depend on efficient destabilisation of particles, efficient coagulation/flocculation remains still requisite to effectively operate DAF and settling alike (Edzwald, 1993b; Vlaški et al., 1996; Teixeira and Rosa, 2006, 2007).

Research addressing the impact of AOM on coagulation of algae-rich waters has so far focused on EOM released in consequence of pre-oxidation as will be discussed in the following section (Ma and Liu, 2002; Plummer and Edzwald, 2002; Ma et al., 2012a,b; Wang et al., 2013) or via metabolic activity into culture media during the harvest of algal biomass (Vandamme et al., 2012; Wu et al., 2012; Garzon-Sanabria et al., 2013). It was proved that the coagulation of algae and cyanobacteria may be disrupted or even suppressed by the presence of AOM (Henderson et al., 2010; Vandamme et al., 2012; Wu et al., 2012; Garzon-Sanabria et al., 2013). For example, Garzon-Sanabria et al. (2013) harvesting the marine microalga *Nannochloropsis salina* (initial ~ $1x10^7$ cells mL^{-1}, OD_{750} ~ 3.0) found that carbohydrate content (8-19 mg L^{-1} C) increased optimal doses of various coagulants. To obtain equal 90% flocculation efficacies after 1h settling, alum coagulation at pH 5.3 needed about 3-fold more $AlCl_3$ (50 vs 160 mg L^{-1}), a 7-fold dose was necessary when using synthetic cationic polymers without pH control (3 vs 20 mg L^{-1}), and almost 10-fold more chitosan was required (5 vs 50 mg L^{-1}, pH 6.5 before and pH 8 after chitosan addition). Similarly, Vandamme et al. (2012), who investigated the importance of AOM in flocculation of the green alga *Chlorella vulgaris* (0.25 g L^{-1} biomass concentration), reported the increased demand of several coagulants in the presence of EOM carbohydrates at 5 mg L^{-1} C.

Equivalent 85% flocculation yields after 30 min sedimentation were reached at almost a 6-fold dose of alum at pH 5.5 (20 vs 115 mg L^{-1} $Al_2(SO_4)_3.18H_2O$), 9-fold in the case of chitosan at pH 7.5 (8 vs 75 mg L^{-1}), about 4.5-fold for cationic starch without pH control (20 vs 90 mg L^{-1}), and for pH-induced magnesium hydroxides flocculation a shift from pH 10.5 to 11.5 (22 vs 49 mg L^{-1} NaOH) was required. Moreover, the AOM impact is highly pH and species specific as supported by evidence of Wu et al. (2012). They recorded significantly affected flocculation (induced by pH increase) of three freshwater and two marine algal species at the presence of EOM polysaccharides. In the case of *Chlorella vulgaris* and its flocculation at pH 10.5, the efficiency dropped from 92% to 40% and 7% due to polysaccharide concentrations of 25 and 70 mg L^{-1} (measured by the phenol-sulfuric acid method, Dubois et al., 1956). For *Scenedesmus* sp. and *Chloroccocum* sp., 12.2 and 30.4 mg L^{-1} decreased approximately 90% rates to 62% and 50%, respectively, at pH 10.5. Further in the case of marine microalgae, concentrations of 21 and 43 mg L^{-1} slightly reduced removal rate of *Nannochloropsis oculata* from nearly 100% to about 80% at pH 9.5 and finally, for *Phaeodactylum tricornutum,* 101 mg L^{-1} at pH 9.5 had, in fact, no effect and flocculation efficiency still ranged above 90%. Nevertheless, Wu et al. (2012) have demonstrated that the adverse polysaccharide effects might be mitigated by the increase in reaction pH (apart from *C. vulgaris* where the pH increase from 10.5 to 11.5 improved the removal efficiency by no more than about 20%). On the other hand, Bernhardt and Clasen (1991) assumed that flocculation of the green alga *Dictyosphaerium pulchellum* might be enhanced, similarly to the quartz dispersion, by low EOM levels (0.1-2.0 mg L^{-1} as C) and accordingly disrupted by increased concentrations (>1-2 mg L^{-1} C).

The AOM interference/improvement in coagulation/flocculation follows from its charge characteristics. AOM may significantly affect the surface charge of algal cells and thus consequently the coagulant demand as illustrated Henderson et al. (2010). EOM presence at concentrations of 1.5±0.15, 0.6±0.01 and 1.0±0.2 mg L^{-1} as C contributed by 84%, 5% and 30% to the electronegative surface charge of *C. vulgaris*, *M. aeruginosa* and *A. formosa* cell suspensions ($5.0x10^5$ ± $5.0x10^4$, $6.0x10^5$ ± $1.5x10^4$ and $5.0x10^4$ ± $1.2x10^4$ cells mL^{-1}, respectively) and required 1.7, 1.9 and 2.2-fold dose of alum for their charge neutralisation (pH 7, $Al_2(SO_4)_3.18H_2O$, cell removals 94-99%) (Henderson et al., 2010). This study further reported that coagulant demand decreased as MWs and charge density of EOM increased. High content of high-MW matter (45% DOC >500 kDa) aided *M. aeruginosa* coagulation at

pH 5 when a far lower Al dose (0.0087 g m^{-2} Al) provided sufficient cell removals and prevented restabilisation of the system compared to suspension of *C. vulgaris* (0.0195 g m^{-2} Al) that has only about 5% of such high-MW DOC and where a restabilisation zone was observed at a transition between the charge neutralisation and sweep flocculation mechanisms.

To control the treatment of algae-laden waters, several studies (Bernhardt and Clasen, 1991, 1994; Henderson et al., 2008a,c, 2010) revealed a stoichiometric relationship between coagulant demand and charge density or cell surface area. The former appears to be more robust for establishing optimum coagulant doses as opposed to surface area, given that charge density measurement (commonly through the zeta potential) would also take into account the charge of associated released organic matter, irrespective of individual species characteristics (Bernhardt et al., 1985, 1987, 1991; Henderson et al., 2008a,c, 2010). Nevertheless, non-spherical species (i.e. large, filamentous, species with protruding appendages or large colonies forming species) do not conform to these rules. They cannot be treated as colloidal entities through charge neutralisation, thereby requiring the mechanism of sweep flocculation through higher coagulant doses to be applied (Bernhadt and Clasen, 1991; Henderson et al., 2008a,c).

1.3 PRE-OXIDATION

To enhance coagulation/flocculation of algae, numerous studies have employed pre-oxidation using various oxidants such as chlorine, chlorine dioxide, ozone, a combination of ozone and hydrogen peroxide (O_3/H_2O_2), potassium permanganate ($KMnO_4$) or potassium ferrate (K_2FeO_4) (Sukenik et al., 1987; Paralkar and Edzwald, 1996; Petruševski et al., 1996; Widrig et al., 1996; Ma and Liu, 2002; Plummer and Edzwald, 2002; Chen and Yeh, 2005; Chen et al., 2009; Ma et al., 2012a,b; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013; Xie et al., 2013; Qi et al., 2016). The pre-oxidation is deemed effective in promoting the coagulation of both algal cells and associated organic matter. The beneficial effect of pre-oxidation lies in inhibition of algal cells and changes in their external structure reducing colloidal cell stability, as shown by the decreasing absolute zeta potential or reduced electrophoretic mobility (Sukenik et al., 1987; Ma and Liu, 2002; Plummer and Edzwald, 2002; Chen et al., 2009; Coral et al., 2013; Pranowo et al., 2013). Moreover, though

maintaining the relative integrity of the cells, pre-oxidation stimulates algae to secrete desirable amounts of algal organic matter (AOM) (Ma and Liu, 2002; Chen and Yeh, 2005; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013) of which particularly macromolecules may favour algal coagulation. High-MW matter easily combines with a coagulant, promotes coagulation and cell removal most likely through adsorptive bridging, and possibly reduces the coagulant demand (Sukenik et al., 1987; Ma and Liu, 2002; Henderson et al., 2010; Ma et al., 2012a; Pranowo et al., 2013; Wang et al., 2013; Xie et al., 2013).

Pre-oxidation is beneficial for the algal removal particularly at low pre-oxidant levels (Plummer and Edzwald, 2002; Wang et al., 2013). On the other hand, extensive pre-oxidant levels lead to physiological stress associated with cells lysis and elevated excretion of extracellular/cellular organic matter (EOM/COM) (including toxins, T&O compounds and DBPs precursors) which may, in turn, stabilise cells by increasing their negative surface charge (Paralkar and Edzwald, 1996; Wang et al., 2013). Pre-oxidation is further accompanied by degradation of released macromolecules into low-MWs, which are less prone to coagulate, and thus not only reduce the coagulation efficiency, but also concurrently increase consumption of the coagulant as mentioned above (Hoyer et al., 1987; Paralkar and Edzwald, 1996; Plummer and Edzwald, 2002; Świetlik et al., 2004; Ma et al., 2012a; Pranowo et al., 2013; Wang et al., 2013; Xie et al., 2013). On the other hand, increased doses of pre-oxidant, especially ozone at longer reaction time, is then capable of oxidising dissolved organic matter to carbon dioxide, which results in turn in residual DOC decrease (Hoyer et al., 1987; Ma and Liu, 2002; Pranowo et al., 2013; Wang et al., 2013). However, the effect of pre-oxidation differs not only with the oxidant dose and type, but also among phytoplankton species, their growth phase and composition of AOM (Hoyer et al., 1987; Widrig et al., 1996; Ma and Liu, 2002; Plummer and Edzwald, 2002; Coral et al., 2013). This specifity was illustrated for instance by Plummer and Edzwald (2002) who compared ozone and chlorine pre-oxidation of two different species. Pre-treatment with ozone at 1.2 mg L^{-1} enhanced the removal of the green alga *Scenedesmus quadricauda* from initial 83% to 94% and 99% reduction of cell counts when followed by coagulation with 0.2 and 1 mg L^{-1} Al (PACl), respectively, and settling (initial $2x10^4$ cells mL^{-1}, pH 6.5-6.8). In the case of chlorination by 1 mg L^{-1} Cl_2, the alum dose as low as 0.25 mg L^{-1} Al already provided effective coagulation (99% removal of cells). On the contrary, neither pre-ozonization nor

pre-chlorination showed any benefit for the diatom *Cyclotella* sp. On the other hand, the dose as high as 3 mg L^{-1} ozone/chlorine did not cause severe damage to *Cyclotella* cell wall ($1x10^5$ cells mL^{-1}), whereas the same dose applied to *Scenedesmus* suspension had already significantly altered cell morphology and caused cell lysis (Plummer and Edzwald, 2002). Moreover, Plummer and Edzwald (2002) demonstrated that AOM released from pre-oxidation of *S. quadricauda* suspension induced flocculation of this alga even without addition of a coagulant obtaining 58%reduction in cell counts (1.2 mg L^{-1} $O_3 \sim 0.058$ mg O_3 per 10^3 cells at pre-oxidation time of 30 min raised DOC concentrations from initial 0.2 to over 1 mg L^{-1}). Nevertheless, single pre-chlorination with 1 mg L^{-1} with no coagulant added reduced only 10% of cells.

Moreover, pre-oxidation using chlorine, chlorine dioxide and ozone is associated with the elevated risk of disinfection by-products (DBPs) (Petruševski et al., 1996; Coral et al., 2013; Xie et al., 2013). To mitigate the formation of DBPs, the permanganate is an effective alternative (Chen et al., 2009) as supported by the findings of Xie et al. (2013). They compared the effect of ozone and permanganate followed by alum coagulation, sedimentation and downstream chlorination on the removal of *M. aeruginosa* and DBPs production. Both pre-oxidants improved the initial cell removal efficacy of 56.7% (initial $(1.01\pm0.06)x10^6$ cell mL^{-1}, 0.8 mg L^{-1} Al, pH 7) by 15-25% depending on the type and dose of pre-oxidant with almost the same maximum cell removal rates (78% and 80.2% at dose of 1 mg L^{-1} $KMnO_4$ and 2 mg L^{-1} O_3, respectively). Analogously in the study by Chen et al. (2009), both ozone and permanganate at optimum doses (1 mg L^{-1} O_3 and 1.25 mg L^{-1} $KMnO_4$) enhanced removal of *Chlorella* sp. from 84% to 91% and 98%, respectively through alum coagulation (70 mg L^{-1} (7.5% Al_2O_3)) followed by sedimentation. However, permanganate has been shown to be less destructive pre-oxidant. Up to 2 mg L^{-1} of $KMnO_4$ induced mainly release of EOM and surface-bonded/retained organic matter (SOM) without causing cell damage or change in cell viability (Chen et al., 2009). Xie et al. (2013) recorded no DOC increase using 2 mg L^{-1} $KMnO_4$ while the same dose of ozone caused DOC increase from 0.42 to 1 mg L^{-1}. Consequently, permanganate did not affect the formation of DBPs while ozone brought about the extensive destruction of cells, loss of their integrity and increase in low-MW COM precursors of DBPs (Chen et al., 2009; Xie et al., 2013). In addition, permanganate produces manganese dioxides (MnO_2) accelerating the flocculation kinetics at higher pH (Chen et al., 2009; Wu et al., 2012). Colloids formed by hydrous MnO_2 serving as initial cores

of precipitation facilitate the formation of primary aggregates, increase particle concentration and thus collision frequency. As another benefit, MnO_2 together with the released EOM/SOM/COM adsorb onto the cell surface and thus promote the growth of flocs with increased size, specific weight and settling velocity (Petruševski et al., 1996; Ma and Liu, 2002; Chen and Yeh, 2005; Chen et al., 2009; Ma et al., 2012a,b; Pranowo et al., 2013).

It follows that research into the pre-oxidation of algae-laden waters has been dealing with the whole algal suspensions rather than with single AOM (Ma et al., 2012a,b; Wang et al., 2013; Xie et al., 2013) and if so, even though COM most likely forms the bulk of AOM in surface waters after the decline of an algal bloom, attention has been given predominantly to the EOM fraction (Hoyer et al., 1987; Paralkar and Edzwald, 1996; Widrig et al., 1996). Similarly to the cells, the effect of pre-oxidation on the removal of EOM is species-specific and changes with a dose of both the coagulant and pre-oxidant as observed in the study by Widrig et al. (1996). At a relatively low dose of 0.8 mg O_3 mg^{-1} C, pre-ozonation performed at pH 8 and followed by coagulation at pH 5 (a dose of 2.96 mmol Fe) improved removal of EOM derived from *M. aeruginosa*, *S. quadricauda* and *D. pulchellum* by about 5-15% depending on specific species (Widrig et al., 1996). On the other hand, they observed an increase in lower-MW fractions indicating the plausible cleavage of polymers to smaller units when pre-oxidation induces the breakage of e.g. glycosidic bonds in saccharide compounds followed by their oxidation (Hoyer et al., 1987; Paralkar and Edzwald, 1996). Either adsorbed or released organics might be oxidised to more polar forms. Pre-oxidation reduces their aromaticity and increases negative charge, thereby enhancing interactions of especially carboxyl groups in surface associations with coagulant species (Reckhow et al., 1986; Paralar and Edzwald, 1996; Shao et al., 2014). In addition, ozonation was observed to break-up organo-metal complexes and re-release oxidized metals (e.g. Fe(III) or Mn(IV)) (Reckhow et al., 1986). Additionally, also microcystins (MCs) having MW as low as about 1 kDa, which usually demand implementation of advanced techniques such as membrane nano-filtration (Teixeira and Rosa, 2005; Dixon et al., 2010) or adsorption onto activated carbon (Ho et al., 2011), were shown to be oxidised and thus consequently degraded by pre-oxidation using potassium permanganate (Chen et al., 2005; Jurczak et al., 2005; Rodríguez et al., 2007; Fan et al., 2013; Shao et al., 2014). In the studies by Jurczak et al. (2005) and Rodríguez et al. (2007), permanganate secured rapid degradation of MCs and maintained their concentrations below the guideline value of 1 μg L^{-1} for MC-LR (-L = leucine, -R = arginine)

established by the World Health Organisation (WHO, 2011) and implemented in the Czech national legislation (the decree No. 252/2004 Coll.). The reaction rate has been reported to accelerate with increasing temperature and oxidant concentration with a negligible effect of pH (Chen et al., 2005); another important factor is also reaction time (Fan et al., 2013). Nevertheless, dependence on the initial MCs concentration is contradictory (Chen et al., 2005; Rodríguez et al., 2007). To keep the balance between avoiding extensive pre-oxidation and improving the removal of algae/AOM, moderate pre-oxidant levels considering specific conditions of both source water and employed water treatment processes should be applied (Hoyer et al., 1987; Rodríguez et al., 2007; Coral et al., 2013; Fan et al., 2013).

2 MOTIVATION AND OBJECTIVES

In the context of eutrophication of aquatic environments, drinking water production faces incidence of algal blooms in surface water resources. Algal organic matter (AOM) is generally believed to disrupt the coagulation and affect even the removal of other impurities naturally occurring in source water. However, only a handful of studies have dealt with their simultaneous coagulation and preferably focused on extracellular organic matter (EOM). Moreover, most existing studies have predominantly discussed coagulation dependence on a coagulant dose rather than pH value and hence have not systematically explained the conditions and mechanisms under which AOM may influence coagulation. What has not received much attention so far is cellular organic matter (COM) released after cell lysis, which may form a majority of dissolved natural organic matter contained in surface waters (Henderson et al., 2008b). COM shows in comparison to EOM higher degree of hydrophilicity reflecting its limited treatability (Fang et al., 2010; Li et al., 2012; Liao et al.; 2015). It was also found to be much more involved in coagulation interference rather than the EOM fraction (Takaara et al., 2007). And furthermore, COM were reported to create higher amounts of DBPs, including nitrogenous ones (except for trichloronitromethane) (Fang et al., 2010), which corresponds to a larger portion of proteinaceous matter comprised in COM when compared with EOM (Pivokonsky et al., 2006; Henderson et al., 2008b).

For these special characteristics, which make the COM fraction by far the most significant, this Ph.D. thesis addresses the challenges that COM poses for water treatment by examining its traits, potential of removal and impact on coagulation of other particles or molecules present in surface waters. The knowledge of the character of COM is essential for understanding its treatability, evaluation of the level of interference, and subsequent optimization of the removal processes. Finally, by looking into interactions between COM, other impurities and coagulants, this thesis indicates the optimum coagulation conditions and presents novel data and insights into the impact of cyanobacterial COM on coagulation.

The emphasis is placed preferentially on the proteinaceous part of COM of the common cyanobacterium *Microcystis aeruginosa* that has been reported to comprise approximately 60% of cellular matter (Pivokonsky et al., 2006, 2009, 2012; Henderson et al., 2008b). To test our hypothesis that COM may enhance coagulation of various impurities under properly designed reaction conditions, we ran the jar tests with mixtures of

M. aeruginosa proteinaceous COM and kaolin particles or humic substances and whole COM and cells of *Merismopedia tenuissima*. The coccoid cyanobacterium *M. tenuissima* has been chosen as it represents a widespread species of a tiny cell size (not exceeding 2 µm in diameter) (Hörnström, 1999; Komárek, 2003; Dantas et al., 2011; McGregor, 2013) allowing cells to break through water treatment plants (WTPs) and pass into treated water. Moreover, unlike other phytoplankton species, and occupying nearly the entire water column (Dantas et al., 2011; Koza and Rederer, 2014), *M. tenuissima* does not create the typical cyanobacterial upper bloom near the water surface. Thus, WTPs cannot prevent *M. tenuissima* from its input by adjusting the depth of the sampled profile. We coagulated this cyanobacterium in the presence of its whole COM to simulate natural conditions after the decay of its populations.

The specific objectives of the study were addressed as follows:

- Characterise AOM derived from various phytoplankton species (cyanobacteria *Microcystis aeruginosa* and *Merismopedia tenuissima*, green alga *Chlamydomonas geitleri*, and diatom *Fragilaria crotonensis*) in terms of molecular weight (MW) distribution, hydrophobicity, charge and protein content, traits important from the perspective of water treatment by means of coagulation (Publications 1, 2, 8).
- Investigate coagulation of hydrophilic proteinaceous COM of *M. aeruginosa* (Publications 3, 4, 5, 7), the entire COM of *M. tenuissima* (Publication 8), hydrophobic kaolin suspension representing clay colloids (Publications 1, 5) and peat humic substances (HS) standing for another fraction of natural organic matter (NOM) (Publication 4), as well as cyanobacterial cells of *M. tenuissima* (Publication 8); assess the impact of COM on the coagulation of aforementioned impurities (Publications 1, 4, 5, 8).
- Investigate the impact of pre-oxidation step on the coagulation of proteinaceous COM of *M. aeruginosa* using potassium permanganate as a pre-oxidant (Publication 7).
- Review the up-to-date knowledge of the AOM with respect to its treatability, coagulation mechanisms, impact on the drinking water treatment techniques and quality of purified water; outline the knowledge gaps for future endeavours (Publications 3, 6).

Those aims were fulfilled by carrying out series of experiments in a laboratory scale. Specific results have been published in eight papers that constitute the main body of this Ph.D. thesis.

3 SUMMARY

The Ph.D. thesis evaluates differences between extracellular (EOM) and cellular (COM) organic matter of four phytoplankton species: *Chlamydomonas geitleri*, *Fragilaria crotonensis*, *Microcystis aeruginosa* and *Merismopedia tenuissima*. Choosing COM as a more challenging issue for water treatment than EOM fraction, we assessed the removability of COM derived from two cyanobacterial species: *M. aeruginosa* and *M. tenuissima*. In the case of *M. aeruginosa*, the emphasis was dominantly placed on the proteinaceous part of COM. We investigated the effect of COM on coagulation of other impurities occurring in surface water sources, i.e. kaolin, humic substances (HS) and cyanobacterial cells. Based on charge relations of single components and coagulants, we discussed the plausible interaction mechanisms between them in relation to a pH value and coagulant dosage. Finally, we reviewed the removability of algal organic matter (AOM) by means of various water treatment techniques and the impact of AOM on those technologies.

The specific results can be summarised as follows:

1) The four microorganisms (*Chlamydomonas geitleri*, *Fragilaria crotonensis*, *Microcystis aeruginosa* and *Merismopedia tenuissima*) demonstrated several similarities; however, their traits changed with both the species and the growth phase. All the microorganisms' AOM shared significant proteinaceous portion, molecular weight (MW) diversity as well as predominantly hydrophilic nature corresponding to low specific UV absorbance (SUVA). The values of all parameters except for SUVA rose as the cultures grew and they also revealed the differences between EOM and COM. Furthermore, both EOM and COM of all four species contained large portions of low-MW (<1 kDa) and high-MW (>100 kDa) non-proteinaceous matter, whereas their proteinaceous fractions exhibited narrower MW distribution and insignificant content of low-MWs. Nevertheless, the proteinaceous low-MW fraction might be underestimated or the non-proteinaceous low-MW fraction in turn overestimated due to the limitations of the salting out method for isolating amino acids (Publications 1, 2, 8).

2) Coagulation of all individual impurities highly depends on the reaction pH that not only governs the charge traits of the components and coagulant species, but also controls coagulation mechanisms accordingly.

Both the proteinaceous COM of *M. aeruginosa* and the entire COM of *M. tenuissima* succumbed to efficient coagulation in the acidic pH (4.0-6.0 with Fe or 5.2-6.7 with Al in the case of proteinaceous COM of *M. aeruginosa* and 5.0-6.0 with Fe for *M. tenuissima* COM) and with the removal rates of 60-85% and 43-53%, respectively, depending on the initial dissolved organic carbon (DOC) concentration. Under those conditions, COM undergoes charge neutralisation by polynuclear cationic Al/Fe-hydroxopolymers. Al/Fe-hydroxopolymers interact with dissociated functional groups in COM (in particular carboxyl ones) and allow for its destabilisation. Moreover, limited removals were obtained at around neutral pH 6-8 when COM can adsorb onto the cationic surface of colloidal Al/Fe-oxide-hydroxides by not only forming hydrogen or covalent bonds, but also facilitating ligand exchange (Bernhardt et al., 1985). Nevertheless, the extent of AOM removal by adsorption mechanism is usually less significant since adsorption is effective only at the low DOC:coagulant ratio (e.g. <0.33 for Fe). Within the given pH range (6-8), gradual restabilisation is more likely to occur instead; COM extensively occupies Fe-oxide-hydroxide surface, raises its negative charge density, and causes consequential charge reversal and stabilisation (Bernhardt et al., 1985; Pivokonsky et al., 2012) (Publications 3-8). Bovine serum albumin (BSA) was proved to be a suitable model protein coagulating in a narrow pH optimum of 5.8-6.2 (for Al) and with a maximum removal rate of 85% under the used conditions (Publication 4).

The kaolin suspension was coagulated much more effectively at about neutral pH (6.4-8.0 for ferric and 7.0-8.5 for aluminium coagulant) with maximum turbidity removal of 99%. The presence of *M. aeruginosa* proteinaceous COM substantially shifted the pH for turbidity removal to the moderately acidic pH as for single proteinaceous COM (4.0-5.5 for Fe and 5.0-6.5 for Al) and altered coagulation mechanisms accordingly. While single kaolin interacted preferably through adsorption of Fe-oxide-hydroxides onto the electronegative surface of kaolin particles (Bernhardt et al., 1985), the proteinaceous COM-kaolin mixture yielded to charge neutralisation by Al/Fe-hydroxopolymers. Moreover, proteinaceous COM induced kaolin flocculation probably through inter-particle bridging even in the absence of a coagulant at acidic pH (approx. <4.5), but only with lower DOC removal rates (about 45%) compared to the tests with Al/Fe. Otherwise, at higher pH when COM contains a higher rate of dissociated carboxyl groups, COM in turn stabilises kaolin suspension (Publications 1, 5).

In the case of humic substances (HS), maximum DOC removal rate of 65% was gained at pH 5.5-6.0 for aluminium by means of charge neutralisation with Al-hydroxopolymers. Similarly to single proteinaceous COM of *M. aeruginosa*, HS were removed to some extent even at pH 6-7 due to their adsorption onto Al-oxide-hydroxides. The addition of BSA or the proteinaceous COM did not almost change the optimum pH for HS removal, but produced higher DOC removals (of about 80% for COM-HS and of 83% for HS-BSA) using even lower coagulant doses than for single HS (almost twice as lower in the case of the COM-HS and almost 3.5 times lower for the BSA-HS mixture). In addition, a noticeable DOC reduction was observed even at pH <5 for both mixtures even though the coagulant remained dissolved and did not participate in coagulation. In the tests without the coagulant, proteinaceous COM induced flocculation of HS through inter-particle bridging at acidic pH below 3.5-4.5 with DOC removals of 55-69%, in the case of BSA at pH below 4.0-4.5 with DOC removal efficiency of 68-77%, depending on DOC initial ratio of those components. However, at higher pH, flocculation was not effective without the addition of the coagulant due to the repulsion between negatively charged functional groups within the organic molecules (Publication 4).

Coagulation tests with cell suspensions of *M. tenuissima* proved maximum cell removals of 99% in the pH range of 6.0-7.7 for ferric coagulant. Simultaneous coagulation of cells and associated COM then considerably changed the pH optimum and consequently the interaction mechanisms. While single electronegative cells readily adsorbed onto Fe-oxide-hydroxides at about neutral pH, the COM-cells mixture succumbed charge neutralisation by Fe-hydroxopolymers within moderately acidic pH corresponding to the pH optimum for single COM (5.0-6.5). Additionally, COM induced cells flocculation also at acidic pH (3.4-3.9 in the presence and 3.6-4.6 in the absence of the coagulant) with nearly the same COM/cell removal rates. Furthermore, the tests with COM-cell mixtures produced similar COM/cell removals using even lower coagulant doses than the single component tests (Publication 8).

Electrostatic interactions, hydrogen or hydrophobic bonding facilitating inter-particle bridging between COM and kaolin particles, humic substances or cells proved to be beneficial for the coagulation/flocculation of those components. Inter-particle bridging is particularly ascribed to high-MW COM (>10 kDa) serving as a cationic polymer flocculant aid (Henderson et al., 2010; Ma et al., 2012a). These polymer chains akin to cationic

polyelectrolytes may protrude from HS, kaolin or cell surfaces, overcome the repelling forces and the distance between those particles, thus triggering their aggregation (Gregory, 1973, 1996; Bernhardt et al., 1985; Henderson et al., 2010) (Publications 1, 4, 5, 8).

3) Relatively low removals of *M. tenuissima* COM and *M. aeruginosa* proteinaceous COM can be ascribed to the prevalence of hydrophilic fraction and to the high portion of low-MWs (<10 kDa) as they proved to be less prone to coagulation if they are either of saccharide (Bernhardt et al., 1985) or proteinaceous nature (Pivokonsky et al., 2012) compared to high-MW and hydrophobic matter (Ghernaout et al., 2010) (Publications 3, 4, 8). Moreover, the proteinaceous COM of *M. aeruginosa* was interfering with the coagulation by the formation of soluble organo-metal complexes at about pH 6.2 for Fe and 6.8 for Al either in the single proteinaceous COM coagulation tests, or in the tests with the proteinaceous COM-kaolin mixture (Publications 1, 3, 5).

When applied permanganate, pre-oxidation followed by coagulation with ferric sulphate enhanced the removal of proteinaceous COM of *M. aeruginosa* by 5-12% (depending on the permanganate dose) compared to coagulation alone. Higher DOC removals probably stem from the decomposition of organics to inorganic carbon; on the other hand, pre-oxidation could simultaneously degrade high-MW matter into low-MW organics that have been proved to be less amenable to coagulation and thus decreasing the coagulation efficacy. Pre-oxidation further slightly lowered coagulant doses (from 7.0 to 6.4 mg L^{-1} Fe for initial 8 mg L^{-1} DOC) and extended pH optimum (from 4.3-6.0 to 4.0-7.3). The shift in the pH is given probably by the formation of hydrous manganese dioxide (MnO_2) that might be involved in adsorption processes. However, considering the acceptable residual manganese levels, the coagulation pH above 5.5 can be recommended (for doses of 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC). Besides, permanganate pre-oxidation eliminated concentrations of toxic microcystins and prevented the formation of organo-metal complexes (Publication 7).

4 CONCLUSIONS

Based on Publications 1-8, the following conclusions can be drawn:

Coagulation was proved to be effective in the treatment of either turbid or algae-laden waters removing both colloid kaolin particles and cells of cyanobacterium *M. tenuissima*. Despite relatively low AOM removability, coagulation remains the key water treatment step that requires proper optimisation of reaction conditions in all cases. Considering the complex nature of AOM reflected by the differences between EOM and COM, the treatment strategy for source water containing algae or cyanobacteria should take into account not only the species and the growth phase of phytoplankton populations, but the COM release as well.

Most notably, the evidence of this study supports the claim that the increased COM levels may not inevitably deteriorate the coagulation process. Provided that COM interacts with other impurities, those interactions can in turn lower coagulant doses and not only enhance the turbidity removal, but also favour the coagulation efficacy of other organics represented by peat humic solution when compared with the treatment of single components alone. Moreover, in particular high-MW COM induces flocculation and aggregation of those impurities even in the absence of the coagulant at acidic pH (approximately <4.5). Low-MW matter, by contrast, withstands coagulation and requires the introduction of additional sophisticated technologies such as membrane filtration or adsorption onto activated carbon. The COM removability itself may be then enhanced by permanganate pre-oxidation that additionally obviates the formation of organo-metal complexes.

Unlike previous studies, our findings are the first to demonstrate that even increased concentrations of AOM may contribute to effective coagulation of algae. This outcome has clear implications for the flocculation-based harvest of algal cultures for e.g. biofuel purposes. In drinking water treatment, a decrease in pH might be a prerequisite for the efficient coagulation of both COM and algal or cyanobacterial cells during the decay of algal blooms. However, considering plausible further cell lysis and subsequent extensive COM release in the acidic pH range, the coagulation pH about 5.0-5.5 can be recommended.

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6 PUBLICATIONS

PUBLICATION 1

Influence of peptides and proteins produced by cyanobacterium *Microcystis aeruginosa* on the coagulation of turbid waters

Jana Safarikova, **Magdalena Baresova**, Martin Pivokonsky and Ivana Kopecka

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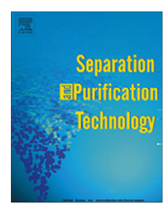

Influence of peptides and proteins produced by cyanobacterium *Microcystis aeruginosa* on the coagulation of turbid waters

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Peptides/proteins
Turbidity removal

ABSTRACT

The study investigated the influence of cellular peptides and proteins derived from cyanobacterium *Microcystis aeruginosa* on the coagulation of kaolin particles during water treatment. To describe the coagulation mechanisms, coagulation system constituents (peptides/proteins, kaolin and coagulant) were characterized in terms of their surface charges. The removal mechanisms of peptides/proteins and kaolin were evaluated by the comparison of the coagulation tests performed with and without coagulant (ferric or aluminum sulfate). We confirmed the peptide/protein inhibiting effect on coagulation through the formation of dissolved complexes with coagulants at a pH value of about 6 for Fe and a pH value of about 6.8 for Al. On the other hand, we demonstrated that cyanobacterial peptides/proteins also have positive effects as they induce the coagulation of hydrophobic kaolin particles within the pH range 4–6 for Fe and 5–6.5 for Al. Interestingly, when peptides/proteins bear a sufficiently low amount of negative charge (pH < 4.5), they can coagulate with kaolin by means of electrostatic interactions even in the absence of a coagulant. The study showed that peptides/proteins produced by *M. aeruginosa* can serve as coagulation aids and contribute to the turbidity removal at pH values below neutral (pH < 6 for Fe and pH < 6.5 for Al).
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1. Introduction

Eutrophication, followed by the growth of cyanobacteria, such as *Microcystis aeruginosa*, brings about several issues in drinking water treatment, especially when algal organic matter (AOM) is released into raw water [1–3]. A number of deleterious effects of AOM on drinking water treatment has been reported: reduction of coagulation efficiency resulting in a rising coagulant demand [1–5], increased membrane fouling [5], filter clogging, higher yield of sludge as a result of an increased coagulant dose [1] and disinfection by-product formation [6]. In addition, AOM affects the color, taste and odor of drinking water [6] and a number of cyanobacterial species also excrete toxic metabolites which can cause health problems [7]. AOM comprises extracellular organic matter (EOM) resulting from the algal metabolic activity and cellular organic matter (COM) released into water during the cell decay. In a simplified way, AOM can be divided into peptide/protein and non-peptide organic matter [8–10].

In surface water, AOM is usually accompanied by other impurities, most commonly by inorganic colloidal particles which need to be removed together with AOM during the water treatment process. Some authors have, therefore, focused on the coagulation inhibition caused by AOM, specifically on the effect of AOM on the coagulation of inorganic colloidal particles, such as quartz or kaolin [1,8,9]. Particular attention has been paid to COM peptides and proteins that are able to form soluble complexes with coagulants, which results in a coagulant consumption and a subsequent decrease in coagulation efficiency [1,8–11]. Moreover, AOM was also reported to interfere with the coagulation using cationic biopolymers such as chitosan and cationic starch [4]. Cationic biopolymer coagulants may interact with oppositely charged polyelectrolytes within the AOM, such as carbohydrates and proteins, which leads to the dispersion restabilization [4]. On the other hand, some studies suggested that cyanobacteria-derived organics might enhance the coagulation of other impurities under specific conditions [1,3], similar to a range of natural polymers commonly used in water treatment (e.g. chitosan, sodium alginate, and seeds of *Moringa oleifera*) [12]. To achieve an efficient coagulation in a system consisting of multiple impurities such as inorganic colloidal particles and AOM, it is necessary to understand the pathways by which these impurities are removed. Although researchers have investigated the coagulation mechanisms of inorganic colloidal particles and organic matter, the majority of studies have focused separately on the interactions between the coagulant and only one of these impurities [11,13–16]. Little work has been carried out to elucidate the coagulation mechanisms when both these impurities (AOM and inorganic colloidal particles) are present in raw water [8,9]. The highest concentrations of dissolved AOM are present in surface water during the algal bloom decay in the form of COM released from damaged cells. At that time, the

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most serious deterioration of coagulation process is usually observed [2]. It was reported that the major portion of cyanobacterial COM is represented by peptides and proteins [10].

Therefore, the purpose of this study was to examine the coagulation behavior of the system consisting of COM peptides/proteins produced by cyanobacterium *M. aeruginosa* and hydrophobic kaolin particles, which represented inorganic colloids. The study focuses on understanding the interaction mechanisms between COM peptides/proteins, kaolin particles and hydrolysis products of coagulants (Al and Fe salts). Because both AOM and kaolin removal has been reported to be highly pH-dependent [11,13–15,17], particular emphasis was put on the effect of pH on the coagulation efficiency.

2. Experimental

2.1. Cultivation of M. aeruginosa

The inoculum of cyanobacterium *M. aeruginosa* was obtained from the Culture Collection of Algal Laboratory, Institute of Botany, AS CR, Czech Republic. The culture of *M. aeruginosa* was harvested on the 16th day of cultivation during the steady-state growth, when the concentration of chlorophyll-a was 2340 $\mu g\ L^{-1}$. Methodologies of cultivation and chlorophyll-a measurements are described in the literature [10].

2.2. COM peptide/protein preparation

The *M. aeruginosa* cells were separated from the growth media by a 0.22 μm membrane filter (Millipore, USA). To obtain COM, the cells were mixed with ultra-pure water (200 ml) and disrupted in ice bath using an ultrasonic homogenizer (UP400S, Hielscher Ultrasonics, Germany) at 60% amplitude of ultrasonication (240 W) in pulse mode for 5 min. Dissolved COM was separated from residual solids by a 0.22 μm membrane filter (Millipore, USA) and thereafter concentrated tenfold in a rotary evaporator (Laborota 4000 HB/G1, Germany) at 20 °C. Peptides/proteins were isolated from the COM by precipitation using $(NH_4)_2SO_4$, the methodology of which is described in our previous studies [10,15]. The peptide/protein precipitate was then separated from the dissolved non-peptide organic matter by filtration through a 0.22 μm membrane filter (Millipore, USA) and dissolved in 200 ml of ultra-pure water. The obtained peptide/protein solution was purified using an ultrafiltration membrane PLAC 1000 Da (Millipore, USA) and a Solvent Resistant Stirred Cell (Millipore, USA). To determine the portion of peptide/protein and non-peptide (comprising mostly saccharides and polysaccharides) matter in COM, DOC concentration was analyzed in the filtrate as non-peptide DOC (DOC_{NP}). The peptide/protein portion DOC_P was calculated as follows [10]:

$$DOC_P = DOC_T - DOC_{NP} \quad (1)$$

where DOC_P is the amount of peptide/protein DOC, DOC_T the total DOC of the COM, and DOC_{NP} the amount of non-peptide (saccharide) DOC. The peptide/protein precipitations were carried out in triplicate and errors of DOC_P calculation were less than 5% and final DOC_P concentrations were around 500 $mg\ L^{-1}$.

2.3. COM peptide/protein characterization

2.3.1. DOC analysis

Concentration of peptide/protein COM was monitored as dissolved organic carbon (DOC) in samples filtered through a 0.22 μm membrane filter (Millipore, USA) using a Shimadzu TOC-V_{CPH} analyzer (Shimadzu Corporation, Japan). TC–IC method, which measures total organic carbon (TOC) as the difference between total carbon (TC) and inorganic carbon (IC) analysis values, was employed. All measurements were conducted in triplicate and errors of measurement were less than 2%.

2.3.2. Molecular weight fractionation

Apparent molecular weights (MW) of COM peptides/proteins were determined by high performance size exclusion chromatography (HPSEC) using Agilent Bio SEC-5 100 Å, 300 Å and 500 Å columns (7.8 × 300 mm, 5 μm) connected in series (separation range 100–1,250,000 Da). The HPLC system (Agilent Technologies, USA) was coupled with a diode array detector (DAD) operated at 280 nm and it was calibrated using peptide and protein SEC standards (Sigma–Aldrich, USA) of MW range from 224 Da to 900 kDa. The apparent MWs were calculated using a semi-log calibration curve ($r = 0.98$). Reproducibility of the MW fractionation of COM peptide/protein samples was very good, with MW deviations of less than 3% from repeated measurements. The methodology of HPSEC is described in detail elsewhere [11].

2.3.3. Charge determination

Isoelectric focusing (IEF) carried out with a Multiphor II electrophoresis system (Pharmacia, Sweden) was performed to determine isoelectric points (p*I*) of isolated COM peptides/proteins. This method is further described in our previous study [11].

Determination of the amount of peptide/protein functional groups which are able to accept proton (or dissociate) and, therefore, bear a charge was undertaken by potentiometric titrations performed under nitrogen atmosphere using an Orion 960 Autotitrator (Thermo Scientific, USA). The samples containing COM peptides/proteins ($DOC_P \approx 500\ mg\ L^{-1}$) were prepared in 150 ml of ultra-pure water with 0.1 M NaCl. After their pH value had been adjusted to 12 by 1 M NaOH, the samples were titrated to pH 1.5 using 0.05 M HCl at a constant temperature 25.0 ± 0.2 °C. A blank titration was also performed under the same conditions. The number of deprotonated functional groups present in peptides/proteins was determined as the difference between the peptide/protein titration curve and the blank curve [18]. It can be assumed that the points of titration curve with the minimum rate of change in pH with added H^+ ions represent dissociation constants of peptide/protein functional groups. Moreover, points of titration curve with the maximum rate of change in pH with added H^+ ions were taken to be equivalence points. They indicate the pH value where the influence of one functional group on the titration process starts and the influence of another one ends [19]. The difference in a number of accepted H^+ ions between the two equivalence points corresponds with the amount of a specific functional group which dissociate in the pH range between these equivalence points. The number of a specific functional group was calculated as follows:

$$N_R = N_H^+(I_1) - N_H^+(I_2) \quad (2)$$

where N_R is the number of a specific functional group, $N_H^+(I_1)$ is the amount of added H^+ ions in equivalence point 1 where dissociation of the given functional group starts, $N_H^+(I_2)$ is the amount of added H^+ ions in equivalence point 2 where dissociation of the given functional group ends.

2.4. Kaolin characterization

The kaolin clay (particles <4 μm) was obtained from the deposit of Sedlec (Sedlecky Kaolin, Czech Republic). Kaolin was dispersed in ultra-pure water, then homogenized using an ultrasonic homogenizer (UP400S, Hielscher Ultrasonics, Germany) at 100% amplitude of ultrasonication (400 W) in pulse mode for 30 min, and immediately after homogenization used in coagulation experiments.

2.4.1. Kaolin charge determination

Its pH-dependent charge was determined by potentiometric titrations performed at three electrolyte concentrations. Specifi-

cally, 40 g of kaolin clay (<4 μm) was mixed with 1.0, 0.1 and 0.01 M solutions of NaCl and the final volume was 400 ml. Then, 0.1 M NaOH was added to reach an initial pH of 12 and the samples were titrated with 0.1 M HCl to pH 2.5 in nitrogen atmosphere using an Orion 960 Autotitrator (Thermo Scientific, USA). Blank titrations were also performed. The relative charge was determined from the difference between titration curves and blank curves and was then plotted against pH. The point of zero charge (pzc) of kaolin was estimated at the pH where titration curves crossed [20].

2.5. Coagulation tests

Jar testing was done with the variable speed eight position paddle stirrer (LMK 8-03, IH ASCR, Czech Republic) and 2L jars. Either aluminum ($Al_2(SO_4)_3{\cdot}18H_2O$; Sigma–Aldrich, USA) or ferric sulfate ($Fe_2(SO_4)_3{\cdot}9H_2O$; Sigma–Aldrich, USA) were used as coagulants. To enable the description of probable coagulation mechanisms, three types of jar tests were compared: (1) with kaolin (25 mg L^{-1}, Tu ≈ 100 NTU), (2) with kaolin (25 mg L^{-1}, Tu ≈ 100 NTU) and COM peptides/proteins (DOC concentrations of 1, 3, 5 and 8 mg L^{-1}) together and (3) with kaolin (25 mg L^{-1}, Tu ≈ 100 NTU) and COM peptides/proteins (DOC concentrations of 1, 3, 5 and 8 mg L^{-1}) without a coagulant (Al or Fe). The model water, into which kaolin and COM peptides/proteins were added, was ultra-pure water with alkalinity adjusted to 1.5 mmol L^{-1} (75 mg L^{-1} $CaCO_3$) by $NaHCO_3$ and with pH 8.5. To describe the effect of COM peptides/proteins on the coagulation of kaolin particles, the dose of the Al/Fe coagulant was optimized for kaolin (25 mg L^{-1}, Tu ≈ 100 NTU) by the tests without pH control and with coagulant doses ranging from 0.037 to 0.370 mmol L^{-1} Al (1–10 mg L^{-1} Al) or 0.018 to 0.180 mmol L^{-1} Fe (1–10 mg L^{-1} Fe). The change in pH was dependent on the Al or Fe dose and ranged from 3 to 10. The influence of coagulant dose on the removal of COM peptides/proteins was determined in our previous studies [11,15]. For experiments with pH control and the optimized coagulant dose (0.075 mmol L^{-1} Al, 0.072 mmol L^{-1} Fe), the target pH (varying between 3 and 10) was adjusted by adding a predetermined amount of 0.1 M NaOH or 0.1 M HCl prior to the addition of coagulated impurities and coagulants. The jar test procedure consisted of 1 min of high intensity agitation (shear rate ~200 s^{-1}, calculated from torque measurement), followed by 15 min of low intensity agitation (shear rate ~50 s^{-1}, calculated from torque measurement) and 60 min of settling. After the sedimentation of suspension, the samples were analyzed for residual Al or Fe, dissolved organic carbon (DOC), turbidity (Tu), pH and alkalinity. Al and Fe concentrations were measured by colorimetric method using an UV–VIS 8453A spectrophotometer (Agilent Technologies, USA). The measurements of aluminum were carried out at a wavelength of 580 nm and pyrocatechol violet was used as the colorimetric agent [21]. Iron was measured with thiocyanate at a wavelength of 480 nm [21]. The residual turbidity was determined nephelometrically by a TURB 555 IR turbidimeter (WTW, Germany). Molecular weights of residual peptides/proteins were also monitored as described in Section 2.3.2.

Moreover, the scanning electron microscopy (SEM) was used to provide additional information for the better description of the underlying coagulation mechanisms. Samples of aggregates settled after jar tests were gradually dried at 25 °C for 30 min and then scanned uncoated by Vega 3 equipped with secondary electron detector (Tescan, Czech Republic).

3. Results and discussion

3.1. COM peptide/protein characterization

Cellular organic matter (COM) of cyanobacterium *M. aeruginosa* was found to comprise about 63% of peptide/protein material determined as DOC_P, which is in agreement with the findings of other studies [11,22]. The COM peptides/proteins consequently used in coagulation experiments were further characterized in terms of molecular weight (MW) distribution. Peptides/proteins of apparent MWs of approximately 1, 2.8, 4, 4.5, 5, 5.7, 6, 6.8, 8, 8.5, 12, 30, 40, 52, 106, 266, 470 and 1077 kDa were detected as components of *M. aeruginosa* COM.

3.2. Charge characteristics of coagulated constituents

It was reported that charge neutralization plays a significant part in the coagulation of organic matter [11,15,17,23]. Therefore, the knowledge of charge characteristics of coagulated constituents can provide a better insight into the removal mechanisms.

3.2.1. COM peptides/proteins

Peptide/protein isoelectric points (p*I*) were ascertained to be approximately 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 (Fig. 1a). The number of peptide/protein p*I*s identified by IEF corresponded to the number of peptides separated by HPSEC. It is widely recognized that peptides/proteins provide a wide range of charged sites on their surfaces due to their various functional groups, some of which are able to release or accept a proton depending on the pH of the solution (—OH, —COOH, —SH, $—NH_3^+$, $=NH_2^+$, etc.) [19]. The total number of functional groups which can dissociate within a COM peptide/protein mixture was quantified by potentiometric titrations [18,24]. The titration curve (Fig. 1a) shows the number of protons, which the peptide/protein mixture is able to accept under a given pH (during titration from pH 12 to the given pH) and which is equal to the amount of dissociated functional groups (in millimoles of H^+ ions per 1 g of DOC). The curve shows the total isoelectric point (pI_T) of the peptide/protein mixture, which lies in the pH region where isoelectric points of single peptides/proteins were identified. Moreover, the curve provides several equivalence points (pEq_1, pEq_2 and pEq_3) and dissociation constants (pK_1, pK_2 and pK_3), which can be attributed to different COM peptide/protein titratable functional groups also depicted in Fig. 1a. However, it should be taken into consideration that the titration was performed with a mixture of compounds containing a number of various functional groups, whose dissociation constants depend not only on the type of group, but also on the overall net charge of the molecule [24]. It is likely that $pK_3 = 9.94$ could be assigned to several functional groups having their dissociation constants in alkaline pH, such as —SH, —OH, $=NH_2^+$ and $—NH_3^+$ (on both side chains, i.e. ε-NH_3^+, and terminal parts of molecules, i.e. α-NH_3^+). On the other hand, dissociation constant $pK_2 = 4.13$ can be attributed exclusively to —COOH groups on COM peptide/protein side chains (β-COOH of aspartic acid with $pK_a = 3.86$ and γ-COOH of glutamic acid with $pK_a = 4.25$). Finally, $pK_1 = 2.26$ coincides with the dissociation constants of —COOH groups of the terminal amino acids in COM peptide/protein molecules (α-COOH) [19]. The amount of a specific functional group in the COM peptide/protein mixture can be determined from the number of H^+ ions added between the two equivalence points that define the beginning and the end of dissociation of this group [19]. Numbers of H^+ ions added to reach equivalence points and points of dissociation constants are depicted in Fig. 1a above the *x*-axis. According to Eq. (2), it can be assumed that the number of titratable groups in the COM peptide/protein mixture is:

$$N_{\beta\text{-and}\gamma\text{-COOH}} = 78 - 36 = 42 \text{ mmol g}^{-1}$$

for β-COOH and γ-COOH

$$N_{\alpha\text{-COOH}} = 100 - 78 = 22 \text{ mmol g}^{-1}$$

for α-COOH

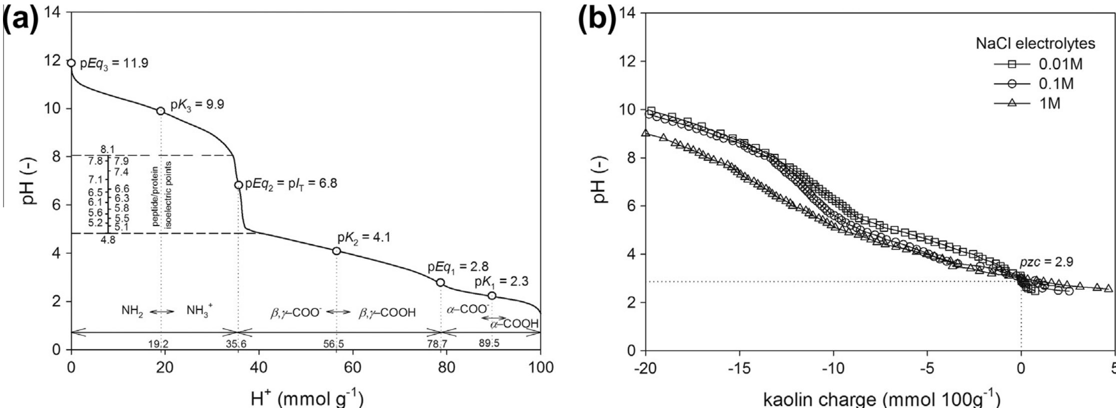

Fig. 1. (a) pH – titration curve for the COM peptide/protein mixture with equivalence points (pEq_1, pEq_2 and pEq_3), dissociation constants (pK_1, pK_2 and pK_3) and total isoelectric point (pI_T). Isoelectric points of single peptides/proteins contained in the mixture are depicted on the left. (b) pH – titration curves (electrolyte concentrations 0.01, 0.1 and 1 M NaCl) for kaolin suspension with point of zero charge (pzc).

$$N_{alk} = 36 - 0 = 36 \text{ mmol g}^{-1}$$

for groups with dissociation constants in alkaline pH.

Humic substances were reported to contain up to 10 mmol of COOH groups per 1 g of DOC [25]. Newcombe [18] reported higher values of acidic groups, i.e. 31 mmol per 1 g of DOC. However, these authors performed the titration experiments in a narrower pH range, Hong and Elimelech [25] from pH 3.6 to 7 and Newcombe [18] from pH 3 to the first equivalence point. As a consequence, they probably did not determine all titratable groups. Alginate, a polysaccharide produced by some algae and bacteria, was estimated to contain about 11 mmol of COOH groups per 1 g of DOC [26]. It is, therefore, evident that COM peptides/proteins bear a considerable amount of titratable functional groups (100 mmol per 1 g of DOC) on their surfaces comparable to other natural organic substances present in surface water.

3.2.2. Kaolin

As well as in the case of peptides/proteins, the charge characteristics of kaolin were evaluated to enable the description of coagulation mechanisms. The point of zero charge (pzc) is observed at the intersection of the three curves in Fig. 1b, at pH $\approx$ 2.9. This is consistent with the values reported in the literature, Coles and Yong [20] found the pzc at pH $\approx$ 2.6. The kaolin pzc value obtained in the current study indicate that, approximately at pH > 2.9, the negative charge of kaolin particles prevails, i.e. kaolin particles are negatively charged over the entire pH range examined in the coagulation tests (pH 3–10). In this pH range, the total kaolin charge is at least two orders of magnitude lower ranging between 0 and 0.2 mmol per 1 g of kaolin when compared with COM peptides/proteins.

3.2.3. Al/Fe coagulants

Aluminum or ferric salts used as coagulants undergo a series of hydrolysis reactions upon addition into water. Al or Fe species produced from these reactions bear a charge depending on pH, similarly to COM peptides/proteins and kaolin. The distributions of Al and Fe species as a function of pH and concentration are described in the literature [27]. In brief, for the coagulant concentrations used in our experiments (Al/Fe in concentrations of 10^{-4} M), at low pH values Al^{3+} (pH < 4.7) and Fe^{3+} (pH < 2.6) ions occur to about 99% as Al/Fe-hexaaquacomplex ($[Al(H_2O)_6]^{3+}$ or $[Fe(H_2O)_6]^{3+}$) in an aqueous medium. As the pH rises, hydrolysis proceeds and the release of protons from hexaaquacomplex leads to the formation of positively charged polynuclear Al/Fe-hydroxopolymers (e.g. $Al_7(OH)_{17}^{4+}$, $Al_{13}(OH)_{34}^{5+}$, $Fe_3(OH)_4^{5+}$, $Fe_4(OH)_6^{6+}$) and consequently of Al/Fe-oxide–hydroxides (AlO(OH) and FeO(OH)). At alkaline pH values, Al (pH > 8.5) and Fe (pH > 8) largely occur as anionic hydroxocomplexes $[Al(OH)_4]^-$ and $[Fe(OH)_4]^-$ [27].

In summary, the charge characterization results show that all coagulated constituents bear a charge changing depending on pH. It is therefore assumed that coagulation by charge neutralization and electrostatically induced adsorption may be effective when performed under appropriate pH conditions. For that reason, coagulation tests were carried at various pH values and changes in the removal of each type of components were observed as a function of pH.

3.3. Coagulation tests with kaolin

To optimize the dose of the aluminum and ferric coagulant, jar tests with raw water samples containing 25 mg L^{-1} of kaolin particles (Tu $\approx$ 100 NTU) were performed without pH control. The highest Tu and Al/Fe removals were achieved at the coagulant doses of 0.075 mmol L^{-1} Al (2 mg L^{-1} Al) and of 0.072 mmol L^{-1} Fe (4 mg L^{-1} Fe). Subsequently, these optimized doses were used in all coagulation tests which were aimed at describing the influence of the pH value on kaolin and COM peptides/proteins coagulation.

The results of coagulation tests with kaolin demonstrated that the lowest residual turbidity was reached in the pH range from 7 to 8.5 for the aluminum coagulant (Fig. 2a) and from 6.4 to 8 for the ferric one (Fig. 2b). A slight difference in optimum pH values for coagulation by Al and Fe stems from their different hydrolysis product distributions [22,27]. Very similar observations were noted by Ching et al. [13] and Kim and Kang [14] who found the optimum pH value for the coagulation of kaolin suspension by ferric coagulants between 6 and 8. The optimum pH values for kaolin removal are close to or slightly below the effective points of zero charge of both Al (pzc of AlO(OH) = 7.7–8.1) and Fe (pzc of FeO(OH) = 6.7–7.6) hydrolysis products [22,27]. The coagulation is induced by the formation of Al/Fe-oxide–hydroxide precipitates and by their adsorption onto the negatively charged surface of kaolin particles, which results in gradual neutralization of kaolin charge and efficient coagulation. Adsorption of kaolin onto Al/Fe-oxide–hydroxide precipitates is explained by electrostatic interactions, exchanging reactions and hydrogen bonding [22]. It should be noted that at sufficiently low coagulant dosages, coagulation of kaolin takes place at lower pH values (4.5–7 for Al and 3.5–6.4 for Fe) due to the adsorption of positively charged Al/Fe hydrolysis species onto kaolin particles [28]. However, the above mentioned optimization tests with kaolin showed that coagulation efficiency at low pH values decreases.

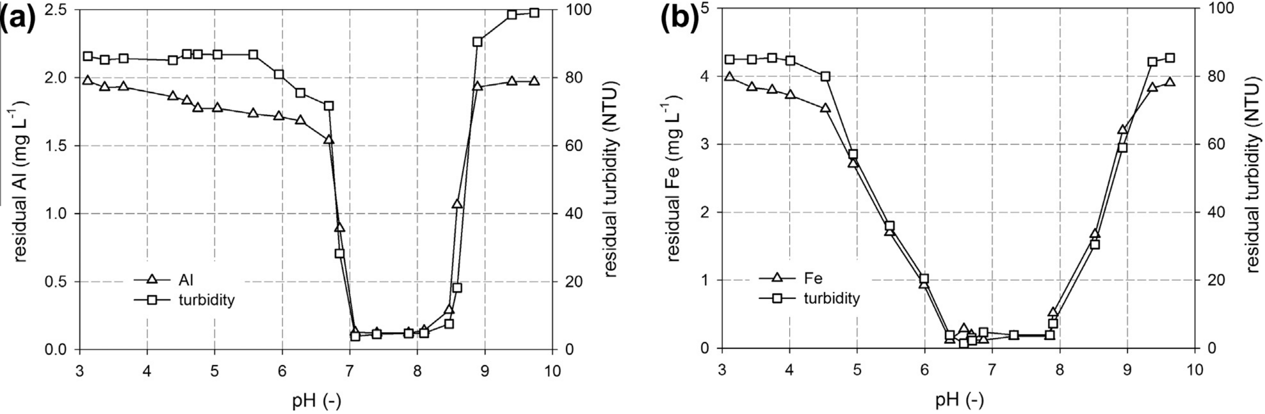

Fig. 2. Coagulation tests with kaolin and either Al (a) or Fe (b) – dependence of residual Al/Fe and turbidity on a pH value.

3.4. Coagulation tests with kaolin, peptides/proteins and Al/Fe coagulant

Mechanisms of COM peptides/proteins removal depending on the coagulant dose and reaction pH have already been described in our previous studies [11,15]. Nevertheless, in the present study, the situation becomes more complicated due to the presence of hydrophobic kaolin particles in a coagulating system. The coagulation tests with raw water containing kaolin and COM peptides/proteins of different initial concentrations (1, 3, 5 and 8 mg L^{-1} DOC) were performed with an optimized dose of either aluminum sulfate (dose of Al = 2 mg L^{-1} = 0.075 mmol L^{-1}) or ferric sulfate (dose of Fe 4 mg L^{-1} = 0.072 mmol L^{-1}) under various pH conditions. Both coagulants showed the same trends and similar results. For a thorough description of removal mechanisms, we have chosen the tests with ferric sulfate, whose results are shown in Fig. 3a–c. It was observed that the optimum pH for the coagulation of COM peptides/proteins and kaolin mixture by ferric coagulant is approximately at pH of 4–6 for all initial DOC concentrations. Interestingly, this optimum tends to shift to higher pH values with decreasing initial DOC concentrations from pH 4–5 for DOC = 8 mg L^{-1} to pH 5–6 for DOC = 1 mg L^{-1}. This shift is given by the ratio of charges in the coagulating system and was also observed in our previous study [11] for the coagulation of COM peptides/proteins by a ferric coagulant.

The capability of COM peptides/proteins to be coagulated stems from the character and content of their functional groups, especially of those which may bear a charge under certain pH conditions. As demonstrated by isoelectric point measurements and potentiometric titrations (Fig. 1a), at pH values of the highest coagulation efficiency (pH = 4–6), COM peptides/proteins carry both positively charged sites on their surfaces due to $-NH_3^+$ and $=NH_2^+$ groups and negatively charged sites due to $-COO^-$ groups which remain in dissociated form even at relatively low pH values. Therefore, COM peptides/proteins are able to electrostatically interact not only with positively charged coagulant hydroxopolymers, but also with negatively charged kaolin particles. The Al/Fe–kaolin coagulation tests (Fig. 2) showed that for the used concentrations of kaolin and Al/Fe coagulants, kaolin does not interact with Al/Fe-hydroxopolymers at pH values below neutral, but only with Al/Fe-oxide–hydroxides that are formed at higher pH values (7–8.5 for Al and 6.4–8 for Fe). This implies that in the case of Fe-peptide/protein–kaolin tests, kaolin removal at pH 4–6 results from the interaction between kaolin and COM peptides/proteins. Positively charged $-NH_3^+$ and $=NH_2^+$ peptide/protein groups interact electrostatically with the negative surface of kaolin. Without any additional agent, this process would lead to the charge stabilization of kaolin particles by peptides/proteins [1,29]. Consequences of this phenomenon are further explained in Section 3.5. When the Al/Fe coagulant is added, formed Al/Fe-hydroxopolymers interact with negatively charged $-COO^-$ groups of COM peptides/proteins and give rise to the formation of uncharged aggregates including not only coagulant and COM peptides/proteins, but also kaolin particles bound to them (Fig. 4). Hence, amphoteric COM peptides/proteins induce the coagulation of kaolin at low pH values, at which kaolin is not coagulated in the absence of them. Moreover, kaolin is also removed by the Fe coagulant at pH 6.4–8 resulting in low residual Tu and Fe concentrations (Fig. 3b and c). This indicates that Fe-oxide–hydroxides adsorb onto the negatively charged kaolin particles, as in the case of Al/Fe–kaolin tests (Fig. 2). However, peptides/proteins obviously did not participate in coagulation process and their residual content remained high.

It has been reported that the mechanism of adsorption may also play its role in peptides/proteins removal [11]. At pH 6–8, peptides/proteins can be adsorbed onto the surface of Fe-oxide–hydroxides by means of electrostatic patch model [30], especially when the DOC/coagulant ratio is low (<0.3) [11]. Nevertheless, when kaolin is involved in the coagulation (as in the case of the present study) it adsorbs onto Fe-oxide–hydroxides while peptides/proteins do not. Negative kaolin particles adsorbed on the surface of Fe-oxide–hydroxides obviously prevent largely negatively charged peptides/proteins from binding to coagulant precipitates.

As seen in Fig. 3b, c, at a pH of around 6, there is a peak of residual turbidity and iron. The peak is likely to be caused by two distinct features. First, it can be attributed to the formation of soluble Fe-peptide/protein complexes, resulting in a significant reduction in Fe available for coagulation by its complexation with COM peptides/proteins. Their complexation properties have been described in our previous study [11] that found the peak of residual Fe and DOC at a pH of about 6 when coagulating COM peptides/proteins by ferric sulfate (without kaolin). Our previous study also quantified the peptide/protein maximum binding capacity for Fe to be 1.38 mmol of Fe per 1 g of DOC at a pH of about 6, i.e. 0.077 mg of Fe per 1 mg of DOC [11]. According to those findings, the dissolved Fe-peptide/protein complexes make up to 30% of residual Fe peak obtained in the Fe–kaolin-peptide/protein coagulation tests performed in the present study. Second, the peak may also represent the transition between two different processes, i.e. the coagulation of peptides/proteins and kaolin together at pH 4–6 and the coagulation of kaolin itself at pH 6.4–8. More than 70% of the rise in residual Fe is probably attributable to this feature.

The coagulation tests performed with the second coagulant – aluminum sulfate (dose of Al = 2 mg L^{-1} = 0.075 mmol L^{-1}) – are summarized in Fig. 3d, where the initial DOC concentration of

Fig. 3. Coagulation tests with Fe, COM peptides/proteins (initial DOC of 1, 3, 5 and 8 mg L^{-1}) and kaolin – dependence of residual DOC (a), turbidity (b) and Fe (c) on a pH value. (d) Coagulation tests with Al, COM peptides/proteins (initial DOC of 5 mg L^{-1}) and kaolin – dependence of residual Al, DOC and turbidity on a pH value.

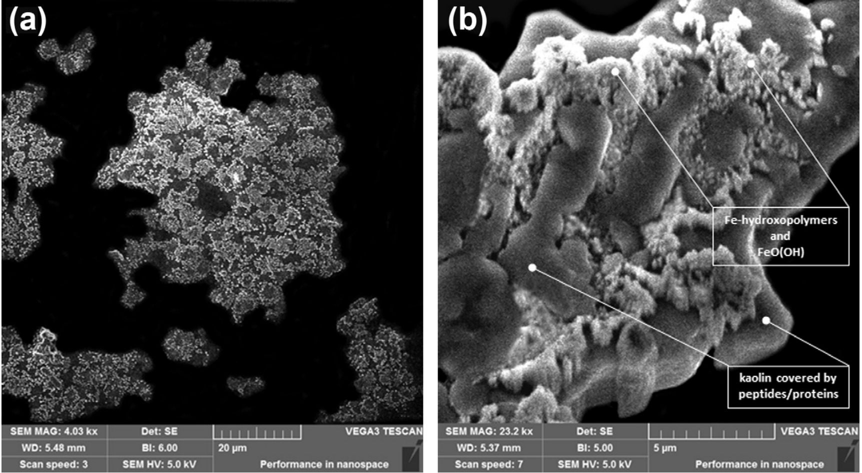

Fig. 4. SEM micrographs of aggregates formed through the interaction between Al/Fe, COM peptides/proteins and kaolin particles – complete (a) and detail (b) picture.

5 mg L^{-1} was chosen as representative. The obtained results are very much similar to those with ferric sulfate. However, there is a slight difference in pH ranges in which particular coagulation pathways occur compared to coagulation tests with ferric sulfate. Similar to Al/Fe–kaolin tests (Fig. 2), this is due to the different hydrolysis product distribution of Al and Fe [27]. Both COM peptides/proteins and kaolin particles are removed at pH values below neutral (pH 5–6.5) as a result of the electrostatic interactions between COM peptides/proteins, kaolin and Al-hydroxopolymers. Kaolin is again removed at the optimum pH for coagulation of kaolin (7–8.5 for Al) by its adsorption onto Al-oxide–hydroxides, but peptides/proteins are not involved in the adsorption. Since it has been demonstrated that aluminum is also able to form soluble complexes with COM peptides/proteins [8–10], the increase in

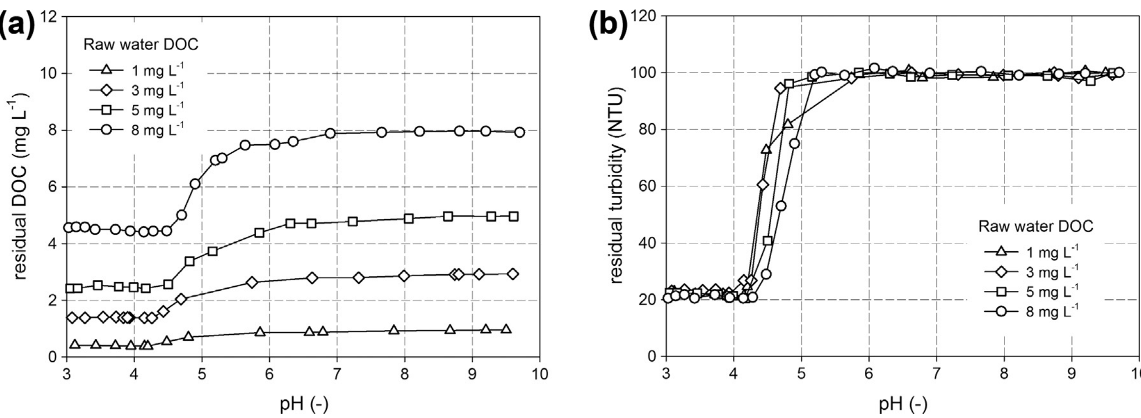

Fig. 5. Coagulation tests with COM peptides/proteins (initial DOC of 1, 3, 5 and 8 mg L^{-1}) and kaolin – dependence of residual DOC (a) and turbidity (b) on a pH value.

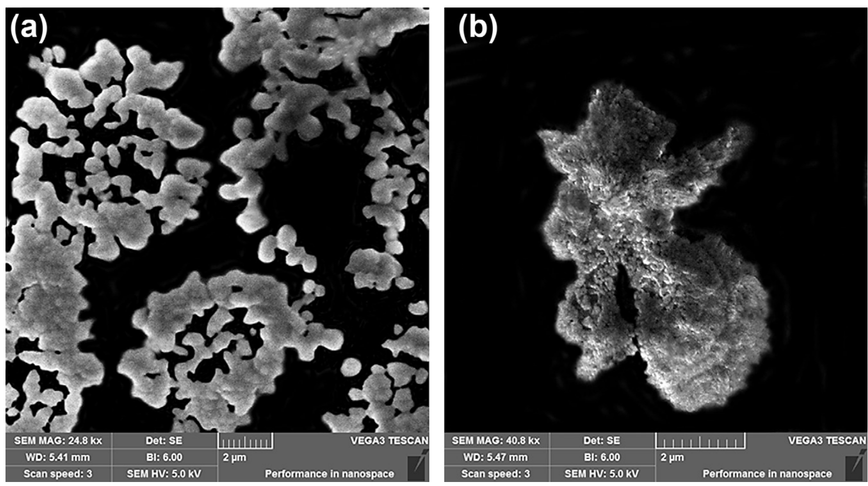

Fig. 6. SEM micrographs of aggregates formed through the interaction between COM peptides/proteins and kaolin particles (without Al/Fe) – complete (a) and detail (b) picture.

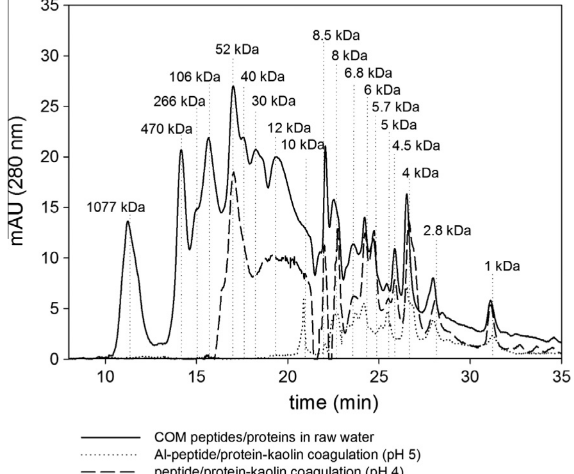

Fig. 7. Molecular weight distributions of COM peptides and proteins in raw water and residual peptides and proteins after coagulation test with: (1) Al, COM peptide/protein (initial DOC = 5 mg L^{-1}) and kaolin (the tests with Fe as a coagulant provided almost the same results; thus, only one of the coagulants is shown) and (2) COM peptides/proteins (initial DOC = 5 mg L^{-1}) and kaolin without a coagulant.

residual Al and turbidity at a pH of around 6.8 (Fig. 3d) may be again attributable to the formation of these complexes. Furthermore, the increase is likely to result from the transition between the coagulation of peptides/proteins and kaolin together at pH 5–6.5 and the coagulation of kaolin itself at pH 7–8.5, as well as in the case of Fe-peptide/protein–kaolin tests described above.

While some studies [1,4,8,9,16] highlighted the inhibitory effect of AOM on coagulation, our results show that COM peptides/proteins inhibit coagulation of hydrophobic particles only in a narrow pH range, in which COM peptides/proteins form dissolved complexes with coagulants [11]. Furthermore, the present results indicate that COM peptides/proteins serve as cationic coagulation aids and enhance the coagulation of other impurities present in raw water at pH values below neutral. Similarly, Bernhardt et al. [1] observed the positive effect of low concentrations of extracellular organic matter (EOM) obtained from green alga *Dictyosphaerium* sp. on the coagulation of quartz particles. The substances contained in EOM are mostly of polysaccharide character [10]. They were reported to behave as anionic polyelectrolytes due to the presence of —COO^- groups in their molecules and to attach themselves to the quartz surface through the formation of hydrogen bonds or ligand exchange and covalent bonding [1]. Furthermore, Ma et al. [3] also reported that COM of *M. aeruginosa* behaved as anionic and

non-ionic polyelectrolytes and its low level mainly benefited the coagulation *M. aeruginosa* cells. However, these studies have not fully explained the mechanisms by which AOM positively influence the removal of other impurities. In order to provide a better understanding of the contributive effect of COM peptides/proteins on coagulation of hydrophobic kaolin particles, the tests without any additional coagulants (Al/Fe sulfate) were carried out.

3.5. Coagulation tests with kaolin and peptides/proteins without coagulants

It is well-documented that natural polyelectrolytes could serve not only as coagulant aids but also as the only coagulants in the treatment of turbid waters [12]. A similar effect has also been observed for COM peptides/proteins. The results of peptide/protein-kaolin coagulation tests showed that they coagulate with kaolin even in the absence of the Al/Fe coagulant. The highest removal efficiencies of both impurities, i.e. COM peptides/proteins and kaolin particles, was accomplished approximately at pH < 4.5 (Fig. 5a and b) as a result of electrostatic interactions between them. As in the case of the Al/Fe-peptide/protein–kaolin tests, positively charged $-NH_3^+$ and $=NH_2^+$ peptide/protein groups interact with the negative charge on the kaolin surface. This can lead to two different features depending on the character of peptide/protein surface charge, thus on the pH. First, if peptides/proteins bound to kaolin bear enough negatively charged $-COO^-$ groups, charge stabilization of kaolin particles by peptides/proteins occurs [1,29]. Second, if the amount of negative charge on peptides/proteins is reduced, i.e. $-COO^-$ groups accept protons, the repulsion between peptide/protein molecules does occur to a lower extent and is overcome by attractive forces [1]. In this case, peptides/proteins bound to kaolin can further interact with other kaolin particles and enable formation of aggregates by interparticle bridges (Fig. 6). As seen from the titration curve of peptides/proteins (Fig. 1a), approximately at pH < 4.5, all the peptide/protein β- and γ-COOH groups are not dissociated yet and hence the non-dissociated ones do not contribute to the repulsion forces. The repulsion forces are thus overcome by attractive ones and aggregation takes place. On the contrary, at pH > 4.5, dissociation of β- and γ-COOH groups proceeds, leading to the excess of negative charge and repulsion between particles in the system. It makes the aggregation impossible and as a consequence, the levels of residual turbidity and residual DOC sharply rise (Fig. 5). However, the efficient removal of peptides/proteins and kaolin without any additional agent suffers from some drawbacks. In practice, the low reaction pH value may be quite problematic. For instance, when cyanobacterial cells are present in raw water low pH value leads to cell lysis and subsequent increase in DOC concentration in treated water [31], which may change parameters of the removal process [3]. Zhang et al. [31] reported that at pH 5 almost all cells of *M. aeruginosa* were dead or destroyed. Besides the low reaction pH (<4.5), the peptide/protein-kaolin coagulation provides lower removal rates for DOC (about 45%) compared to tests with Al/Fe.

3.6. Residual COM peptides/proteins

The changes in peptide/protein composition after coagulation tests were determined by HPSEC analysis. Chromatograms in Fig. 7 compare MW profiles of COM peptides/proteins in raw water with the ones after the coagulant-peptide/protein-kaolin tests at pH 5 (maximum removal efficiency for DOC, Al/Fe and Tu) and after peptide/protein-kaolin tests at pH 4 (maximum removal efficiency for DOC and Tu) for initial DOC concentration of 5 mg L^{-1}. It demonstrates that under optimum reaction conditions (pH 4–5.5 for Fe and 5–6.5 for Al), high-MW proteins of MW > 10 kDa are completely removed, whereas low-MW peptides of MW of approximately 1, 2.8, 4, 5, 6, 6.8, 8, 8.5 and 10 kDa remain in the solution. This observation is consistent with the findings of other studies [3,11,15], in which high-MW COM compounds were removed with higher efficiency than low-MW ones. Furthermore, in the case of peptide/protein-kaolin tests, residual peptides/proteins of MW of 1–52 kDa were identified in the solution after coagulation at pH 4 (Fig. 7). These results indicate that high-MW proteins interact with kaolin particles more readily than low-MW peptides and enables partial coagulation of kaolin particles without the Al/Fe coagulant (Fig. 5).

4. Conclusion

The occurrence of cyanobacterial peptides/proteins in turbid waters substantially changes the optimum conditions for their treatment. The removal process is highly pH dependent since the charge of removed impurities as well as of traditional coagulants used (Al and Fe salts) changes with pH value. Though kaolin particles, which represent the clay colloids in turbid waters, are removed at pH about neutral (7–8.5 for Al and 6.4–8 for Fe) due to adsorption mechanism, the optimum pH for coagulation of peptide/protein-kaolin mixture is significantly lower (4–6 for Fe, 5–6.5 for Al). At this pH, electrostatic interactions between amphoteric peptides/proteins, kaolin and coagulant hydroxopolymers lead to the formation of aggregates. Peptides/proteins interact electrostatically with kaolin even in the absence of a coagulant, but they coagulate only at quite low pH values (pH < 4.5). The present findings suggest that during the decay of algal bloom comprising *M. aeruginosa*, a decrease in the coagulation pH is a prerequisite for the efficient removal of both clay colloids and COM peptides/proteins produced by these cyanobacteria.

Acknowledgments

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PUBLICATION 2

A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga

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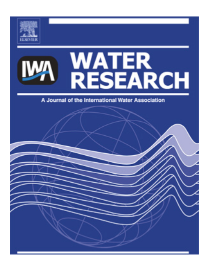

A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga

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ABSTRACT

This study investigated characteristics of algal organic matter (AOM) derived from three species (cyanobacterium *Microcystis aeruginosa*, diatom *Fragilaria crotonensis* and green alga *Chlamydomonas geitleri*) which dominate phytoplanktonic populations in reservoirs supplying drinking water treatment plants. Algal growth was monitored by cell counting, optical density and dissolved organic carbon concentration measurements. Extracellular organic matter (EOM) released at exponential and stationary growth phases and cellular organic matter (COM) were characterised in terms of specific UV absorbance (SUVA), peptide/protein and non-peptide content, hydrophobicity and molecular weight (MW). It was found that both EOM and COM were predominantly hydrophilic with low SUVA. COM was richer in peptides/proteins, more hydrophilic (with about 89% of hydrophilic fraction for all three species) and had lower SUVA than EOM. MW fractionation showed that both EOM and COM of all three species contain large portions of low-MW (<1 kDa) compounds and high-MW (>100 kDa) polysaccharides. Peptides/proteins exhibited narrower MW distribution than non-peptide fraction and it widened as the cultures grew. The highest amount of peptides/proteins with a significant portion of high-MW ones (22%) was observed in COM of *M. aeruginosa*. The results imply that the knowledge of AOM composition and characteristics predetermine which processes would be effective in the treatment of AOM laden water.

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1. Introduction

Algal organic matter (AOM) may comprise an essential part of natural organic matter in surface waters as a consequence of eutrophication of aquatic environments followed by a phytoplankton population increase. The AOM, including extracellular organic matter (EOM) and cellular organic matter (COM), is composed of a wide spectrum of chemical compounds such as saccharides and polysaccharides, nitrogen-

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containing compounds (amino acids, peptides, proteins, nucleic acids), organic acids (e.g. glycolic acid), lipids and fatty acids (Henderson et al., 2008) and also cyanobacterial toxins (Harada, 2004). Dominant AOM constituents are peptide/protein and polysaccharide compounds (Pivokonsky et al., 2006), imparting to AOM a highly hydrophilic character. The AOM composition strongly depends on the species, its growth phase, the age of the culture, and on the culture conditions (Pivokonsky et al., 2006; Henderson et al., 2008).

When algal populations increase in reservoirs supplying drinking water treatment facilities, water treatment technology has to deal not only with increased cell concentration but also with EOM derived from algal metabolic activity and excreted from the cells into the surrounding environment and with COM released by cell lysis during the population growth and decline (Nguyen et al., 2005; Pivokonsky et al., 2006). It should be noted that during the decay of algal bloom, COM release causes a considerable rise in dissolved organic matter (DOC) concentration of which COM comprises a great majority (Pivokonsky et al., 2006; Leloup et al., 2013). COM can be released to the water even during the water treatment process, for instance when pre-oxidation methods are used (Ma et al., 2012). Both EOM and COM may adversely affect drinking water production by disinfection by-product formation (Nguyen et al., 2005; Fang et al., 2010; Li et al., 2012), membrane fouling (Amy, 2008; Qu et al., 2012; Zhang et al., 2013), decreased adsorption efficiency for micropollutants onto activated carbon (AC) (Hnatukova et al., 2011) or reduction in coagulation efficiency (Ma et al., 2012; Pivokonsky et al., 2012). One of the most important factors influencing the efficiency of AOM removal processes is the character of compounds comprising AOM. For example, membrane fouling is dependent on the charge of organic molecules, on their hydrophobicity as well as on their molecular weight (MW) (Amy, 2008; Qu et al., 2012; Zhang et al., 2013). Adsorption of organic molecules onto AC is also affected by their MW, surface charge and the presence and type of functional groups (Velten et al., 2011). In general, low-MW organics are easily adsorbed onto granular AC, while the removal of high-MW protein- and polysaccharide-like biopolymers is poor because their large size may prevent access to the internal pore structure of granular AC (Hnatukova et al., 2011; Velten et al., 2011). On the other hand, coagulation was shown to be highly effective in removing large organic molecules, whereas low-MW ones remain in the solution and require further treatment (Safarikova et al., 2013).

The knowledge of AOM composition and characteristics (MW distribution, charge, hydrophobicity, protein content) is essential for understanding its fates and treatability in water supplies and subsequent optimization of the removal process. Although the AOM gains importance in connection with eutrophication of aquatic environments, there are only a handful of studies investigating AOM characteristics (Nguyen et al., 2005; Pivokonsky et al., 2006; Henderson et al., 2008; Fang et al., 2010; Li et al., 2012; Leloup et al., 2013). Therefore, the aim of this study is to characterise the AOM originating from three algal species of different natures: cyanobacterium *Microcystis aeruginosa*, diatom *Fragilaria crotonensis* and green alga *Chlamydomonas geitleri*. These species represent the dominant phytoplanktonic organisms in the Svihov water reservoir which provides source water for one of the biggest water treatment plants in Europe (Zelivka Waterworks, Czech Republic) producing about 3700 L s^{-1} (with maximum possible production of 6900 L s^{-1}) and supplying 1.5 million people. *F. crotonensis* dominates the spring phytoplankton, *C. geitleri* prevails at the turn of spring and summer and *M. aeruginosa* forms cyanobacterial bloom in late summer. The study evaluates and compares the properties of EOM at both exponential and stationary growth phases and COM separately. Furthermore, it deals with the characteristics of peptide/protein and non-peptide fractions of EOM and COM because peptide/protein compounds were found to possess some specific properties, such as good removability by coagulation caused by their high content of charged functional groups (Safarikova et al., 2013) and ability to form dissolved complexes with coagulant metals (Al and Fe) (Takaara et al., 2007; Pivokonsky et al., 2012). The influence of EOM and COM composition and also of COM release into raw water on water treatment processes was discussed.

2. Material and methods

2.1. *Algal species*

Three species: cyanobacterium *M. aeruginosa* (strain Zap. 2006/2), diatom *F. crotonensis* (strain SAG 28.96), and green alga *C. geitleri* (strain Ettl. 1982/3) were used in this study. *M. aeruginosa* is a bloom-forming species which can produce harmful hepatotoxins such as microcystins (Harada, 2004) and is characterized by small prokaryotic spherical cells (2.5–5.5 μm in diameter) usually organized into irregularly shaped colonies. *F. crotonensis* is an important component of the spring bloom, typically declining when the water reservoir stratifies. Its growth is wholly dependent on the presence of soluble silicon oxides in water. Cells are substantially bigger (about 40–170 μm long and 2–5 μm wide) than those of other two species used in this study, are enclosed within siliceous lanceolate frustules and can be joined in large ribbon-like colonies. *C. geitleri* is a unicellular green alga which swims with two flagella. Its cells are about 10 μm in diameter and has a multilayer cell wall made of hydroxyproline-rich glycoproteins (Lee, 2008). Axenic inoculums of the three strains were supplied by the Culture Collection of Algal Laboratory, Institute of Botany, Academy of Sciences of the Czech Republic, Prague (*M. aeruginosa* and *C. geitleri*) and by the Experimental Phycology and Culture Collection of Algae at the University of Goettingen, Germany (*F. crotonensis*).

2.2. *Culture conditions*

All microorganisms were cultivated in aquarium tanks at 40 L volumes. The cultures of *M. aeruginosa* and *C. geitleri* were grown at 20 °C and 16 h-light/8 h-dark cycle in WC medium (Guillard and Lorenzen, 1972) and mixed by shaking apparatus at 20 rpm. *F. crotonensis* was grown in diatom culture medium (Beakes et al., 1988) at 15 °C and a 12 h-light/12 h-dark cycle, with continuous mixing by shaking apparatus at 10 rpm. Inorganic carbon (CO_2) was continuously added into the growth media using self-regulating CO_2 dosing apparatus with

pH control (Linde Gas, Czech Republic). The cultures were illuminated using eight Sun Glo (Hagen, Germany) 40 W aquatic fluorescent lamps (white balance 4200 K) supplying about 2400 lux (cd sr m^{-2}). All materials and media were sterilised by autoclaving before assembly and operation. Cultivations of all microorganisms were performed four times from samples grown on separate occasions.

2.3. Growth monitoring

Microorganisms' growths were monitored daily until the beginning of the decline phase by cell counting (CC) and measuring optical density (OD) at 560, 675 and 720 nm. Cell counts were performed by a Z2 Coulter Counter particle count and a size analyser (Beckman Coulter, USA). Optical densities of cell suspensions at 675 nm for *M. aeruginosa*, 560 nm for *F. crotonensis* and 720 nm for *C. geitleri* were determined by a UV/VIS 8453A spectrophotometer (Agilent Technologies, USA) with 1 cm-long quartz cells. The used wavelengths corresponded to absorption maximums for individual microorganisms' cultures in a spectrum ranging from 190 to 860 nm.

2.4. AOM characterization

Microorganisms' exudates excreted into the cultivation media (i.e. EOM) were evaluated every day of cultivation as total dissolved organic carbon (DOC_T) concentrations. DOC was determined in samples filtered through a 0.22 μm membrane filter (Millipore, USA) by a Shimadzu TOC-V_{CPH} analyser (Shimadzu, Japan). EOM for MW fractionations (described in Section 2.4.7.) was extracted from algal cultures during both the exponential and the stationary growth phases corresponding to 8th and 16th day of *F. crotonensis* and *C. geitleri* cultivation periods, and 10th and 18th day for cultivation of *M. aeruginosa*, respectively. Cultures of all microorganisms were harvested on the 20th day of cultivation during the stationary growth phase to assess COM samples, which include intracellular organic matter (IOM) and surface-retained organic matter (SOM) (Takaara et al., 2007).

2.4.1. SUVA analyses

UV absorbance at 254 nm (UV_{254}) was measured using a UV-VIS 8453A spectrophotometer (Agilent Technologies, USA) with 1 cm-long quartz cells. Specific UV absorbance (SUVA) was then calculated as a ratio of UV_{254} to DOC (Edzwald, 1993).

2.4.2. Preparation of EOM samples

EOM was extracted by filtering the cell suspension through a 0.22 μm membrane filter (MF, Millipore). Tri- and divalent cations (Fe^{3+}, Ca^{2+}, Mg^{2+} etc.) in the filtrate were replaced with monovalent cations by passing the filtrate through the Na^+ cation-exchange resin Dowex 50-WX-8 (Sigma–Aldrich, USA). The eluents were then concentrated tenfold with a rotary evaporator (Laborota 4000 HB/G1) at 20 °C and was stored at −18 °C.

2.4.3. Preparation of COM samples

The microorganisms' cells were separated from the culture media by filtering through a 0.22 μm membrane filter (Millipore, USA). The separated cells were stirred with ultra-pure water (200 mL) and disrupted in ice bath using an ultrasonic homogeniser (UP400S, Hielscher Ultrasonics, Germany) at 60% amplitude of ultrasonication (240 W) in pulse mode for 5 min. The residual solids were removed by a 0.22 μm membrane filter, and filtrates were concentrated tenfold in a rotary evaporator (Laborota 4000 HB/G1, Germany) at 20 °C. The concentrated COM was stored at −18 °C.

2.4.4. Determination of peptide/protein and non-peptide content

The content of peptide/protein and non-peptide fractions was determined as described in our previous study (Safarikova et al., 2013). After their separation, the peptide/protein (dissolved in 200 mL of ultra-pure water) and non-peptide fractions were purified using cation (Dowex 50-WX-8, Sigma–Aldrich, USA) and anion (Dowex 1-X-2, Sigma–Aldrich, USA) exchange resins.

2.4.5. Determination of hydrophilic and hydrophobic fractions

The fractionation technique described by Malcolm and MacCarthy (1992) was used for the division of the AOM into hydrophilic (HPI), hydrophobic (HPO) and transphilic (TPI) fractions. AOM samples of 250 mL with pH adjusted to 2 (with 2 M HCl) were passed consecutively through the two 15 mm columns connected in series and filled with 50 mL of DAX-8 and XAD-4 resin, respectively. The HPO fraction was adsorbed on DAX-8 resin, the TPI fraction on XAD-4 resin and non-retained compounds represented HPI fraction. Adsorbed HPO and TPI fractions were eluted from the resins with 150 mL of 0.1 M NaOH. Flow rates of filtration and elution were the same, fixed at 1 mL min^{-1}. Concentrations of each fraction were determined by DOC measurements.

2.4.6. Molecular weight fractionation

Peptide/protein and non-peptide AOM fractions were characterised in terms of MW distribution. Centrifugation (4000 rpm, $T = 40$ min) in ice bath was used to drive the AOM fraction through Amicon Ultra-15 centrifugal filters of 100, 50, 30, 10, 3 NMWL and 1 kDa PLAC ultrafiltration membrane (Millipore, USA) resulting in AOM division into fractions of >100, 50–100, 30–50, 10–30, 3–10, 1–3 and <1 kDa. The MW distribution was expressed as a DOC portion of each MW fraction. Each MW fractionation was conducted for EOM samples at the exponential and the stationary growth phase and for COM samples.

MW distribution of peptide/protein AOM was also determined by a high performance size exclusion chromatography (HPSEC) using Agilent Bio SEC-5 100 Å, 300 Å and 500 Å columns (7.8 × 300 mm, 5 μm) connected in series (separation range 100–5,000,000 Da). The HPLC system (Agilent Technologies, USA) was coupled with a diode array detector (DAD) operating at 280 nm and calibrated using peptide and protein SEC standards (Sigma–Aldrich, USA) of MW range from 220 Da to 1500 kDa.

3. Results and discussion

3.1. Algal growth

Growth curves of *M. aeruginosa*, *F. crotonensis* and *C. geitleri* are depicted in Fig. 1a–c where cell counts (CC), optical densities

(OD) and DOC_T concentrations are plotted as a function of cultivation time. The growth of all three species over a 20-day period, expressed by CC and OD, exhibited three growth phases: lag, exponential and stationary phase. Lag phases lasted for about 3–4 days for all microorganisms. *M. aeruginosa* exhibited the longest exponential phase of 12 days, while the durations of exponential phases for *F. crotonensis* and *C. geitleri* were 8 and 10 days, respectively. Based on cell counts, the exponential-phase specific growth rates (calculated according to a formula used by Huang et al. (2007)) were 0.31 day^{-1} for *M. aeruginosa*, 0.43 day^{-1} for *F. crotonensis* and 0.38 day^{-1} for *C. geitleri*. In the stationary phase, populations reached their maximums of about 18.7×10^6 cells mL^{-1} for *M. aeruginosa*, 11.8×10^4 cells mL^{-1} for *F. crotonensis* and 11.4×10^6 cells mL^{-1} for *C. geitleri*.

Unlike CC and OD the concentrations of organics in the cultivation media (DOC_T, titled as EOM in the following sections) grew almost linearly in the course of the exponential and the stationary phases. During the exponential phase DOC_T was mainly comprised of EOM excreted from living cells. In the stationary phase, the population growth stagnated but DOC_T concentration kept rising due to the continuing production of EOM and higher cell mortality rate compared with the exponential phase leading to the release of COM into the cultivation media (Pivokonsky et al., 2006; Henderson et al., 2008). The relative amount of DOC_T released during the cultivation period depended on the species (Pivokonsky et al., 2006; Henderson et al., 2008). Specifically, DOC_T productions of cyanobacterium *M. aeruginosa* and green alga *C. geitleri* were quite similar (0.0029 and 0.0011 ng $cell^{-1}$ at the beginning of stationary phase, respectively) while diatom *F. crotonensis* with considerably bigger cells and also less numerous population (two orders of magnitude lower) produced substantially more DOC_T per cell (0.75 ng $cell^{-1}$ at the beginning of stationary phase) than the other two species. Comparable observations were done by Henderson et al. (2008) who reported DOC released per cell at the stationary phase to be 0.00095 ng $cell^{-1}$ for cyanobacterium *M. aeruginosa*, 0.0029 ng $cell^{-1}$ for green alga *Chlorella vulgaris* ng $cell^{-1}$, 0.019 ng $cell^{-1}$ for diatom *Asterionella formosa* and 0.65 ng $cell^{-1}$ for diatom *Melosira* sp.

Good linear correlations between OD and CC were observed for all cultivated microorganisms with correlation constants of 0.991 for *M. aeruginosa*, 0.993 for *F. crotonensis* and 0.989 for *C. geitleri* (Fig. 1d). The study of Zhang et al. (2013) also verified the linear correlation between OD_{684} and CC ($R^2 > 0.99$) for *M. aeruginosa*. Moreover, a good linear correlation between OD_{730} and dry algal biomass was observed by Nguyen et al. (2005) for diatom *Chaetoceros muelleri*, cyanobaterium *Oscillatoria prolifera* and green alga *Scenedesmus quadricauda*.

3.2. Peptide/protein and non-peptide content

The portions of peptide/protein (DOC_P) and non-peptide (DOC_{NP}) organic matter from the total amount of organics released into cultivation media (DOC_T) are depicted in Fig. 2. The data clearly demonstrate the increase in peptide/protein portion throughout the cultivations of all microorganisms. At the 20th day of cultivation, peptide/protein portion reached

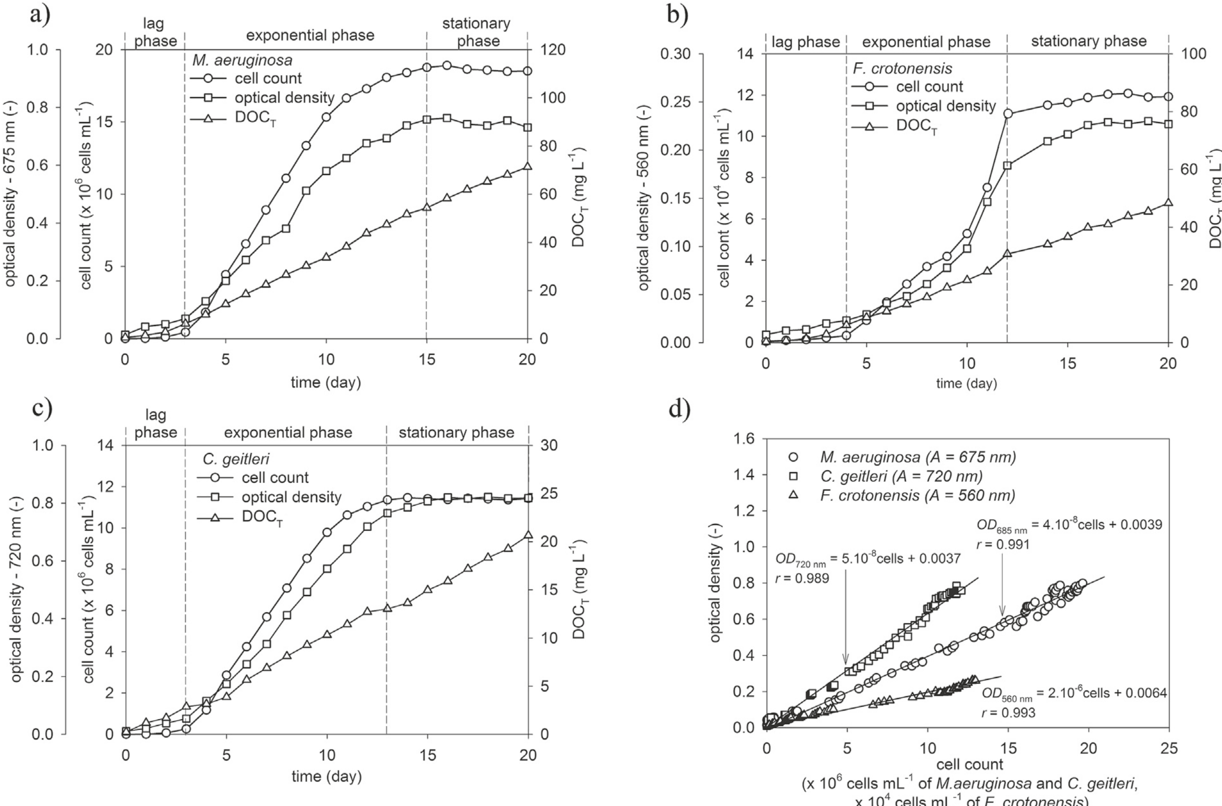
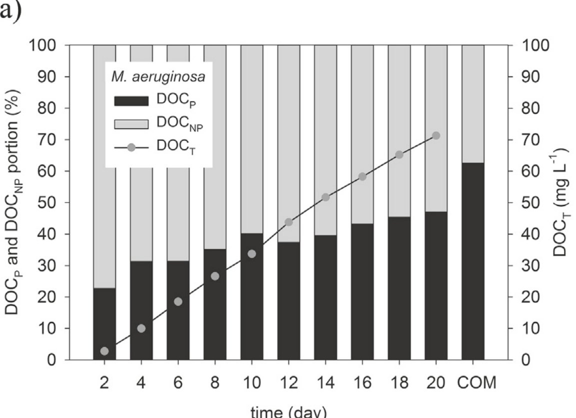
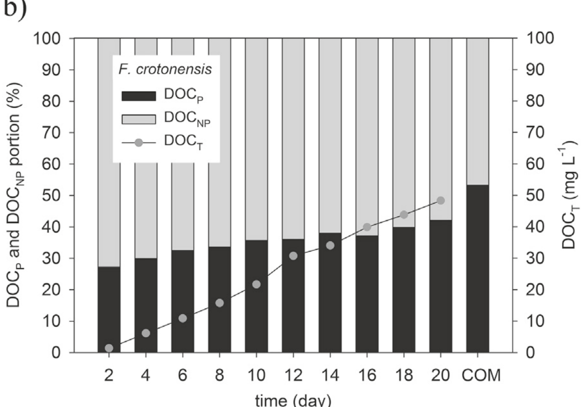


Fig. 1 – Growth curves of *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c) with growth phases marked, and linear correlations between optical densities and cell counts (d).

47% for *M. aeruginosa*, 42% for *F. crotonensis* and 28% for *C. geitleri*. The rise in peptide/protein content at the stationary phase of *M. aeruginosa* growth compared to the exponential phase was ascertained also by Pivokonsky et al. (2006), Henderson et al. (2008) and Huang et al. (2012). This may be caused by the release of COM into the cultivation media during the stationary growth phase as noted earlier. Furthermore, Fig. 2 shows that COM contained a higher peptide/protein portion (63% for *M. aeruginosa*, 53% for *F. crotonensis* and 33% for *C. geitleri*) than EOM did, which is consistent with the other studies investigating EOM and IOM (intracellular organic





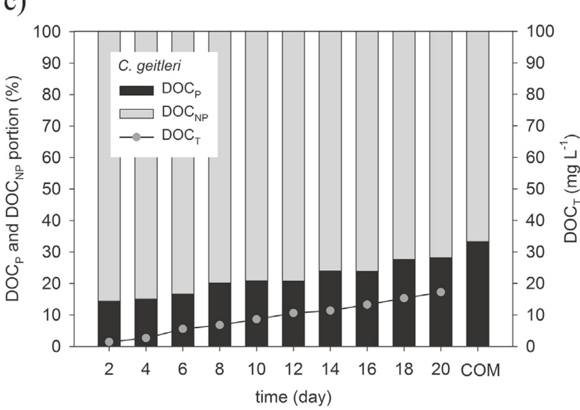


Fig. 2 – Portions of peptide/protein (DOC_P) and non-peptide (DOC_{NP}) organic matter from the total amount of organics released into cultivation media (DOC_T) as a function of cultivation time, and from COM of *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c).

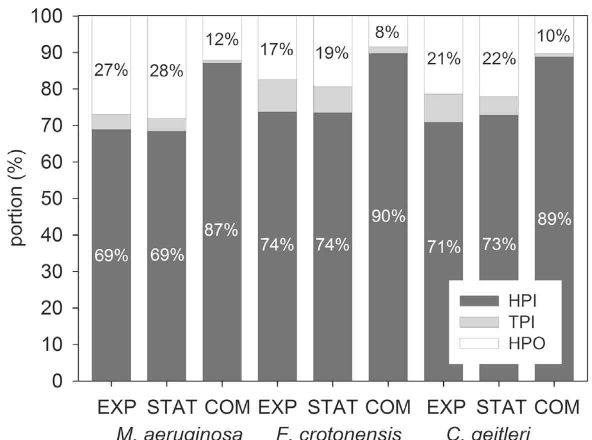


Fig. 3 – Hydrophilic (HPI), transphilic (TPI) and hydrophobic (HPO) fractions of EOM at exponential (EXP) and stationary (STAT) growth phases and of COM.

matter) composition (Pivokonsky et al., 2006; Fang et al., 2010). In addition, the study of Fang et al. (2010) reported that IOM of *M. aeruginosa* consisted of more nitrogen-containing high-MW substances, had a higher concentration of free amino acids, in which arginine, lysine and glycine were the most abundant, and had lower concentrations of aliphatic amines (diethylamine and ethylamine) than EOM. In our study, the highest peptide/protein portion was detected in COM of *M. aeruginosa* since cyanobacteria tend to produce COM relatively rich in peptides/proteins compared to eukaryotic microalgae (Pivokonsky et al., 2006). This is given by the fact that cyanobacterial cells include cyanophycin granules, a protein-like storage material consisting of high-MW copolymers of aspartic acid and arginine, used as an intracellular nitrogen reserve (Lee, 2008).

The release of COM containing a high portion of proteins is likely to have a considerable influence on the efficiency of water treatment process, specifically on coagulation. Studies of Pivokonsky et al. (2012) and Safarikova et al. (2013) showed that proteins of *M. aeruginosa* can, due to their amphoteric properties and the high content of charged functional groups (Safarikova et al., 2013), be easily removed by coagulation and can even act as coagulation aids under appropriate reaction conditions, especially pH value. It can be concluded that with increasing age of culture and particularly with COM release (occurring after pre-oxidation or during the cyanobacterial bloom decay) the coagulant dose may rise as a consequence of increased DOC concentration in raw water (Ma et al., 2012), but a bigger portion of DOC would be coagulated, particularly in case of cyanobacteria. On the other hand, some cyanobacterial peptides and proteins, mainly low-MW ones, were found to bind coagulant metals (Al and Fe) through their dissociated carboxyl (–COO-) groups (Takaara et al., 2007; Pivokonsky et al., 2006, 2012; Safarikova et al., 2013). This leads to the rise in coagulant demand and also to a low removal efficiency of metal-complexing organics which tend to remain in solution after the coagulation (Pivokonsky et al., 2006; Takaara et al., 2007). Nevertheless, the study of Pivokonsky et al. (2012) showed that the inhibitory potential of complex-forming peptides and proteins of *M. aeruginosa* could be significantly

Table 1 – SUVA values of EOM released at exponential and stationary growth phases and for COM for different algal species.

Microorganism	Growth phase	Measured data	Nguyen et al., 2005	Henderson et al., 2008
Microcystis aeruginosa	Exponential	1.6 ± 0.4	–	1.7
	Stationary	0.7 ± 0.3	–	0.5
	COM	0.4 ± 0.2	–	–
Fragilaria crotonensis	Exponential	1.8 ± 0.4	–	–
	Stationary	0.8 ± 0.3	–	–
	COM	0.4 ± 0.1	–	–
Asterionella formosa	Exponential	–	–	1.7
	Stationary	–	–	0.5
Melosira sp.	Exponential	–	–	0.6
Oscillatoria prolifera	Exponential	–	0.8 ± 0.1	–
Chaetoceros muelleri	Exponential	–	1.0 ± 0.4	–
Chlamydomonas geitleri	Exponential	1.2 ± 0.3	–	–
	Stationary	0.6 ± 0.2	–	–
	COM	0.3 ± 0.1	–	–
Chlorella vulgaris	Exponential	–	–	1.3
	Stationary	–	–	0.5
Scenedesmus quadricauda	Exponential	–	1.5 ± 0.5	–

SUVA (m^{-1} mg^{-1} L).

lowered by the appropriate choice of coagulation conditions. Moreover, it was found that COM forms larger quantities of DBPs than EOM does, especially of nitrogen-containing ones (Fang et al., 2010), which is consistent with higher peptide/protein portion in COM compared to EOM.

3.3. Hydrophilic and hydrophobic fractions and the SUVA values

Fig. 3 shows the hydrophilic (HPI) and hydrophobic (HPO) properties of EOM at exponential and stationary growth phases and of COM. In terms of DOC, HPI material dominated both EOM and COM of all three microorganisms. HPI fractions constituted about 70% of EOM at both the exponential and the stationary phases, which is in accordance with the values (57–80%) reported by other researchers (Henderson et al., 2008; Huang et al., 2012; Li et al., 2012; Qu et al., 2012; Leloup et al., 2013). COM produced by all three microorganisms was even more hydrophilic than EOM. HPI fractions of COM accounted for 87% for *M. aeruginosa*, 90% for *F. crotonensis* and 89% for *C. geitleri*. These results compare well with the findings of Li et al. (2012) that IOM and EOM of *M. aeruginosa* contained 86% and 63% of the HPI compounds, respectively. In our study, the transphilic (TPI) fractions comprise only 3.4–9% of EOM with the lowest values for *M. aeruginosa* and the highest ones for *F. crotonensis* and took just a very small part in COM (<2%). HPO fraction content in EOM varied according to species and growth phase from a minimum of 17% for *F. crotonensis* at the exponential phase to a maximum of 28% for *M. aeruginosa* at the stationary phase. In the case of COM, HPO fractions were significantly lower than in EOM, comprising 12% for *M. aeruginosa*, 10% for *C. geitleri* and 8% for *F. crotonensis*. Likewise, Li et al. (2012) reported that *M. aeruginosa* EOM contained 31% of HPO compounds compared to 9% in IOM. This may be given by the high peptide/protein content in COM (see Section 3.2.) contributing to COM lower hydrophobicity. It should be noted that some authors classify peptides/proteins as hydrophilic compounds (Li et al., 2012) while the others classify them as hydrophobic ones (Edzwald, 1993; Henderson et al., 2008). Nevertheless, peptides and proteins are composed of both hydrophilic (e.g. glutamine, asparagine, glutamic and aspartic acid, lysine, arginine) and hydrophobic (e.g. alanine, tryptophan, leucine, valine and phenylalanine) amino acids. In aqueous solutions the latter are clustered together within the core of the peptide/protein leading to peptide/protein folding while the hydrophilic amino acids are situated on the water-exposed surface where they interact with surrounding water molecules (Creighton, 1993). It was demonstrated that cyanobacterial peptides and proteins bear a considerable amount of polar and also charged functional groups on their surfaces (Safarikova et al., 2013), which enables them to behave as hydrophilic compounds. The presence of peptide/protein-like compounds in HPI fraction was ascertained by Qu et al. (2012), who identified peptide/protein-like (using modified Lowry method) and polysaccharide-like substances (using phenol-sulphuric method) in both HPI and HPO fractions of *M. aeruginosa* EOM. This observation could result from the fact that each of these two groups (peptide/protein- and polysaccharide-like) comprises a wide spectrum of organic substances with different MWs and hydrophilic/hydrophobic properties. Moreover, cyanobacteria and some algae produce compounds of both peptide/protein and polysaccharide nature such as peptidoglycans in cyanobacteria or hydroxyproline-rich glycoproteins in cell walls of *Chlamydomonas* sp. (Lee, 2008). In addition, the way AOM compounds divide into fractions depends on fractionation conditions, such as pH value, as it influences the structure and surface characteristics of organics, especially those of peptides/proteins (Safarikova et al., 2013). In general, HPI fraction is believed to contain carbohydrates, hydroxy acids, low-MW carboxylic acids, amino acids, amino sugars, peptides, low-MW alkyl alcohols, aldehydes and ketones, while HPO fraction includes hydrocarbons, high-MW alkyl amines, high-MW alkyl carboxylic (fatty) acids and aromatic acids, phenols and humic substances (Edzwald, 1993).

The results of XAD-resin fractionation correlate well with SUVA values which similarly corresponded to the hydrophilic

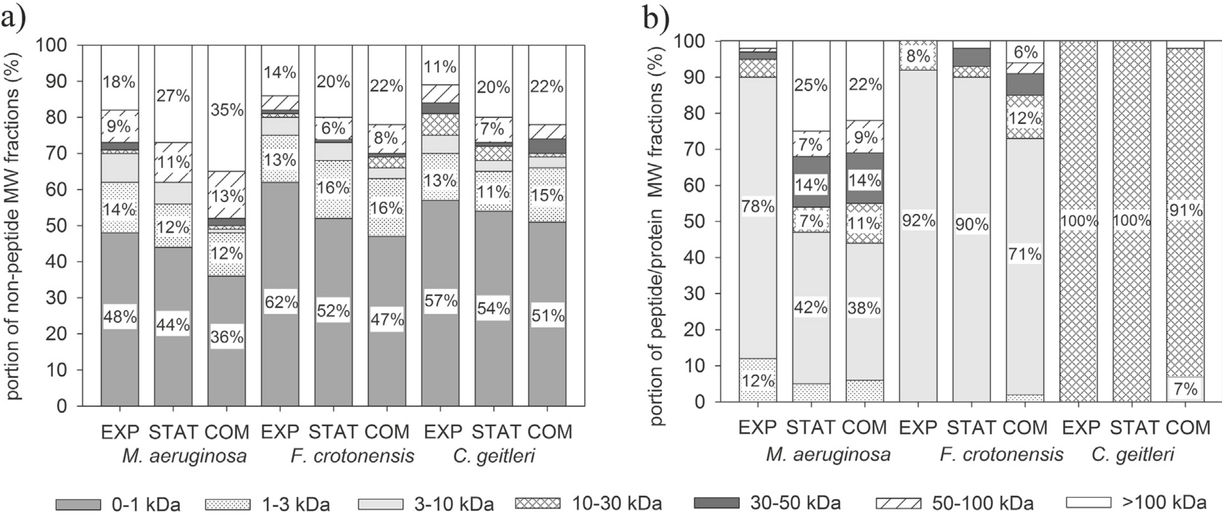


Fig. 4 – Molecular weight (MW) fractionation of non-peptide (a) and peptide/protein (b) portions of EOM at exponential (EXP) and stationary (STAT) growth phases, and of COM determined by centrifugation-driven filtration.

nature of the AOM. SUVA indexes of EOM at exponential and stationary phases as well as of COM and their comparison with values obtained by other studies (Nguyen et al., 2005; Henderson et al., 2008) are reported in Table 1. SUVA decreases in the order exponential-phase EOM > stationary-phase EOM > COM, which is in agreement with Fang et al. (2010), who determined lower SUVA values for IOM than EOM of *M. aeruginosa* and also with Henderson et al. (2008) and Huang et al. (2012) reporting lower SUVA values for the stationary-phase EOM than for the exponential-phase one. It could, therefore, be concluded that SUVA tends to decrease with increasing content of cellular/intracellular organics, whose concentration rises throughout the cultivation period due to increasing cell mortality rate (see Section 3.1.).

Low hydrophobicity of AOM may adversely affect its treatment, especially when applying membrane filtration. Hydrophilic algal organics cause significant flux decline and membrane fouling. On the other hand, membrane fouling is believed to be negligible for hydrophobic substances (Qu et al., 2012; Zhang et al., 2013). This implies that both EOM and COM will substantially reduce the performance of membrane filtration while the adverse effect of COM will be more pronounced since COM of all three studied species contains about 20% more hydrophilic compounds than their EOM does.

3.4. *Molecular weight fractionation*

Peptide/protein and non-peptide portions of EOM at exponential and stationary growth phases and of COM were fractionated by centrifugation-driven filtration into fractions of MW of 0–1, 1–3, 3–10, 10–30, 30–50, 50–100 and >100 kDa. Non-peptide compounds in all three microorganisms exhibited bimodal distributions (Fig. 4a). In terms of DOC, the largest portion of non-peptide compounds was determined in 0–1 kDa fraction which decreased with the age of culture reaching its minimum in COM (36% for *M. aeruginosa*, 47% for *F. crotonensis* and 51% for *C. geitleri*). This fraction represents low-MW intermediate products of metabolism such as aldehydes, hydrocarbons, amines, glycolic acids and amino acids as well as mono- and oligosaccharides (Nguyen et al., 2005; Huang et al., 2007). Molecules with MW of 1–3 kDa and character similar to those in 0–1 kDa fraction (i.e. aldehydes, carboxylic acids, hydrocarbons, oligosaccharides etc.) also formed a significant portion of both non-peptide EOM and COM (11–16%). The second largest non-peptide fraction was the one with compounds larger than 100 kDa. It probably includes high-MW and storage polysaccharides, i.e. cyanobacterial starch in cyanobacteria, chrysolaminarin in diatoms and starch in the form of amylose and amylopectin in green algae, and also polysaccharide-like structures from the cell walls like peptidoglycans in cyanobacteria and glycoproteins in *C. geitleri* (Lee, 2008). Contrary to 0–1 kDa fraction, the portion of >100 kDa fraction increased with the age of culture, was highest for *M. aeruginosa* and reached its maximum in COM of all three microorganisms (35% for *M. aeruginosa*, 22% for *F. crotonensis* and 22% for *C. geitleri*) which is likely to be related with the release of storage polysaccharides from cells. These results are well consistent with Henderson et al. (2008) and Qu et al. (2012) who demonstrated bimodal distributions for a polysaccharide-like portion of EOM of *M. aeruginosa* (Henderson et al., 2008; Qu et al., 2012) as well as of green alga *C. vulgaris* and diatoms *A. formosa* and *Melosira* sp. (Henderson et al., 2008). The latter study also showed that *M. aeruginosa* had a higher portion of polysaccharide-like >100 kDa fraction compared to green alga and both diatoms.

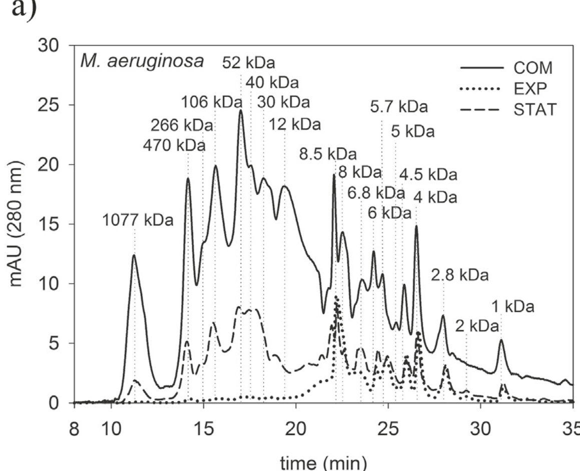
The production of peptide/protein compounds in all three microorganisms showed a different pattern (Fig. 4b). At the exponential growth phase, the highest portions of peptide/protein DOC were determined in 3–10 kDa fraction for *M. aeruginosa* (78%) and *F. crotonensis* (92%) and in 10–30 kDa fraction for *C. geitleri* (100%) indicating that the metabolic products of all three microorganisms contain some polypeptide molecules, for example, extracellular enzymes or their components (Chróst et al., 1989). The exponential-phase EOM of *M. aeruginosa* also comprised 1–3 kDa fraction (12%) which can be attributed to oligopeptides like cyanobacterial

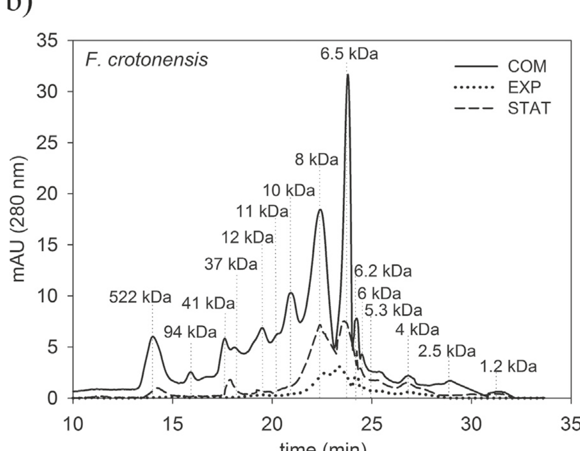
toxins (Harada, 2004) or siderophores, extracellular ligands that aid in solubilisation and assimilation of Fe^{3+} (Lee, 2008). At the stationary growth phase, *F. crotonensis* and *C. geitleri* followed the same trends as at the exponential phase, whereas *M. aeruginosa* exhibited a lower portion of 3–10 kDa (42%) and an increase in higher-MW fractions, particularly in >100 kDa. This fraction accounted for 25% as a result of the release of intracellular storage proteins into the culture media (as described in Section 3.1.) and thus formed a significant portion also in *M. aeruginosa* COM (22%). A slight increase in the higher-MW fractions of COM was ascertained for *F. crotonensis* and *C. geitleri* as well. No compounds were determined in any of 0–1 kDa peptide/protein fraction, which implies that the method of peptide/protein precipitation by $(NH_4)_2SO_4$ (based on salting out effect) used in this study does not isolate amino acids, which are thus contained in the low-MW non-peptide fraction. On the other hand, researchers who used modified Lowry method (based on the reactions of copper ions with the peptide bonds and the oxidation of aromatic amino acid residues) obtained some 0–1 kDa peptide/protein fraction in algal EOM (Henderson et al., 2008; Qu et al., 2012).

Fig. 5 shows the MW distributions of peptide/protein portions of EOM at exponential and stationary growth phases and of COM determined by HPSEC. In general, the quantity and diversity of peptides/proteins in all three microorganisms increased as the cultures grew, i.e. in the order exponential-phase EOM < stationary-phase EOM, and reached their maximums in COM. *M. aeruginosa* produced the highest amount of COM peptides/proteins, especially of high-MW ones, which is consistent with the results of protein portion determination described in Section 3.1. Our findings are in agreement with Pivokonsky et al. (2006), Fang et al. (2010) and Li et al. (2012) who concluded that algal IOM consisted of more high-MW peptide/protein substances than EOM. Furthermore, the study of Pivokonsky et al. (2006) observed similar trends in increasing amount and diversity of proteins during the growth of cyanobacteria *M. aeruginosa*, *Anabaena flos-aqua* and green alga *Scenedesmus quadricauda*.

To compare the two fractionation methods correlations were developed between peptide/protein HPSEC peak heights (Fig. 5) and peptide/protein portions in >100, 50–100, 30–50, 10–30, 3–10, 1–3 and <1 kDa fractions expressed as DOC (Fig. 4b). Very good correlations were observed with correlation constants (*r*) of 0.989, 0.994 and 0.991 (exponential growth phase, stationary growth phase and COM) for *M. aeruginosa*; 0.995, 0.993 and 0.989 for *F. crotonensis*; and 0.999, 0.998 and 0.997 for *C. geitleri*.

The rise in the portion of high-MW organics (mainly of polysaccharide and protein-like character) throughout the cultivations and particularly their high content in COM, which is most pronounced for *M. aeruginosa*, will affect treatment processes. High-MW algal compounds are easily removed by coagulation as shown by Bernhardt et al., (1985) for EOM polysaccharides of several algal species (green algae *Dictyosphaerium* sp., *Scenedesmus* sp., *Chlorella* sp., and cyanobacterium *Pseudanabaena* sp.) and for alginic acid, a model compound for EOM polysaccharides, and by Pivokonsky et al. (2012) for COM proteins of cyanobacterium *M. aeruginosa*. On the other hand, the removal of low-MW AOM compounds by coagulation is negligible, as shown for low-MW peptides and proteins (<10 kDa) of *M. aeruginosa* (Pivokonsky et al., 2012; Safarikova et al., 2013) and thus other processes, such as membrane filtration or adsorption onto activated carbon, are necessary for their treatment. It should be noted that the

a)


b)


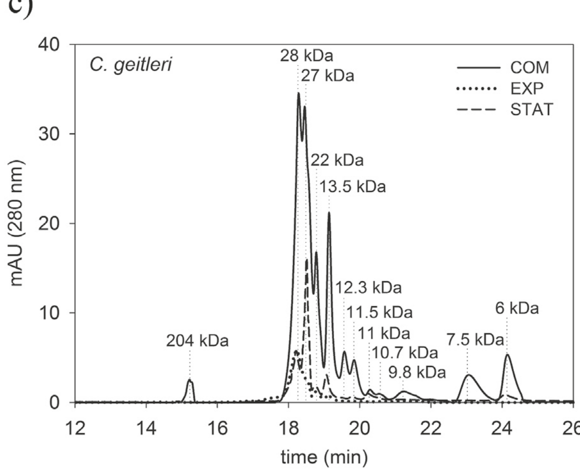
c)


Fig. 5 – Molecular weight (MW) distributions of peptide/protein portions of EOM at exponential (EXP) and stationary (STAT) growth phases, and of COM determined by HPSEC for *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c).

performance of those techniques is substantially worsened by the occurrence of polymers. High-MW organics were found to cause significant flux decline and membrane fouling by the reversible formation of cake layer and also by irreversible pore plugging (Amy, 2008; Qu et al., 2012; Zhang et al., 2013). Moreover, high-MW compounds are difficult to remove by adsorption onto activated carbon (AC) while low-MW AOM fractions are preferentially adsorbed, probably due to the better accessibility to micropores of the AC (Hnatukova et al., 2011; Velten et al., 2011). For this reason, the coagulation is an essential pre-treatment process, especially when COM containing a larger portion of high-MW substances than EOM does is present in raw water.

4. Conclusions

The AOM of all three microorganisms demonstrated several similarities such as significant portion of peptide/protein material, low SUVA and high content of hydrophilic compounds. However, the AOM composition and characteristics changed with both the species and the growth phase and important differences between EOM and COM were found. Specifically, a portion of peptides and proteins as well as their quantity and diversity, a portion of hydrophilic fraction and of high-MW compounds in EOM rose with the age of culture and were noticeably bigger in COM than in EOM of all three microorganisms. This phenomenon was most evident for cyanobacterium *M. aeruginosa*, which is probably given by a different nature of prokaryotic cells and cyanobacterial metabolism. The results imply that the water treatment processes should be adapted not only to the species composition and the age of algal populations occurring in the source water, but also to the release of COM to source water. During the algal bloom decay when COM forms a majority of DOC in water, coagulation effective in removal of high-MW, protein-rich organic matter should be employed to improve the performance of down-stream processes. Low-MW organics which are difficult to coagulate can be subsequently removed by techniques, such as membrane filtration and/or adsorption onto activated carbon, sensitive to the presence of polymers. The results indicate that the monitoring of algal growth as well as its decline is important for the successful prediction of AOM composition and for the effective adjustment of the water treatment process.

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PUBLICATION 3

Mechanismy koagulace při odstraňování peptidů a proteinů produkovaných fytoplanktonem

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MECHANISMY KOAGULACE PŘI ODSTRAŇOVÁNÍ PEPTIDŮ A PROTEINŮ PRODUKOVANÝCH FYTOPLANKTONEM

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1. Úvod

Významnou součástí většiny povrchových vod jsou přírodní organické látky (NOM – Natural Organic Matter). Z chemického hlediska tyto látky představují složitou směs alifatických i aromatických uhlovodíkových struktur s mnoha typy funkčních skupin, např. amidových, karboxylových, hydroxylových, ketonických a dalších funkčních skupin. NOM jsou tvořeny převážně huminovými látkami (huminové kyseliny a fulvokyseliny) a látkami nehuminového charakteru (především proteiny a polysacharidy)[1]. V povrchových vodách je zpravidla obsah huminových látek ve srovnání s látkami nehuminového charakteru vyšší. Nehuminové látky se v našich podmínkách ve zvýšených koncentracích vyskytují převážně ve vegetačním období při masovém rozvoji sinic a řas – jedná se o tzv. AOM (Algal Organic Matter). V případě využití povrchových zdrojů vody pro pitné účely mohou mít právě AOM značný dopad na proces úpravy vody[2,3]. Již při velmi nízkých koncentracích (řádově ng l^{-1}) jsou zdrojem nepříjemného zápachu a negativně ovlivňují i chuťové vlastnosti vody, jedná se o tzv. T&O (Taste and Odor) sloučeniny. Například 2-methylisoborneol a *trans*-1,10-dimethyl-*trans*-9-decalol, dva z nejvýznamnějších netoxických metabolitů, jsou příčinou zatuchlé zemité pachuti a zápachu vody[4]. AOM tvoří také řadu vedlejších produktů desinfekce vody, a to zejména při hygienickém zabezpečení vody chlorem a jeho sloučeninami, kdy dochází k tvorbě halogenovaných organických sloučenin, především trihalogenmethanů – THM (např. chloroform a dibromchlormethan) a halogenderivátů kyseliny octové – HAA (např. kyselina dichloroctová a trichloroctová). Do současné doby bylo detegováno přes 700 takových sloučenin vyskytujících se v pitné vodě zpravidla v koncentracích v řádech μg l^{-1}, z nichž některé jsou toxické nebo karcinogenní[5]. Vzniku těchto sekundárních kontaminantů lze účinně předcházet maximálně možným odstraněním organických látek při úpravě vody. Zatížení upravované vody zvýšeným obsahem AOM je nežádoucí také z důvodu výskytu obtížně odstranitelných cyanotoxinů[6]. Dále bylo prokázáno, že AOM mají inhibiční vliv na koagulaci ostatních ve vodě přítomných znečišťujících příměsí (např. zákalotvorné hlinitokřemičitany)[7]. Tato skutečnost vede k poruchám koagulace doprovázeným zvýšenými koncentracemi zákalotvorných a organických látek, ale i hliníku a železa ve filtrátu[7,8]. Navzdory skutečnosti, že v období rozvoje fytoplanktonu ve vodárenských nádržích mohou tyto látky významně komplikovat technologii úpravy vody, principy koagulace AOM ani vliv AOM na procesy probíhající při úpravě vody nebyly dosud uspokojivě objasněny. Cílem tohoto referátu je popis dosud známých mechanismů interakcí peptidové/proteinové složky AOM s produkty hydrolýzy hlinitých a železitých koagulačních činidel.

2. Původ a charakterizace AOM

AOM jsou produkovány dvěma základními mechanismy. Metabolickými pochody sinic a řas se AOM do povrchových vod dostávají jako tzv. extracelulární organické látky (EOM – Extracellular Organic Matter), při odumírání a rozkladu vlastních buněk fytoplanktonu jsou pak uvolňovány tzv. intracelulární organické látky (IOM – Intracellular Organic Matter)[9,10] a organické látky vázané na povrchu buněk (SOM – Surface-bonded/Surface-retained Organic Matter)[11]. Společně lze označit IOM a SOM jako tzv. celulární organické látky (COM – Cellular Organic Matter)[7]. AOM jsou do vody uvolňovány rovněž v důsledku mechanického nebo chemického poškození buněk sinic a řas při úpravě vody[10].

Chemické složení AOM je velmi různorodé, závisí především na druhu a růstové fázi organismu[2,12], fyziolo-

gických podmínkách[12], fotosyntetické aktivitě vlastních buněk mikroorganismů a případně na podmínkách jejich kultivace[13]. Významný vliv mají především hodnota pH, iontová síla, teplota[1], intenzita a doba slunečního záření, obsah živin a organických látek a koncentrace O_2 a CO_2 ve vodě či kultivačním médiu[12]. Množství a diverzita AOM narůstá během růstové křivky fytoplanktonu[11,14]. V průběhu exponenciální fáze růstu je odumírání buněk relativně nízké a AOM jsou uvolňovány především metabolickou činností organismů ve formě EOM. Ve stacionární fázi růstu již dochází k úhynu organismů a nárůst koncentrací organických látek je částečně způsoben i autolytickými procesy[14]. Se stářím kultury se tak postupně zvyšuje podíl COM na celkové koncentraci AOM[7,14].

Dominantní složkou AOM jsou peptidy/proteiny a sacharidy[12]. Jak již bylo uvedeno, složení AOM se značně liší v závislosti na růstové fázi fytoplanktonu. V exponenciální a stacionární růstové fázi převládají sacharidy nad peptidy a proteiny, během stárnutí kultury fytoplanktonu však dochází k postupnému nárůstu koncentrace peptidů a proteinů[7,14,15]. Například u sinice *Microcystis aeruginosa* byl prokázán nárůst jejich podílu z 10 % na počátku kultivace až na cca 40 % ve stacionární fázi růstu. Peptidy a proteiny pak skladbě AOM dominují ve fázi odumírání fytoplanktonu. U sinice *M. aeruginosa* tvoří peptidová/proteinová složka až cca 66% podíl z celkových AOM, u *Anabaena flos-aquae* 51% podíl a pouze 29% podíl peptidů/proteinů byl stanoven v případě zelené řasy *Scenedesmus quadricauda*[14]. Zelená řasa *Chlorella vulgaris* pak obsahuje cca 40% podíl peptidů a proteinů z celkového množství produkované organické hmoty, *Asterionella formosa* 19% a *Melosira* sp. pak 16% podíl[9]. Spolu s druhem mikroorganismu a jeho růstovou fází se nemění pouze zastoupení peptidové/proteinové složky AOM, ale roste také celkové množství a rozšiřuje se i diverzita peptidů/proteinů[11,14,16].

3. Koagulace AOM

Dosavadní výzkum v oblasti koagulace AOM prokázal jejich relativně nízkou míru odstranitelnosti při konvenční úpravě vody[17,18]. Účinnost koagulace AOM je ovlivněna především druhem a růstovou fází organismu, typem a dávkou koagulantu a především reakčním pH (cit.[16,18]). AOM ovlivňují koagulační procesy pomocí řady mechanismů. V závislosti na koncentraci, složení a vlastnostech, jako je např. distribuce molekulových hmotností, velikost povrchového náboje či hodnota izoelektrického bodu, vykazují AOM pozitivní i negativní účinky na koagulaci[10]. Při nízkých koncentracích mohou AOM účinnost koagulace zvyšovat, naopak jejich vysoké koncentrace koagulaci významným způsobem narušují[3,16,19]. Vliv polysacharidových převážně nenabitých AOM na koagulaci částic může být při jejich nízkých koncentracích (1–2 mg l^{-1} DOC) podobný efektu neiontových polymerních flokulačních činidel, kdy adhezí na povrchu znečišťujících částic umožňují tvorbu tzv. mezičásticových polymerních můstků[19]. Naopak při vysokých koncentracích především nabitých proteinových složek AOM může v závislosti na hodnotě pH docházet vlivem odpudivých elektrostatických interakcí k inhibici koagulačních procesů[16,19,20].

Z výsledků studia vlastního odstranění AOM při úpravě vody je patrné, že s vyšší účinností jsou koagulovány proteinové než neproteinové látky (např. polysacharidy)[10,18]. Odstranění AOM peptidů a proteinů dosahuje cca 75% účinnosti. Naproti tomu účinnost odstranění neproteinových AOM dosahuje pouhých 22 % (cit.[18]). Vyšší účinnosti koagulace především u nabitých proteinových složek AOM je dosahováno při použití železitých činidel, a to pravděpodobně díky skutečnosti, že hydroxid železitý vznikající hydrolýzou železitých solí je mnohem méně rozpustný a hydrolyzuje ve výrazně širším rozsahu pH než hydroxid hlinitý[21].

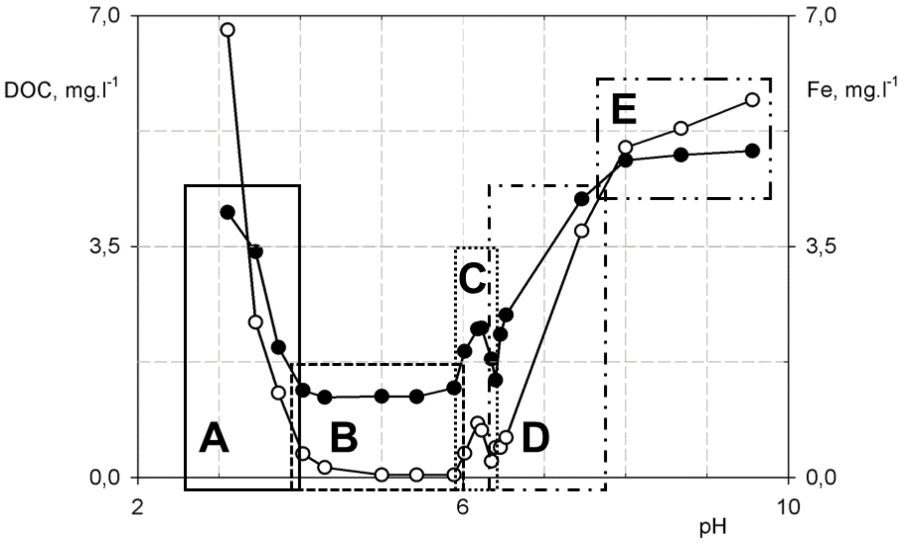
Z výsledků většiny doposud provedených experimentů[3,10,16–18,22] je patrné, že limitována je především schopnost koagulace nízkomolekulárních organických látek, které jsou obtížněji odstranitelné koagulací než vysokomolekulární frakce. Obtížně koagulovatelné jsou především AOM peptidy s molekulovými hmotnostmi (MH) < 10 kDa (cit.[16]). Je zřejmé, že tyto nízkomolekulární peptidy je nutné odstranit jinými mechanismy, např. adsorpcí na aktivním uhlí[23].

3.1. Vliv pH na odstranitelnost AOM peptidů/proteinů – mechanismy interakcí peptidové/proteinové složky s produkty hydrolýzy koagulačního činidla

Jak již bylo řečeno, odstranitelnost organických látek produkovaných fytoplanktonem je úzce závislá na reakčních podmínkách, především na hodnotě pH (cit.[16,18]). Peptidy a proteiny obsahují řadu funkčních skupin (–OH, –COOH, –SH, $–NH_2$, $–CONH_2$ atd.) nesoucích v určitém rozsahu pH náboj, který je příčinou jejich reaktivity a vede k elektrostatickým interakcím s hydroxopolymery a hydratovanými oxidy kovů koagulačních činidel[24,25]. Se změnami nábojových charakteristik AOM peptidů/proteinů, hydroxopolymerů a hydratovaných oxidů kovů se v závislosti na reakčním pH mění i mechanismy interakcí mezi těmito látkami. Příklad průběhu koagulace AOM peptidů/proteinů pomocí síranu železitého v závislosti na hodnotě pH je uveden na obr. 1. Na grafu je zvýrazněno pět reakčních oblastí, ve kterých dochází k odlišné účinnosti koagulace. Vyznačeným oblastem odpovídají jednotlivé typy mechanismů a interakcí.

A – Oblast neúčinné koagulace

V rozsahu pH oblasti A (pH < 4) nedochází k odstranění peptidů/proteinů ani Fe. V této oblasti pH převládá v systému výrazný kladný náboj peptidů/proteinů daný množstvím protonizovaných aminoskupin $–NH_3^+$ (počet deprotonizovaných kyselých funkčních skupin je naopak velmi malý) a železo se vyskytuje v rozpuštěném

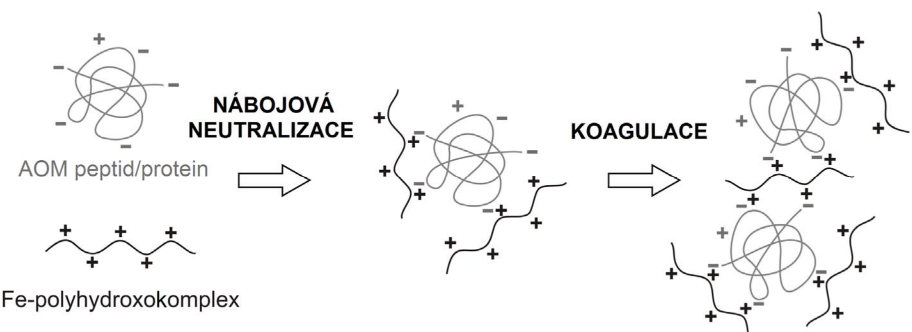


Obr. 1. **Průběh a mechanismy koagulace peptidů/proteinů v závislosti na pH**

stavu jako kladně nabité kationtové komplexy $[Fe(H_2O)_6]^{3+}$, $[FeOH(H_2O)_5]^{2+}$, případně $[Fe(OH)_2(H_2O)_4]^{+}$. V důsledku převažujícího kladného náboje dochází k elektrostatickým repulzím mezi peptidy/proteiny na jedné straně a formami Fe na straně druhé, v důsledku čehož koagulace prakticky neprobíhá[16,22], viz obr. 1.

B – Oblast účinné koagulace mechanismem nábojové neutralizace

Oblast B (pH 4–6) znázorňuje případ, kdy koagulace probíhá naopak s maximální účinností. V této oblasti pH nesou peptidy a proteiny již dostatečné množství záporného náboje daného disociací kyselých karboxylových skupin ($-COO^-$), díky čemuž mohou elektrostaticky interagovat s kladně nabitými Fe-hydroxopolymery nebo Fe-hydratovanými oxidy/hydroxidy – $[Fe(OH)_3(H_2O)_3(aq)]^0$. Tyto přitažlivé interakce vedou k postupné neutralizaci povrchového náboje peptidových a proteinových řetězců a následně tak umožňují vznik nenabitých agregátů (obr. 2)[16,19,22]. Bylo prokázáno, že oblast s nejvyšší účinností koagulace se částečně posouvá k vyšším hodnotám pH s klesající počáteční koncentrací AOM peptidů/proteinů, a to z rozmezí hodnot pH 4–6 při počáteční koncentraci rozpuštěného organického uhlíku (DOC – dissolved organic matter) 8 mg l^{-1} na rozmezí pH 5,5–7,5 při počáteční koncentraci DOC 1 mg l^{-1} (cit.[16,22]). Zvýšení dolní hranice optimálního rozsahu pH pro odstraňování Fe ze 4 (DOC 8 mg l^{-1}) na 5,5 (DOC 1 mg l^{-1}) lze vysvětlit nízkými kon-



Obr. 2. **Tvorba mikroagregátů mechanismem nábojové neutralizace peptidů/proteinů Fe-polyhydroxokomplexy**

centracemi peptidů a proteinů, které neposkytují na svých vedlejších řetězcích dostatečné množství záporně nabitých míst k reakci s kladně nabitými železitými hydroxopolymery[16,22]. V této souvislosti je třeba zdůraznit, že v případě hlinitých koagulačních činidel je vzhledem k rozdílné hydrolýze Al v závislosti na pH oblast účinné koagulace mechanismem nábojové neutralizace posunuta do rozsahu pH 5–6,5 (cit.[26]).

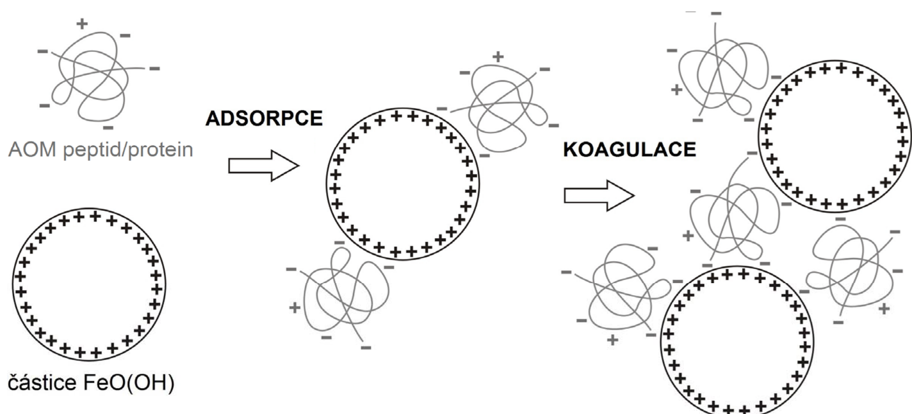
C – Oblast narušení koagulace tvorbou komplexních látek

Oblast C zvýrazňuje nárůst koncentrací DOC i Fe při pH okolo 6 (max. při 6,2). Snížení účinnosti koagulace je v této oblasti způsobeno tvorbou polynukleárních směsných ligandových povrchových komplexních sloučenin[11,14,16,26], k jejichž vzniku vede interakce mezi $–COO^−$ skupinami na povrchu peptidů/proteinů a rozpuštěným či mikrokoloidním Fe. Vznik povrchových Fe-organických komplexů a zejména významná komplexační kapacita AOM peptidů/proteinů kolem pH 6 (obvyklá hodnota reakčního pH při koagulaci většiny povrchových vod s obsahem NOM)[21,27] brání hydrolýze koagulačního činidla a tvorbě kladně nabitých hlinitých či železitých polyhydroxokomplexů nutných k destabilizaci znečišťujících příměsí. Počáteční stádia hydrolýzy iontů kovů (Al/Fe-monohydroxokomplexy) jsou prostřednictvím těchto interakcí vázány v organické hmotě ve formě stabilních rozpuštěných či mikrokoloidních Al/Fe-peptidových/proteinových komplexních sloučenin a nemohou se dále účastnit koagulačního procesu[14,16,26]. Kromě toho hliník či železo vázané v molekulách proteinů blokují negativně nabitá místa na jejich povrchu, čímž zabraňují jejich koagulaci mechanismem nábojové neutralizace. Tvorba komplexních sloučenin tak vede k poklesu účinnosti koagulace, neúměrnému nárůstu účinné dávky koagulačního činidla a zvýšení zbytkových koncentrací organických látek a koagulantu (Al či Fe) v upravované vodě[3,11,14,16,26]. Nicméně, je třeba zdůraznit, že při dostatečně vysoké dávce koagulantu a pH > 7 lze Al-, resp. Fe-peptidové/proteinové komplexy účinně odstranit mechanismem tzv. enmeshmentu, při kterém dochází v důsledku vysoké dávky koagulantu k velmi rychlé tvorbě sraženiny hydratovaných oxidů kovů, která do své struktury strhává ostatní ve vodě obsažené koloidní příměsi[21]. Takto vysoké dávky koagulačního činidla však mají za následek vznik nadměrného množství odpadního kalu a vyšší ekonomickou náročnost procesu[16]. Mechanismy a hodnocení tvorby komplexních látek jsou podrobněji popsány v kap. 3.2.

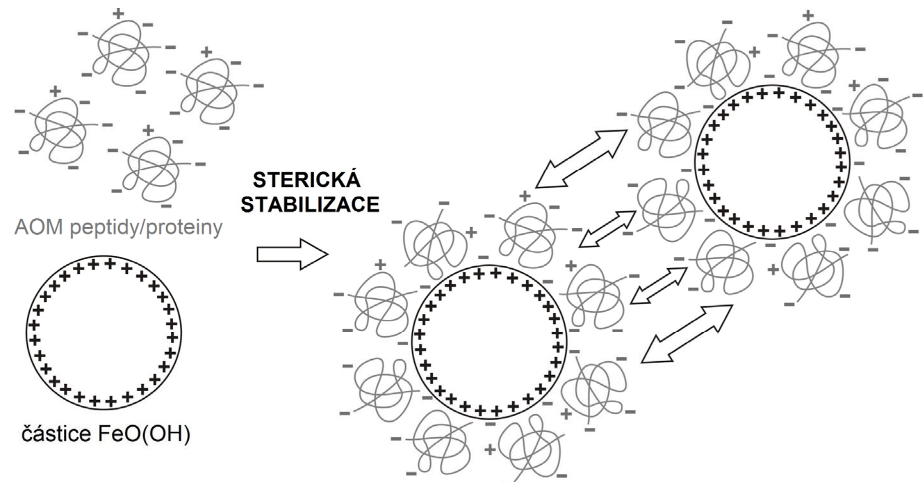
D – Oblast nespecifické adsorpce a sterické stabilizace

Z obr. 1 je dále patrné, že v oblasti D (pH 6,5–8) dochází k poměrně prudkému nárůstu zbytkových koncentrací DOC i Fe (cit.[16,22]). V této oblasti pH se již obvykle tak výrazně neuplatňují klasické elektrostatické interakce vedoucí k postupné nábojové neutralizaci systému, naopak začíná převládat mechanismus nespecifické adsorpce. Účinnost adsorpce při koagulaci organických látek je obvykle ve srovnání s nábojovou neutralizací elektrostatickými interakcemi výrazně nižší[16,19]. Bylo zjištěno, že její účinnost je úzce závislá na poměru koncentrací peptidových/proteinových látek a Al či Fe. Adsorpce jako koagulační mechanismus hraje důležitou roli především v případech, kdy poměr koncentrací AOM peptidů/proteinů a Al/Fe je nízký[16,19,22]. Peptidy/proteiny mohou být adsorbovány na povrchu koloidních hydratovaných oxidů hliníku/železa a mohou tak zvyšovat účinnost koagulace tvorbou mezičásticových můstků (obr. 3). Naopak při vysokém poměru koncentrací DOC peptidové/proteinové frakce a Al či Fe dochází k obsazení prakticky celého povrchu částic hydratovaných oxidů Al/Fe peptidy a proteiny (obr. 4). Silná adsorpce tak může vést k tzv. sterické stabilizaci a následné inhibici koagulačních procesů[16,19,22]. Mechanismem sterické stabilizace dochází ke zvýšení záporného náboje na povrchu částic hydratovaných oxidů hliníku/železa a následně k elektrostatické repulzi takto stabilizovaných částic[19,28,29]. Jako hraniční byl stanoven poměr DOC peptidů/proteinů a Fe = 0,33 (cit.[16,22]).

Mechanismus koagulace adsorpcí je obvykle vysvětlován pomocí tzv. elektrostatického patch modelu[30]. Podle této teorie se mohou záporně nabité peptidy a proteiny



Obr. 3. **Koagulace mechanismem elektrostatického patch modelu při nízkém poměru koncentrací DOC peptidů/proteinů a Fe**



Obr. 4. **Inhibice koagulace mechanismem sterické stabilizace při vysokém poměru koncentrací DOC peptidů/proteinů a Fe**

poutat ke koloidním částicím hydratovaných oxidů kovů (pH nulového bodu náboje FeO(OH) ~ 7,5), což vede ke vzniku záporně nabitých míst na povrchu koloidních částic hydratovaných kovů (obr. 3). Při vzájemné srážce takto nabitých částic dochází současně k upevnění opačně nabitých ploch na povrchu těchto částic. Dalším možným vysvětlením mechanismu nespecifické adsorpce je vznik vodíkových vazeb mezi funkčními skupinami peptidů/proteinů a povrchem hydratovaných oxidů kovů[24,25].

E – Oblast neúčinné koagulace

Při pH > 8 (oblast E) již koagulace prakticky neprobíhá[16,22]. Peptidy/proteiny nesou již značné množství ionizovaných funkčních skupin a železité hydroxopolymery postupně přechází do aniontové podoby, např. $[Fe(OH)_4]^-$ (cit.[31]). Mezi těmito částicemi se tak začínají uplatňovat odpudivé elektrostatické interakce, které vedou k postupnému snížení účinnosti a následně úplnému zastavení koagulace[16,22]. Aniontové formy hliníku v systému již zcela převažují od hodnoty pH 8,5 (cit.[31]).

3.2. Mechanismy a hodnocení tvorby komplexních látek

Afinita hliníku a železa k peptidovým a proteinovým AOM se obvykle vysvětluje pomocí modelu povrchových komplexů[31]. Tento model popisuje tvorbu Al/Fe-peptidových/proteinových povrchových komplexních sloučenin vznikem koordinační vazby mezi disociovanými karboxylovými skupinami na povrchu řetězců biopolymerů a pozitivně nabitými povrchovými skupinami hlinitých/železitých hydroxopolymerů a hydratovaných oxidů kovů[14,16]. Tyto reakce lze znázornit např. následujícím způsobem, viz rovnice (*1*).

$$M(H_2O)_m^{n+} + n\,RCOO^- \leftrightarrow M(H_2O)_{m-n}(RCOO)_n + nH_2O \quad (1)$$

Míra tvorby Al/Fe-peptidových/proteinových komplexů úzce souvisí s hodnotou disociační konstanty karboxylové skupiny vázané na povrchu AOM peptidů a proteinů, resp. s hodnotou pH systému. Hodnota pH ovlivňuje nejen disociaci –COOH skupiny, má ale také zásadní vliv na formy výskytu Al^{3+}/Fe^{3+} iontů. Rozpuštěné nebo mikrokoloidní Al/Fe-peptidové/proteinové komplexy mohou vznikat pouze v případě, kdy jsou karboxylové skupiny peptidových postranních řetězců deprotonizované (β-,γ-RCOOH pK_a = 3,0–4,7)[32]. Ostatní kyselé funkční skupiny na povrchu peptidů/proteinů, např. –SH a –OH, mají hodnoty pK_a konstant ve značně alkalické oblasti (–SH pK_a = 8,3–8,6 a –OH pK_a = 9,8–10,8)[32], kdy Al/Fe-hydroxokomplexy jsou již převážně záporně nabité ($[Al(OH)_4]^-/[Fe(OH)_4]^-$)[31]. Z tohoto důvodu se –SH a –OH skupiny prakticky nemohou (nebo jen malou měrou) účastnit tvorby povrchových Al/Fe-peptidových/proteinových komplexů. Z předchozího je zřejmé, že vazebná kapacita Al/Fe je přímo závislá na hodnotě pH. Maximální komplexotvorná kapacita Fe byla pozorována při pH okolo 6, za těchto reakčních podmínek kapacita peptidů a proteinů vázat kladně nabité železité hydroxopolymery koordinačními elektrostatickými interakcemi dosahuje hodnoty 1,38 mmol Fe g^{-1} DOC. Pro Al byla nejvyšší vazebná kapacita (BC – Binding Capacity = 2,88 mmol Al g^{-1} DOC) zjištěna při pH 5 (cit.[16,22]). S poklesem či vzrůstem hodnoty pH bylo pozorováno, že komplexotvorná kapacita klesá[16,22]. V alkalické oblasti pH je navíc tvorba Fe-peptidových/proteinových komplexů rušena přítomností OH^- iontů jakožto kompetitivních ligandů[31]. Jiné práce uvádí maximální vazebnou kapacitu Al 1,6 mmol g^{-1} při hodnotě pH 6 (cit.[33]) či maximální vazebnou kapacitu Fe 1,2 mmol g^{-1} pro fulvokyseliny[34]. AOM peptidy a proteiny tedy vykazují prakticky stejnou BC železa jako fulvokyseliny, které bývají obvykle označovány za NOM s největší

vazebnou kapacitou pro vícemocné kationty[16,22].

Bylo zjištěno, že na tvorbě komplexních sloučenin se podílejí především nízkomolekulární peptidy a jen některé proteiny. V případě AOM produkovaných sinicí *M. aeruginosa* se jedná o peptidy s MH 1; 2,8; 6; 8; 8,5 a 10 kDa (cit.[16,22]) a proteiny o MH 43; 52 a 67 kDa (cit.[11,16,22]). Mezi nízkomolekulární komplexotvorné sinicové peptidy patří především lineární a cyklické oligopeptidy, jako jsou cyanopeptolin (MH přibližně 950 Da), aeruginopeptin (MH 1022 až 1072 Da), mikropeptin (MH 885 až 1110 Da), ale i mikrocystiny (MH 985 až 1024 Da) a cyanochlorofyl (MH přibližně 900 Da). Dále se také jedná o látky umožňující přenos železa přes buněčné membrány, tzv. sinicové siderofory s MH v rozmezí od 500 do 1500 Da, a vysokomolekulární proteiny s afinitou k Al a Fe (především sinicové metaloenzymy (hydrogenasy) s MH okolo 35 až 60 kDa)[16].

4. Závěr a praktický význam

Zásadní význam pro účinnou koagulaci AOM peptidů/proteinů má hodnota reakčního pH. V úpravárenské praxi je koagulace běžně prováděna při pH okolo 7 (hodnoty se mohou částečně lišit v závislosti na použitém koagulačním činidle a charakteru surové vody). Neutrální oblast pH (pH 6,5–7,5) je ale vhodná především pro úpravu zakalených vod[26]. Peptidová/proteinová složka AOM má maximální účinnost odstranění při pH v rozsahu 4–6 (viz kap. 3.1.)[16,22]. Podle dalších prací jsou rozpuštěné organické látky nejúčinněji odstranitelné při pH v rozmezí 5–6,5 (někdy dokonce i < 5)[18,21,27]. Pro odstraňování přírodních organických látek je tedy obecně výhodné, opět v závislosti na jejich charakteru a použitém koagulačním činidle, snížit pH do oblasti okolo 5,5. Skutečností je, že v této oblasti pH se v praxi obvykle úprava pitné vody neprovádí. Důvodem jsou především obavy o zvýšení zbytkových koncentrací koagulantu, především hliníku, v upravené vodě, a to zejména při odstraňování huminových látek mechanismem neutralizace jejich povrchového náboje. V případě výskytu produktů sinic a řas je však výrazné snížení pH do oblasti pod 6 nezbytným předpokladem jejich účinného odstranění. Z tohoto důvodu je výhodné k odstraňování peptidových/proteinových AOM použít železitá koagulační činidla, která poskytují dostatek kladného náboje nutného k elektrostatickým interakcím s $–COO^−$ skupinami i v oblasti pH 4–5 (cit.[18]).

Dalším možným problémem úpravy vody s obsahem peptidových/proteinových AOM může být skutečnost, že na tyto látky obvykle není optimalizována dávka koagulačního činidla. Ta je velmi často optimalizována na zákal anebo na koncentraci organických látek vyskytujících se ve vodě v průběhu celého roku (huminové látky). Sinicové AOM jsou v našich klimatických podmínkách obvykle sezónní záležitostí a jsou tudíž často při optimalizacích koagulačních procesů opomíjeny, a to i přesto, že v době rozvoje vodního květu mohou tvořit dominantní podíl organických látek obsažených v surové vodě[8]. V případě výskytu sinicových AOM není hlavním problémem ani tak nárůst koncentrace organických látek (ten ostatně ani vzrůst nemusí, protože právě v letních měsících bývají naopak koncentrace huminových látek na relativně nízké úrovni), ale zásadní je především změna jejich charakteru (molekulová hmotnost, nábojové poměry atd.). Tato změna pak obvykle vede k nárůstu zbytkových koncentrací DOC, Al/Fe, snížení účinnosti filtrace, nárůstu koncentrací vedlejších produktů desinfekce vody, tzv. DBPs (Disinfection By-Products) atd. Řešení této situace je možné, viz kap. 3.1., pouze změnou reakčního pH do oblasti 4–6 (u železitého) či do oblasti pH 5–6,5 (u hlinitého činidla), kde se začne uplatňovat mechanismus nábojové neutralizace AOM peptidů/proteinů Al/Fe-polyhydroxokomplexy[16,22]. V této oblasti pH navíc dochází v přítomnosti peptidových/proteinových AOM k velmi účinnému odstranění zákalu a zbytkového hliníku/železa[26], upravená voda tak dosahuje vysoké kvality.

Závěrem je, bohužel, nutné konstatovat, že v praxi jsou problémy s výskytem sinicových AOM velmi často řešeny prostým zvýšením dávky koagulačního činidla, což však samo o sobě je krok zcela nedostačující a bez zásadní změny reakčních podmínek vyplývající z charakteru sinicových AOM (ostatně to platí pro všechny organické látky) nemá opodstatnění.

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M. Barešová[a,b], J. Načeradská[a,b], I. Kopecká[a,b], and M. Pivokonský[a] *([a] Institute of Hydrodynamics, Academy of Sciences of the Czech Republic, Prague, [b]Institute for Environmental Studies, Faculty of Science, Charles University in Prague):* **Coagulation Mechanisms for Removal of Peptides and Proteins Produced by Phytoplankton**

Increased seasonal concentrations of algal organic matter (AOM) in raw water can disrupt production of drinking water. The aim of the present study is to elucidate the mechanisms involved in coagulation of peptides and proteins in AOM. The available data suggest that charge neutralization and nonspecific adsorption are the dominant mechanisms effective in the coagulation. The highest removal of peptides and proteins is achieved in the pH range 4–6, when positively charged hydrolysis products of a Fe(III) coagulant neutralize the negative surface of peptides/proteins. At a low dissolved organic carbon (DOC) and Fe concentration ratio, nonspecific adsorption of peptides/proteins on Fe(III) oxide-hydroxide particles, as described by the electrostatic patch model, enables coagulation at pH 6–8. In contrast, at high concentration ratios of DOC/Fe, steric stabilization reduces the effectiveness of coagulation in the same pH range. At pH around 6, the coagulation is disrupted due to the formation of Fe-peptide/protein complexes. The study shows that the coagulation effectiveness and removability of peptides/proteins are strongly dependent on pH, which determines the charge of peptides/proteins and hydrolysis products of the coagulant and, therefore, the prevailing interactions between them.

PUBLICATION 4

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

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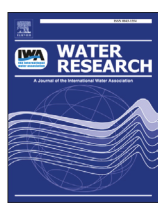

The impact of interactions between algal organic matter and humic substances on coagulation

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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.

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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

DOC of 3 + 5, 5 + 5 and 5 + 8 mg L^{-1}) were investigated. The protein/HS samples were rapidly mixed at shear rate of 200 s^{-1} for 1 min, followed by a slow mixing at shear rate of 50 s^{-1} for 30 min and 60 min of settling. The slow mixing period was lengthened in comparison with tests with coagulant, because protein/HS aggregates required longer periods to grow. The supernatants were analysed for pH, dissolved organic carbon (DOC) and UV_{254}.

3. Results and discussion

3.1. Coagulation of single compounds

The optimum $Al_2(SO_4)_3 \cdot 18H_2O$ coagulant doses for the removal of single BSA, MA proteins and HS of initial DOC = 5 mg L^{-1}were set to 1.25 mg L^{-1} (0.046 mmol L^{-1}) Al, 2 mg L^{-1} (0.074 mmol L^{-1}) Al and 5.5 mg L^{-1} (0.204 mmol L^{-1}) Al, respectively. The optimum pH ranges for these optimized coagulant doses are depicted in Fig. 1.

BSA exhibited a quite sharp and narrow pH optimum of 5.8–6.2 (Fig. 1a), typical for a single compound coagulation (Polasek and Mutl, 2005). The percentage DOC removal was 85%. Similarly, Jiao et al. (2015) found that the optimum pH for coagulation of BSA-kaolin mixture (initial DOC = 2 mg L^{-1}, turbidity = 60 NTU) by alum was at pH 6. Optimum coagulant dose for DOC removal (removal rate about 75%), optimised at pH 7, was higher (i. e. 0.1 mmol L^{-1} Al) than in the present study, probably due to the presence of kaolin and dose optimisation performed under pH 7.

MA proteins were removed within a broader pH range of 5.2–6.7 with removal rate of 75% (Fig. 1b). As well as BSA protein with isoelectric point of 4.7, MA proteins with isoelectric points of 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, which were determined by Safarikova et al. (2013), bear negatively charged functional groups (mainly $-COO^-$) at these pH values, through which they able to interact electrostatically with positively charged medium and high-MW Al-hydroxopolymers. This is consistent with the study of Pivokonsky et al. (2012), where MA proteins were coagulated by ferric sulphate at acidic pH values (pH 4–6) due to electrostatic interactions between proteins and the coagulant hydrolysis species. Moreover, Safarikova et al. (2013) demonstrated that a mixture of MA proteins and kaolin particles were removed by alum at pH values 5–6.5. The difference between optimum pH values for coagulation by aluminium and ferric coagulants stems from the different hydrolysis species distribution of Al and Fe. When compared with BSA, the wider pH optimum for MA proteins is due to the fact that *M. aeruginosa* produces a wide range of peptides and proteins with various MWs and isoelectric points (Pivokonsky et al., 2012, 2014). At pH about 6.8–6.9, peaks of residual DOC and Al concentrations occurred (Fig. 1b). These result from the formation of complexes between MA proteins and Al as demonstrated in the studies of Pivokonsky et al. (2006) and Safarikova et al. (2013).

Maximum HS removal was achieved between pH 5 and 6 with a removal efficacy of 65%, whilst minimum aluminium residuals occurred at pH 5.5–6 (Fig. 1c). At these optimum pH values, which are typical of the HS coagulation by alum, positively charged Al-hydroxopolymers interact with ionized carboxylic and phenolic groups in HS (Matilainen et al., 2010). Both HS and Al were removed to some extent at pH 6–7. As shown by Liu et al. (2009), adsorption of HS onto Al precipitates, i.e. $Al(OH)_3(s)$, dominates HS removal beyond pH 6, but does not achieve satisfactory DOC removals. Moreover, the impact of adsorption on coagulation of HS is dependent on the coagulant/DOC ratio (Cheng and Chi, 2002; Liu et al., 2009).

As stated above, the optimum dose for HS removal was significantly higher than for BSA and MA proteins. This may be due to the fact that 5 mg L^{-1} of DOC formed by HS includes a greater amount of molecules than 5 mg L^{-1} of DOC formed by proteins which contain large molecules. The greater the number of molecules to be destabilised, the higher the coagulant dose required. Furthermore, HS bear more negatively charged functional groups than proteins and, therefore, a higher coagulant dose is required for charge neutralization of HS. As shown by potentiometric titrations of HS (Fig. S1 in Supplementary data), 1 g of HS DOC contains about 7 mmol of acidic carboxylic groups. On the other hand, 1 g of BSA contains only about 1.5 mmol of carboxylic groups (calculated from the known number of $-COOH$ groups in BSA molecule), which is about 2.7 mmol $-COOH$ per 1 g of BSA DOC. It can be assumed that MA proteins also contain a lesser amount of $-COOH$ groups than HS.

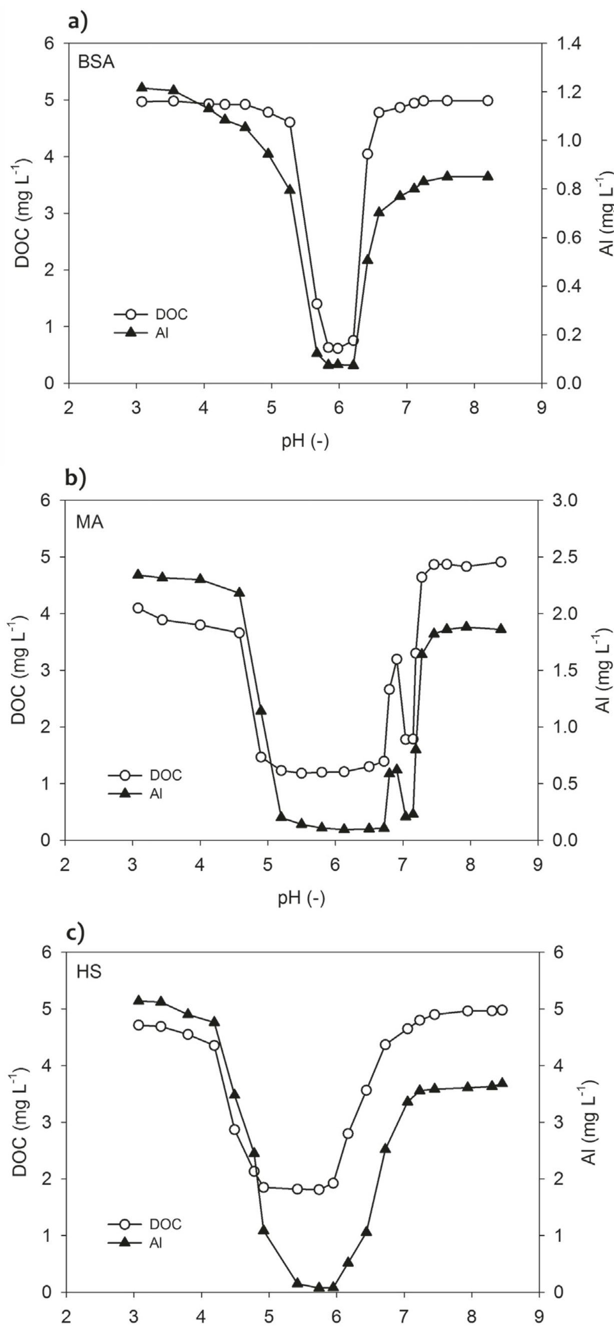


Fig. 1. Coagulation tests with optimized dose of alum and BSA (a), MA proteins (b) and HS (c) of initial DOC = 5 mg L^{-1}.

3.2. Coagulation of peptides/proteins in the presence of humic substances

For coagulation tests with a mixture of MA proteins and HS (initial DOC = 5 + 5 mg L^{-1}), the optimum dose of coagulant was set to 2.84 mg L^{-1} (0.105 mmol L^{-1}) Al. Interestingly, this dose was almost two times lower than the optimum dose obtained for coagulation of HS of initial DOC concentration 5 mg L^{-1} (see Section 3.1.). The effect of pH on MA proteins/HS coagulation is presented in Fig. 2a. Maximum DOC removal occurred at pH levels ranging between 5 and 6.2 with removal rate of 80%, while the lowest aluminium concentrations were detected at pH 5.5–6.3. UV_{254} measurements showed that MA proteins are more amenable to removal than HS as proteins formed only about 25% of residual DOC. A noticeable reduction in DOC concentration was observed even at pH below 5, although aluminium concentrations sharply rose, indicating that coagulant did not take a part in DOC reduction. This observation, together with low optimum coagulant dose, point to the existence of interactions between MA proteins and HS.

Almost identical trends were observed for the coagulation of humic substances in the presence of model protein BSA (initial DOC = 5 + 5 mg L^{-1}), shown in Fig. 2b. For these tests, optimum coagulant dose was 1.58 mg L^{-1} (0.059 mmol L^{-1}) Al which is about 3.5 times lower than the optimum dose obtained for coagulation of HS of initial DOC concentration 5 mg L^{-1} and by only 26% higher than in coagulation tests with single BSA (see Section 3.1. and Table 1). The pH levels for optimum removal of DOC ranged between 5.0 and 6.2 with removal rate of 83%. Aluminium reached the lowest levels at pH 5.5–6.2. As well as in the tests with MA proteins/HS mixture, BSA protein was more readily removed than HS as about 28% of residual DOC was formed by BSA. Moreover, reduction in DOC concentration was also observed at pH below 5, indicating the impact of BSA-HS interactions. The comparison between coagulation of MA protein/HS and BSA/HS mixtures is shown in Table 1. Xiao et al. (2013) also found that removal of NOM by coagulation may enhance removal of perfluorooctane sulfonate (PFOS – a persistent organic pollutant that has been found to be ubiquitous in the environment) in a slightly acidic region due to a strong association between PFOS and NOM.

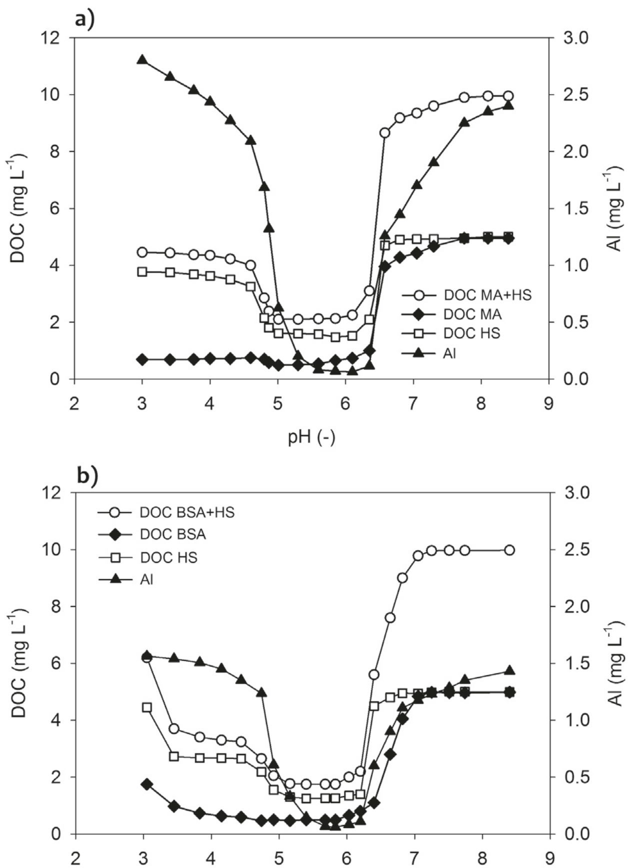


Fig. 2. Coagulation tests with optimized dose of alum and MA proteins and HS (a) and BSA and HS (b) mixtures of initial DOC = 5 + 5 mg L^{-1}.

Fig. 3 shows the HPSEC analyses which were performed after the treatment under optimum pH values. In the case of MA proteins/HS mixture (Fig. 3a), HPSEC indicated coagulation preferentially removed higher-MW compounds whilst those remaining in the treated waters had lower molecular weights. This finding is consistent with the studies on the coagulation of MA proteins (Pivokonsky et al., 2012) and a mixture of MA proteins and inorganic kaolin particles (Safarikova et al., 2013). In Fig. 3b, the peak of residual BSA is much smaller than the peak of HS. It is firstly because BSA has lower UV absorbance compared with HS and secondly because BSA is better removed than HS which is in accordance with results presented in Fig. 2b.

To provide deeper insight into the influence of protein-HS interactions on coagulation, tests with MA proteins/HS and BSA/HS mixtures in the absence of coagulant were carried out. Proteins were dosed at three different DOC concentrations (3, 5 and 8 mg L^{-1}) while DOC concentration of HS was kept at 5 mg L^{-1}. In the case of MA proteins/HS mixture, noticeable DOC removals were observed at pH values below 3.5–4.5 depending on the initial protein:HS ratio (Fig. 4a). DOC reductions of 55%, 63% and 69% were achieved for initial MA proteins/HS concentrations 3 + 5, 5 + 5 and 8 + 5 mg L^{-1}, respectively. The differences in removal rates are given by the fact that proteins were again more amenable to coagulation than HS as also demonstrated by Fig. 5, which shows residual DOC concentrations for MA proteins and HS separately.

Similar observations were made for BSA/HS mixture (Fig. 4b and Fig. 6). A substantial decrease in DOC concentrations occurred at pH levels below 4–4.5 with removal rates 68%, 75% and 77% for initial BSA/HS concentrations 3 + 5, 5 + 5 and 8 + 5 mg L^{-1}, respectively. However, for higher BSA concentrations, residual DOC increased at low pH values, i.e. below pH 3 for 5 + 5 mg L^{-1} and below pH 3.5 for 8 + 5 mg L^{-1}. This is probably a result of the conformational changes in BSA molecule. In solution, different isomeric forms of BSA exist depending on pH value. These correspond to different α-helix contents. The 'N' (normal) form, in which BSA has the most compact state, is predominant in the pH range 4.3–8. Between pH 4.5 and 4.0 the N–F (Normal–Fast; 'F' – fast migrating) transition occurs and partly open 'F' form is produced upon lowering the pH to <4. At pH lower than 3.5, BSA undergoes another expansion with a loss of the intra-domain α-helices and the 'E' (expanded) form appears (Peters, 1985; Carter and Ho, 1994). With increasing concentration of BSA and decreasing pH value, expanded BSA molecules, attached to HS, extend out into solution and prevent molecules from approaching. Electrosteric stabilization (a combination of electrostatic and steric stabilization) of BSA-HS particles is induced and coagulation inhibited. This is not the case of MA proteins as they contain lower amount of large molecules than the corresponding amount (DOC concentration) of BSA.

The observed tendency of proteins and HS to interact at low pH values is supported by the findings of several studies on adsorption of fulvic and humic acids on algal cell surfaces. Cell walls of microalgae (e.g. *Chlorella* sp.) consist of a polysaccharide and glycoprotein matrix, while cyanobacterial cell walls are composed of a peptidoglycan layer, often complexed with specific

Table 1
Optimum coagulation conditions and removal rates for BSA and MA proteins.

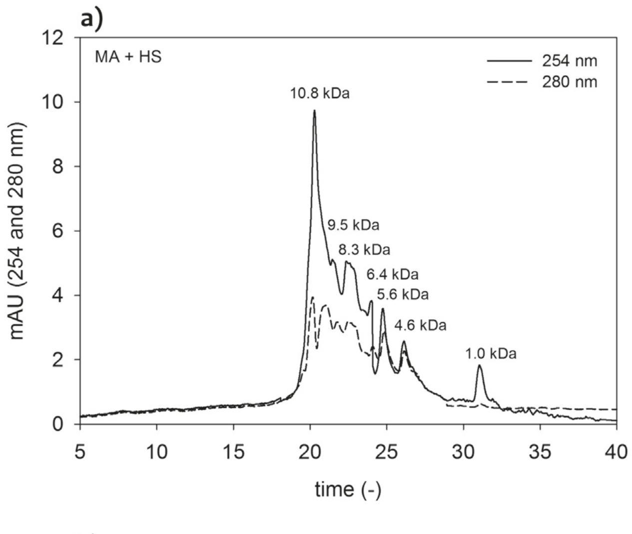
BSA	With $Al_2(SO_4)_3$	With $Al_2(SO_4)_3$ and HS	With HS
pH for DOC removal	5.8–6.2	5.0–6.2	(2.5–3.5) – (4–4.5)[a]
DOC removal rate	85%	83%	68–77%[a]
pH for Al removal	5.8–6.2	5.5–6.2	–
Al dose (mg L^{-1})	1.25	1.6	–
MA proteins			
pH for DOC removal	5.0–6.7	5.0–6.2	<(3.5–4.5)[a]
DOC removal rate	75%	80%	55–69%[a]
pH for Al removal	5.2–6.7	5.5–6.3	–
Al dose (mg L^{-1})	2	2.8	–

[a] Depending on protein/HS ratio.

polysaccharides, and an outer membrane which includes proteins, lipids and carotenoids (Lee, 2008). Knauer and Buffle (2001), who investigated the adsorption of Suwannee River fulvic acid (SRFA) to algal surfaces of three green algae (*Scenedesmus subspicatus*, *Chlamydomonas reinhardtii* and *Chlorella* sp.), observed no adsorption at pH values 6 and 7, whereas at pH 4, up to 31 mg SRFA m^{-2} and at pH 5 up to 4 mg SRFA m^{-2} was adsorbed to the algal surfaces. Similarly, Vigneault et al. (2000) found that the association of Suwannee River humic and fulvic acids with the cell surface of the green alga *Selenastrum capricornutum* was greater at pH 5 than at pH 7. Campbell et al. (1997) also showed that humic and fulvic acids tend to accumulate at the cell surface of green alga *Chlorella pyrenoidosa* and cyanobacterium *Synechococcus leopoliensis*. The amount of HS associated with the phytoplankton cells increased markedly as the pH decreased from 7 to 4.

3.3. Interactions between peptides/proteins and humic substances

It is likely that proteins and HS interact in several ways. Campbell et al. (1997) have proposed two adsorption mechanisms



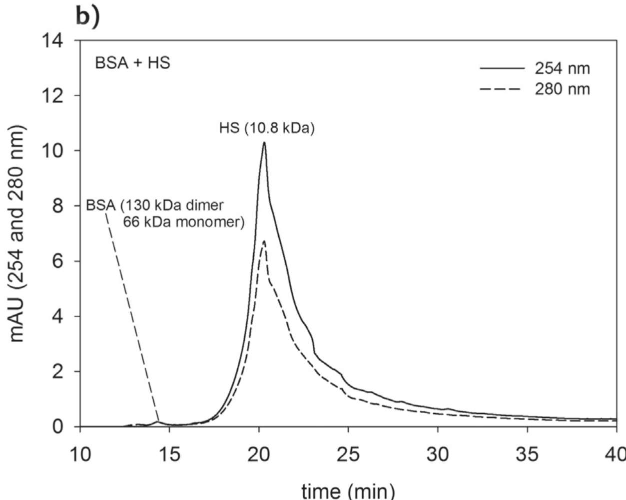
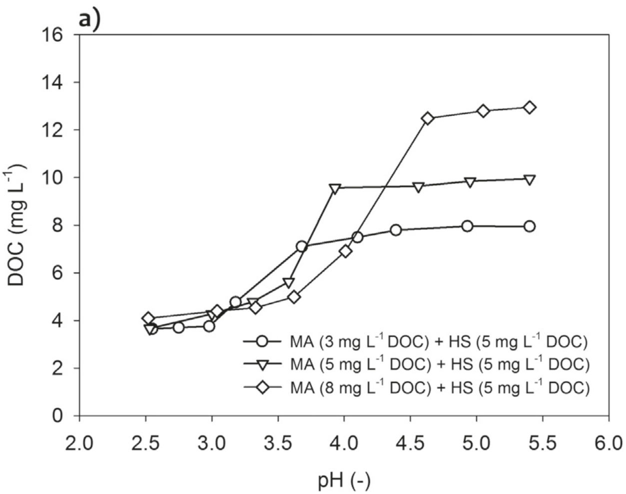


Fig. 3. HPSEC analyses after coagulation of MA proteins and HS (sample DOC = 100 mg L^{-1}) (a) and BSA and HS (sample DOC = 100 mg L^{-1}) (b) mixtures.



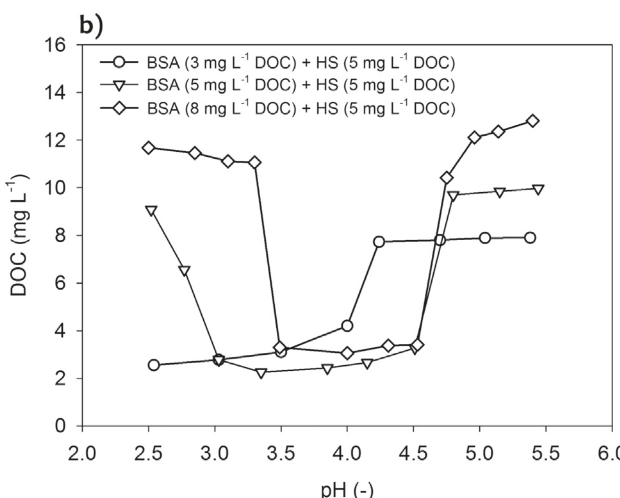


Fig. 4. Coagulation tests with MA proteins and HS (a) and BSA and HS (b) mixtures of initial DOC of 3 + 5, 5 + 5 and 8 + 5 mg L^{-1} without coagulant.

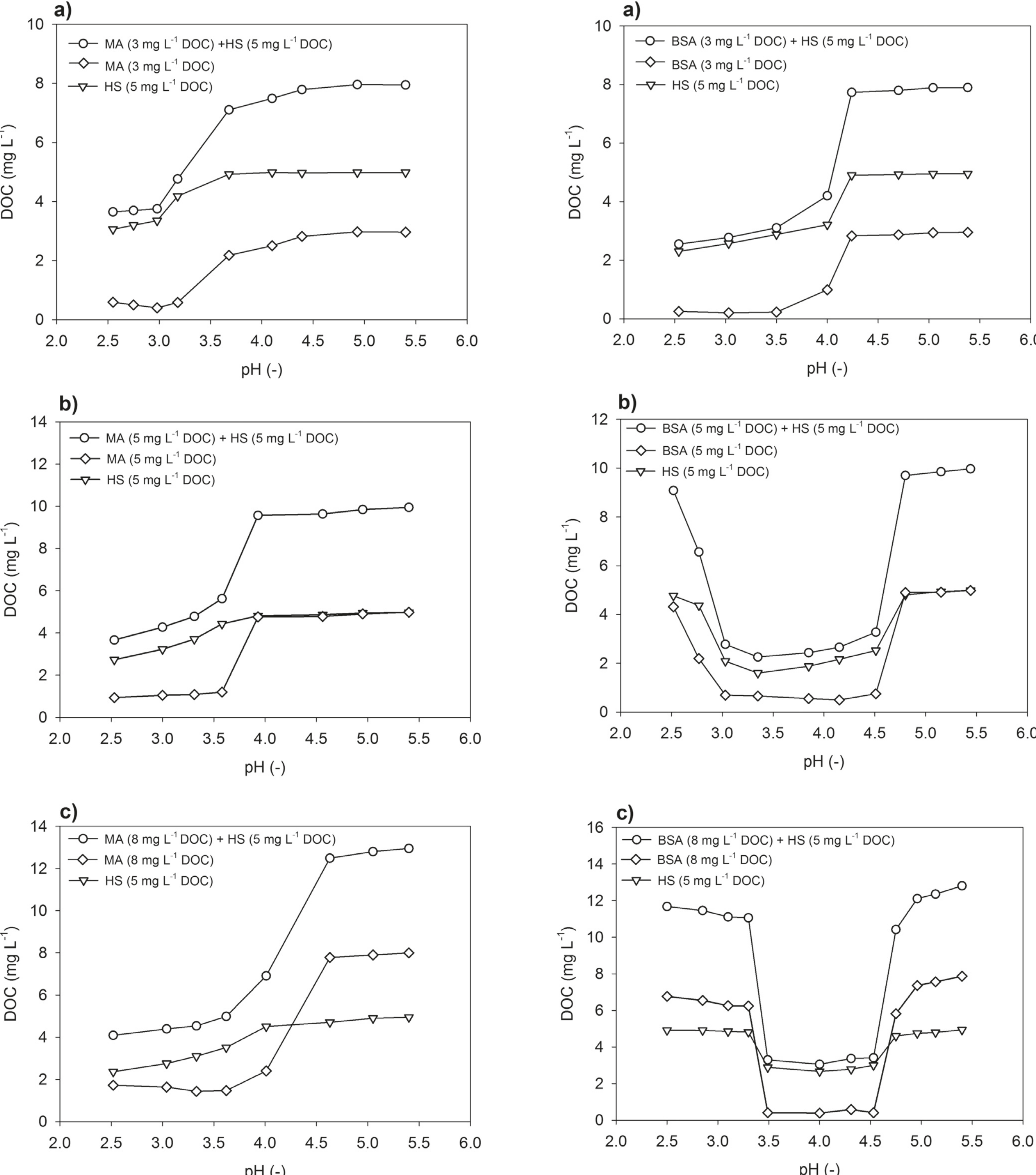


Fig. 5. Coagulation tests with MA proteins/HS mixture without coagulant – residual DOC of mixture, MA proteins and HS for three different MA proteins/HS initial DOC concentrations: 3 + 5 (a), 5 + 5 (b) and 8 + 5 (c) mg L^{-1}.

Fig. 6. Coagulation tests with BSA/HS mixture without coagulant – residual DOC of mixture, BSA and HS for different BSA/HS initial DOC concentrations: 3 + 5 (a), 5 + 5 (b) and 8 + 5 (c) mg L^{-1}.

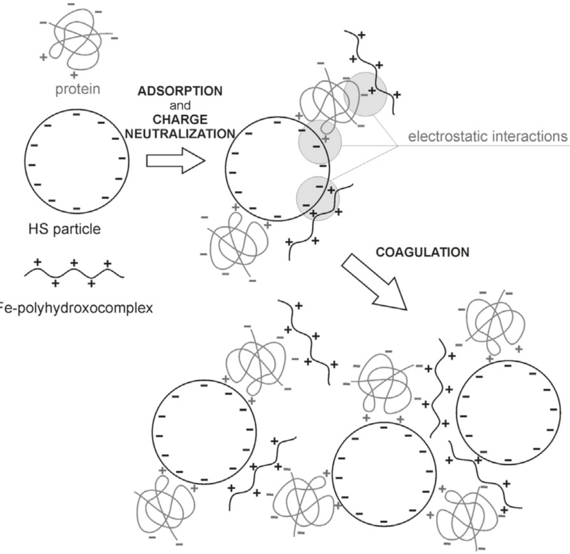
for the adsorption of humic and fulvic acids onto the phytoplankton cell surfaces. Hydrogen bonding and the formation of hydrophobic bonds between the cell surface and the hydrophobic domain of the HS. Myat et al. (2014) showed that BSA interacted with humic acid during membrane filtration. They employed molecular dynamics modelling to provide insights into these interactions and showed that electrostatic, hydrophobic and hydrogen bonds were dominant types of interaction predicted. The most common type of interaction observed throughout this simulation was hydrophobic, involving both aliphatic and

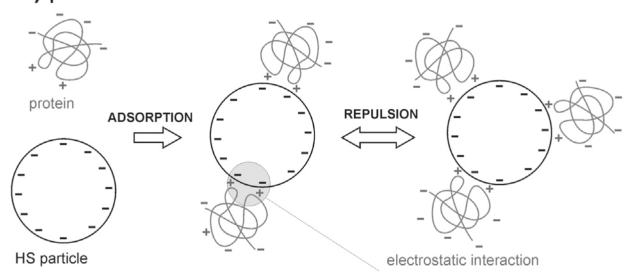
aromatic moieties of the humic acid interacting with hydrophobic regions on the protein surface. However, it is questionable to what extent the hydrophobic interaction occurs at circumneutral pH values or at pH values close to proteins' isoelectric points. Both HS and proteins tend to adopt a conformation in which hydrophilic ionisable functional groups (–COOH, –OH, $-NH_2$) are prevalent on the outside of molecules in the polar aqueous solution whereas hydrophobic moieties (e.g. aliphatic chains, aromatic groups) are self-associated in the interior. In order for the hydrophobic bonding to occur, the molecules would have to undergo major conformational changes to expose the hydrophobic interior region. For example, BSA is in the compact 'N' (normal) form in the pH 4.3–8, as described in the previous section. The hydrophobic moieties of BSA can be exposed at lower pH values where 'F' (fast migrating) and 'E' (expanded) forms occur. Similarly, conformation of HS macromolecules also changes with pH value. With decrease in pH, HS acquire a coiled configuration due to lower intermolecular electrostatic repulsion, thus they may be involved in hydrophobic interactions (Ghernaut et al., 2009). It can be therefore assumed that the hydrophobic interactions between peptides/proteins and HS will gain importance at low pH values.

The next most prevalent interaction type identified by Myat et al. (2014) in BSA – humic acid system was hydrogen bonding. It is understandable since both peptides/proteins and HS contain a large number of polar and hydrophilic functional groups that can be involved in dipole–dipole interactions.

The third common interaction identified through molecular dynamics simulations by Myat et al. (2014) was salt bridge, a combination of a hydrogen bond plus an electrostatic cation–anion interaction. These interactions are stronger compared to the hydrogen bonding. Moreover, the simulations by Myat et al. (2014) identified the salt bridge as the longest lasting interaction. Since the ionization of functional groups is pH dependent, the significance and the consequences of electrostatic interactions will change with the pH value. In case of HS, negative charge prevails over a wide pH range – the ionization of carboxylic groups take place at pH between 3.1 and 6.9 and the ionization of phenolic groups at pH between 6.9 and 8.8 (see Fig. S1 in Supplementary data). The charge of peptides/proteins is defined by their isoelectric points, the values of which are mentioned in Section 3.1.

As stated above, in the coagulation tests with single compounds and alum, the main coagulation mechanism is charge neutralization – positively charged Al-hydroxopolymers interact with negatively charged functional groups of peptides/proteins or HS. This is also the case in the experiments with MA proteins/HS and BSA/HS mixtures. In these tests, the above mentioned protein-HS interactions lead to significant reductions in coagulant dose. The interactions of proteins and HS with coagulant within optimum pH range 5.5–6.2 are depicted in Fig. 7a. Even if organic molecules tend to attach to one another and form groups, coagulation does not take place without the addition of the coagulant at about pH > 4 (Fig. 7b), because the electrostatic repulsion between negatively charged functional groups within the organic molecules does not allow them to approach. The situation changes with decrease in pH value. As the pH is lowered to approximately 4, acidic functional groups present in both peptide/protein and HS molecules are progressively protonated. Electrostatic repulsion between peptides/proteins and HS is suppressed, the conformation of the molecules changes and the possibility of hydrophobic and hydrogen bonding is thus enhanced. Electrostatic interactions are also likely to occur since molecules still bear some charged functional groups, both positive (especially in peptides/proteins) and negative. The suppression of the negative charge at low pH values thus leads to formation of aggregates (Fig. 7c).





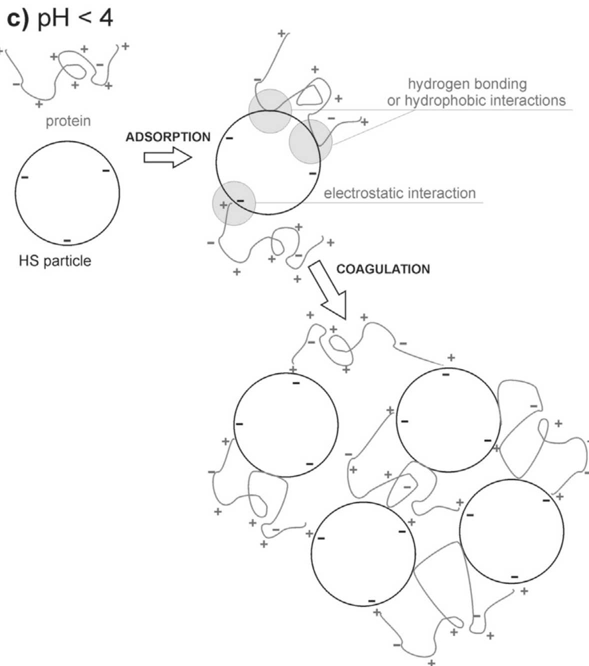


Fig. 7. The interactions between proteins, HS and coagulant (Al) at pH 5.5–6.2 (a), between proteins and HS without coagulant at pH > 4 (b), and between proteins and HS without coagulant at pH < 4 (c).

4. Conclusions

The study showed that the molecular interactions between humic substances and peptides/proteins of *M. aeruginosa* substantially impact on the coagulation process, as these interactions lead to a noticeable decrease in coagulant dose and increase in coagulation effectiveness when compared with coagulation of HS alone. The optimum pH for coagulation of MA protein/HS mixture by alum did not basically differ from the optimum pH for single HS coagulation and ranged between pH 5.5–6.2. Charge neutralization of proteins and HS by coagulant hydroxopolymers was the main coagulation mechanism. MA proteins and HS interacted, probably through hydrophobic, dipole–dipole and electrostatic interactions, even in the absence the coagulant, but these interactions resulted in coagulation only at low pH values ($pH < 4$), where the negative charge of organic molecules was suppressed. Bovine serum albumin behaved in the coagulation experiments in the same way as MA proteins, showing that it is a suitable model for MA proteins. It is generally believed that increases in organic content in water treatment plant source water due to the release of AOM entail deterioration of the coagulation process and a rise in coagulant demand. However, the present findings suggest that this does not have to be the case, when released AOM interact with other particles or molecules present in source water. Further work is required to identify the possible interactions between AOM and other impurities and to assess the influence of these interactions on water treatment processes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.07.047.

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Supplementary Data for

The impact of interactions between AOM and humic substances on coagulation

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This supplementary data contains the following sections and figures:

S1. Potentiometric titrations of humic substances
S2. Acidity of humic substances
S3. HPSEC of humic substances
Fig. S1 Titration curve of humic substances
Fig. S2 HPSEC of humic substances
Fig. S3 HPSEC of peptides/proteins of *M. aeruginosa*
Fig. S4 HPSEC of BSA protein

S1. Potentiometric titrations of humic substances

Titration experiments were carried out under nitrogen atmosphere using an Orion 960 Autotitrator (Thermo Scientific, USA). Before the experiments, the system was standardized with dilute NaOH solutions to pH 11. The ionic strength (I = 0.05 mol L^{-1}) was controlled with NaCl. The HS solutions containing 100 mg C L^{-1} were titrated to pH 2 using 0.05 M HCl at a constant temperature 25.0 ± 0.2 °C. A blank titration was performed under the same conditions. The resultant titration curve (transformed for 1 g C L^{-1}) was determined as the difference between the humic substances (HS) and the blank titration.

S2. Acidity of humic substances

In the titration experiments, two pH buffer regions were identified, as is evident in Fig. S1. Region A in the moderately basic pH range is attributed to the neutralization of phenolic groups and region B in the acid pH range to neutralization of carboxylic groups (Fernandes et al., 2009). The titration curve shows that 1 g of dissolved organic carbon (DOC) formed by humic substances contains almost 7 mmol of carboxylic groups that become protonated/dissociate in the pH range 3.1-6.9 and about 1 mmol of phenolic groups that become protonated/dissociate in the pH range 6.9-8.8, depending on their respective pK_a values.

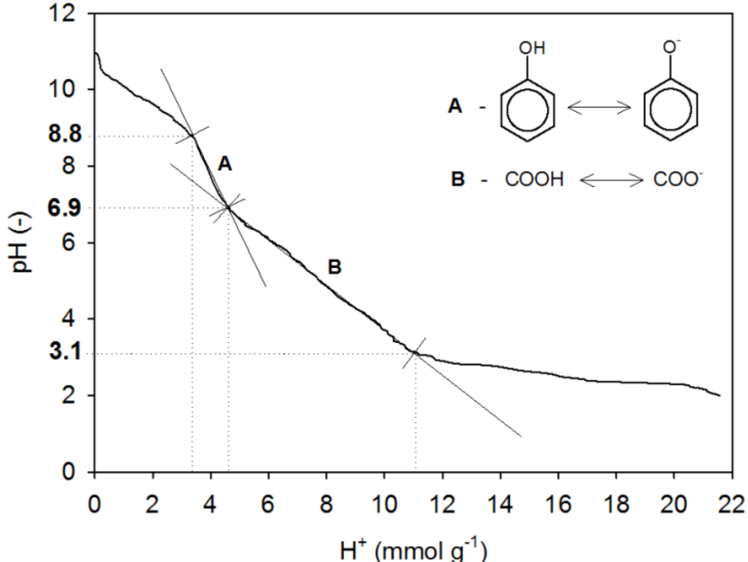

Fig. S1 Titration curve of humic substances.

S3. HPSEC of humic substances

The apparent molecular weight distribution of HS, determined by high-performance size exclusion chromatography (HPSEC), shows that the majority of the molecules fall in the range 4.3 - 15.8 kDa with the highest UV signal for 10.8 kDa molecules (Fig. S2).

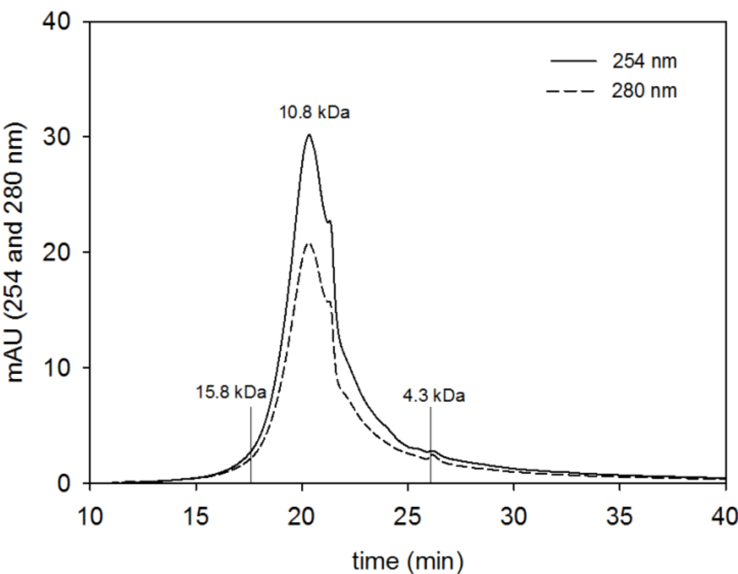

Fig. S2 HPSEC of humic substances (sample DOC = 100 mg L^{-1}).

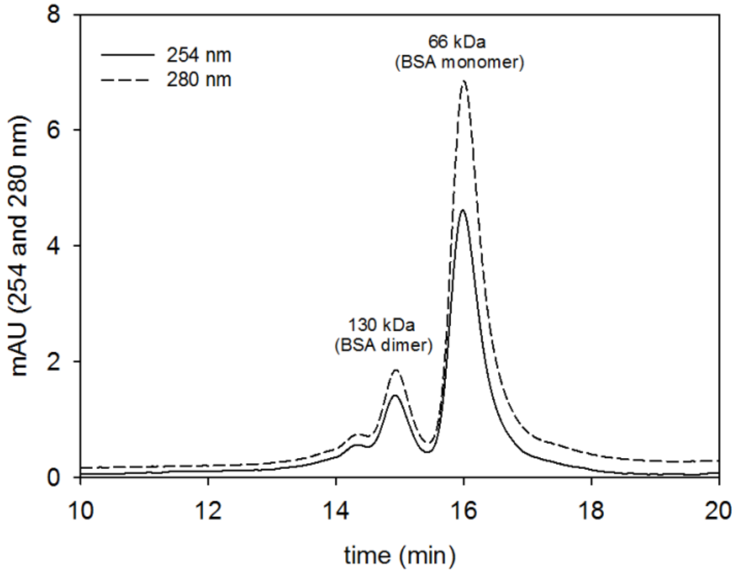

Fig. S3 HPSEC of peptides/proteins of *M. aeruginosa* (sample DOC = 100 mg L^{-1}).

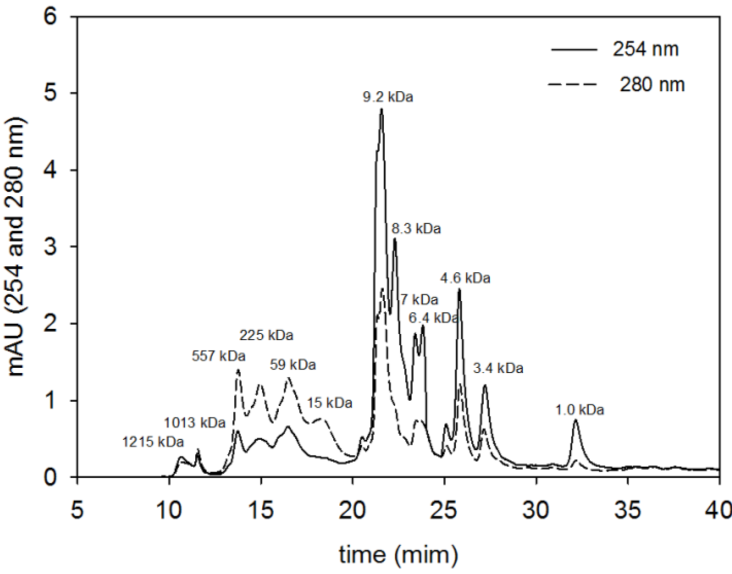

Fig. S4 HPSEC of BSA protein (sample DOC = 100 mg L^{-1}).

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PUBLICATION 5

Effect of cyanobacterial peptides and proteins on coagulation of kaolinite

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EFFECT OF CYANOBACTERIAL PEPTIDES AND PROTEINS ON COAGULATION OF KAOLINITE

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ABSTRACT

Coagulation of peptides and proteins produced by the cyanobacterium *Microcystis aeruginosa* and their influence on the coagulation of hydrophobic kaolinite particles were investigated. For this purpose, the dose of ferric sulphate used as the coagulant was optimized and jar tests with kaolinite, peptides/proteins and both kaolinite and peptides/proteins were carried out under different pH conditions. At pH 4–5.5, the peptides/proteins were efficiently coagulated and peptides/proteins were also found to contribute to the coagulation of kaolinite particles at this pH. Charge neutralization and adsorption were found to be the dominant coagulation mechanisms. The coagulation efficiency and the character of the prevailing coagulation mechanism were strongly dependent on the charge characteristics of the peptides/proteins, kaolinite and hydrolysis products of iron, thus on the pH value. At a pH of about 6, the coagulation process deteriorated due to the formation of soluble Fe-peptide/protein complexes.

Keywords: cellular organic matter (COM), coagulation, complex formation, *Microcystis aeruginosa*, water treatment

Introduction

Eutrophication of surface waters leads to seasonal occurrence of algal blooms and excess growth of cyanobacteria. Algal organic matter (AOM) interferes with the water treatment process, which includes a reduction in the coagulation efficiency resulting in increased coagulant demand (Bernhardt et al. 1985; Takaara et al. 2007; Ma et al. 2012), membrane fouling (Campinas and Rosa 2010), higher production of hazardous disinfection by-products (Huang et al. 2009; Fang et al. 2010) and disagreeable odour and taste compounds (Li et al. 2012). Cyanobacteria, usually a prevailing component of algal blooms, also produce many toxins (Harada 2004). The current knowledge of the influence of AOM on water quality and the water treatment processes are reviewed in detail by Pivokonsky et al. (2016). AOM includes extracellular organic matter (EOM) derived from the metabolic activity of algae and cellular organic matter (COM) released when cells are damaged. COM is released into water at higher rates when pre-oxidation methods are used to enhance the coagulation of algal cells (Ma et al. 2012; Xie et al. 2013) and especially during the decay of algal blooms, which usually lead to a sudden decrease in coagulation efficiency (Pivokonsky et al. 2009a). This could be temporarily overcome by increasing coagulant dose. However, this generates subsidiary problems, e.g. an increase in operating cost and yield of sludge.

COM includes a wide spectrum of substances such as oligosaccharides, polysaccharides, proteins, peptides, amino acids and some other organic acids (Lüsse et al. 1985; Maksimova et al. 2004). In general, the composition of COM can be characterized as non-protein and protein organic matter (Pivokonsky et al. 2006). Some studies have demonstrated that, for instance, proteins produced by the cyanobacterium *Microcystis aeruginosa* during its stationary growth phase make up about 60–65% of its COM (Pivokonsky et al. 2006; Henderson et al. 2008). As a result of the release of COM during algal growth, there are increasing amounts of proteins in the cultivation media of several species. The peptide/protein portion reached up to 47% of the total organics for *Microcystis aeruginosa*, 42% for the diatom *Fragilaria crotonensis* and 28% for the green alga *Chlamydomonas geitleri* (Pivokonsky et al. 2014).

COM peptides and proteins may have either a positive or negative effect on coagulation. They can improve coagulation by acting as polymer aids under specific conditions (Bernhardt et al. 1985; Ma et al. 2012). On the other hand, COM peptides and proteins may adversely affect the coagulation process and increase the consumption of coagulant by forming soluble complexes with metal ions (Fe and Al) used as coagulants (Pivokonsky et al. 2006, 2012; Takaara et al. 2007). In addition, COM influences the coagulation of other impurities present in inpurified water, e.g. inorganic colloids or humic substances (HS). The COM peptides/proteins produced by *M. aeruginosa* can enhance coagulation of humic substances if the process is operated within a certain pH range. The positive effect occurs due to electrostatic, hydrophobic and

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dipole-dipole interactions between proteins and HS (Pivokonsky et al. 2015). Some studies have also revealed the effect of AOM components on coagulation of quartz (Bernhardt et al. 1985) or kaolin particles (Takaara et al. 2007, 2010). However, the effect of reaction conditions (pH, coagulant dose etc.) and the character of COM (molecular weight, surface charge of molecules, functional group content etc.) on coagulation of particulate impurities has not been adequately explained. Therefore, this study is aimed at the elucidation of the effect of COM peptides/proteins (isolated from cyanobacterium *Microcystis aeruginosa*) on the coagulation of a hydrophobic kaolinite suspension and description of the mechanisms of the interactions between peptides/proteins, kaolinite particles and hydrolysis products of the coagulant.

Materials and Methods

Kaolinite Samples

The kaolinite clay (particles < 4 μm) was obtained from the deposit at Sedlec (Sedlecky kaolin a.s., Czech Republic). Aqueous suspension of kaolinite particles was homogenized using an ultrasonic homogenizer (UP400S, Hielscher Ultrasonics, Germany) at 100% amplitude of ultra-sonication (400 W) in pulse mode for 30 min, and subsequently used in coagulation experiments.

Kaolinite Surface Charge Determination

The surface charge of kaolinite was determined using potenciometric titrations performed at three electrolyte concentrations. Specifically, 40 g of kaolinite clay (< 4 μm) was mixed with 1.0, 0.1 and 0.01 M solutions of NaCl so that the final volume was 400 ml. The samples were homogenized using an ultrasonic homogenizer (UP400S, Hielscher Ultrasonics, Germany) at 100% amplitude of ultra-sonication (400 W) in pulse mode for 30 min. Then, 0.1M NaOH was added to obtain an initial pH of 12 and the samples were titrated with 0.1 M HCl to pH 2.5 in a nitrogen atmosphere using an Orion 960 Autotitrator (Thermo Scientific, USA). Blank titrations were also performed. Relative surface charge was determined from the difference between the surface titration curves and the blank curves. Relative surface charge was then plotted against pH. The pH at which the curves of three electrolyte concentrations crossed was the pH at which kaolinite particles exhibit zero net charge at the surface (pH of point of zero charge, i.e. pH_{pzc}). This method is described in detail in the literature (van Raij and Peech 1972; Coles and Yong 2002).

COM peptide/protein preparation

Cyanobacterium *Microcystis aeruginosa* used in this study was harvested at the Svihov water reservoir (Czech Republic). Sampling was done using a plankton net with a mesh size of 0.01 mm. The sampled cells were separated from coarse impurities by washing in ultra-pure water and passing the sample through a sieve with mesh size of 0.1 mm. Subsequently, the cells were separated from water by filtration through 0.22 μm membrane filter (Millipore, USA). Quantitative microscopic analysis of the separated cells showed that samples consisted of approximately 99% *M. aeruginosa*. Thereafter, the cells were stirred with ultra-pure water and disrupted in an ice bath using an ultrasonic homogenizer (UP400S, Hielscher Ultrasonics, Germany) at 60% amplitude of ultra-sonication (240 W) in pulse mode for 5 min. The residual solids were removed by centrifugation and subsequently by a 0.22 μm membrane filter (Millipore, USA). Peptides/proteins were isolated from the COM using $(NH_4)_2SO_4$ to precipitate protein (Dawson et al. 1986). The peptide/protein precipitate was then separated from the non-protein organic matter by filtration through a 0.22 μm membrane filter (Millipore, USA), dissolved in 200 ml of ultra-pure water and purified using an ultrafiltration membrane PLAC 1000 Da (Millipore, USA) and a Solvent Resistant Stirred Cell (Millipore, USA).

COM peptide/protein characterization

Dissolved organic carbon (DOC) concentration was monitored in samples filtered through 0.22 μm membrane filter (Millipore, USA) using a Shimadzu TOC-V_{CPH} analyzer (Shimadzu Corporation, Japan) that measured the organic carbon content by the TC-IC method as the difference between TC (total carbon) and IC (inorganic carbon). The average DOC value for each sample was determined from three measurements and the error was less than 2%.

Apparent molecular weights (MW) of COM peptides/proteins were determined by high performance size exclusion chromatography (HPSEC) using the method described in the literature (Hnatukova et al. 2011; Pivokonsky et al. 2012). Reproducibility of the MW fractionation of COM protein samples was very good, with MW deviations of less than 3% between repeated measurements.

Isoelectric points (pI) of COM peptides/proteins were determined by isoelectric focusing (IEF) using a Multiphor II electrophoresis system (Pharmacia, Sweden) according to the method described in the literature (Hnatukova et al. 2011).

Peptides/proteins, which are able to form soluble complexes with iron, were isolated by affinity chromatography. The samples of peptides/proteins were passed through an affinity column (1 ml HiTrap™, Amersham Bioscience Corp., Japan) in which Fe^{3+} ions were immobilized as ligands. Binding buffer was prepared with 0.02 M sodium phosphate and 0.5 M NaCl (pH 6). Elution buffer was of the same composition as the binding buffer, but its pH was set to 9. The elution strategy was developed in order not to damage peptides/proteins and prepare a sample suitable for UV detection. The flow rate of buffer was 1 ml min^{-1} and the volume of the fractions

collected was 15 ml. Then, the eluted fractions were desalted and concentrated using an ultrafiltration membrane PLAC 1000 Da (Millipore, USA) and a Solvent Resistant Stirred Cell (Millipore, USA). MWs of isolated peptides/proteins were determined using HPSEC. All analyses were done in triplicate.

Coagulation Tests

The influence of COM peptides/proteins on the coagulation process was investigated using standard jar tests (Bratby 2006). Jar testing was done with a variable speed eight paddle stirrer LMK 8–03 (IH AS CR, Czech Republic) in 2 l jars. Ferric sulphate (Sigma-Aldrich, USA) was used as a coagulant because the study of Pivokonsky et al. (2009b) has shown that its efficiency in COM peptide/protein coagulation is higher than that of aluminium coagulants. Its optimum dose for efficient peptide/protein removal was determined by using tests without pH control and with coagulant doses ranging from 0.025 to 0.250 mmol l^{-1} of Fe (1.4–14.0 mg l^{-1}). For experiments in which the pH was controlled and coagulant dose optimized, 0.1 M HCl and 0.1 M NaOH were added to achieve the target pH varying between 3 and 9. The jar test procedure consisted of 1 minute of high intensity agitation (G = 300 s^{-1}), 15 minutes of low intensity agitation (G = 80 s^{-1}) and 60 minutes of settling. The results of coagulation tests were evaluated by water analysis after sedimentation of the suspension. Residual Fe, dissolved organic carbon (DOC), turbidity, pH, alkalinity and molecular weight of residual peptides/proteins were monitored. In order to evaluate the influence of COM peptides/proteins on coagulation of kaolinite suspension, three types of jar tests were operated within the pH range 3–9 using the optimum coagulant dose of 7 mg l^{-1}, i.e. 0.125 mmol l^{-1} of Fe. Moreover, the comparison of jar testing results enabled the description of probable coagulation mechanisms. To facilitate the comparison between different types of jar tests, the optimum dose for peptide/protein removal was also used in other types of jar tests. The jar tests were as follows:

1) coagulation of suspended kaolinite particles,
2) coagulation of COM peptides/proteins,
3) coagulation of suspended kaolinite particles together with COM peptides/proteins.

The corresponding samples of synthetic raw water were used:

1) Ultra-pure water with an alkalinity of 1.5 mmol l^{-1} (75 mg l^{-1} $CaCO_3$) with $NaHCO_3$ + 25 mg l^{-1} of kaolinite particles < 4 μm.
2) Ultra-pure water with an alkalinity of 1.5 mmol l^{-1} (75 mg l^{-1} $CaCO_3$) with $NaHCO_3$ + COM peptides/proteins of DOC concentration 8 mg l^{-1}.
3) Ultra-pure water with an alkalinity of 1.5 mmol l^{-1} (75 mg l^{-1} $CaCO_3$) with $NaHCO_3$ + 25 mg l^{-1} of kaolinite particles < 4 μm + COM peptides/proteins of DOC concentration 8 mg l^{-1}.

DOC concentration of 8 mg l^{-1} is the common COM peptide/protein content in natural surface water (Pivokonska et al. 2008).

Results and Discussion

COM Peptide/Protein Characterization

The COM peptides/proteins were characterized in terms of MW distribution. Fig. 1 shows peptides/proteins isolated from COM produced by the cyanobacterium *M. aeruginosa* of apparent MWs of 1, 2.8, 4, 4.5, 5, 5.7, 6, 6.8, 8, 8.5, 12, 30, 40, 52, 106, 266, 470 and 1077 kDa. The values of peptide/protein isoelectric points (pI) determined by isoelectric focusing (IEF) were 4.79, 5.12, 5.25, 5.45, 5.62, 5.80, 6.10, 6.33, 6.47, 6.63, 7.05, 7.39, 7.82, 7.93 and 8.05. The character of COM peptides/proteins of *M. aeruginosa* has been discussed in our previous studies (Pivokonsky et al. 2006, 2012, 2014; Hnatukova et al. 2011).

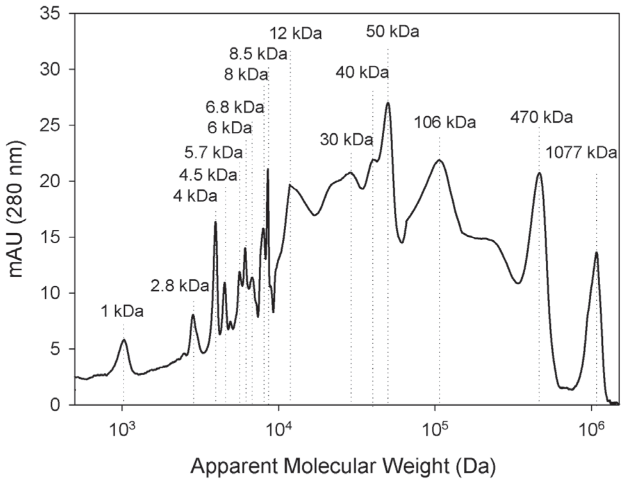

Fig. 1 HPSEC profile of COM peptides/proteins.

Coagulation of Kaolinite Suspension

Fundamental mechanisms of kaolinite coagulation are charge neutralization and adsorption (Bache and Gregory 2007). Their efficiency is closely related to the surface charge value (of both kaolinite particles and hydrolysis products of coagulation agent), which is pH dependent. As for ferric hydroxocomplexes, at pH < 2, Fe^{3+} ions occur in a water environment as Fe-hexaaquacomplex $[Fe(H_2O)_6]^{3+}$. At pH ~ 2, double nuclear Fe-hydroxocomplex $[Fe_2(OH)_2]^{4+}$ ions are formed due to the release of protons from the ion. As the pH rises (pH > 2), hydrolysis proceeds and the positively charged polynuclear Fe-hydroxopolymers (i.e. $[Fe_2(OH)_3(H_2O)_7]^{3+}$, $[Fe_2(OH)_4(H_2O)_5]^{5+}$ and $[Fe_4(OH)_6(H_2O)_{12}]^{6+}$) and Fe-oxide-hydroxides α-FeO(OH) or γ-FeO(OH) are formed. At pH > 8, iron largely occurs as anionic hydroxocomplexes, e.g. $[Fe(OH)_4]^-$ (Stumm and Morgan 1996). The surface charge of a kaolinite suspension, specifically

its point of zero charge (pH_{pzc}), depends on the chemical composition of kaolinite. In this study, pH_{pzc} was determined by potentiometric titrations at three different electrolyte concentrations (0.01, 0.1 and 1.0 M NaCl) and was set to approximately pH = 3. It implies that negatively charged sites on the kaolinite surface prevail at pH > 3 and that the total kaolinite charge is negative.

The coagulation tests performed with aqueous suspension containing 25 mg l^{-1} of kaolinite particles and with an iron dose of 7 mg l^{-1} (i.e. 0.125 mmol l^{-1} Fe) showed that the lowest residual concentrations of both kaolinite (expressed as residual turbidity) and iron were reached in the pH range 6.5 to 8 (Fig. 2). Very similar results for kaolinite coagulation are reported by Kim and Kang (1998) who recorded the highest kaolinite removal by ferric nitrate (initial kaolinite concentration 25 mg l^{-1}) at a pH between 6.7 and 8.2. Within this pH range, positively charged iron hydroxocomplexes and iron oxides-hydroxides are adsorbed on negatively charged kaolinite particles, which results in a gradual neutralization of the kaolinite surface charge and efficient coagulation. Adsorption of iron constituents is explained by electrostatic interactions, exchanging reactions and hydrogen bonding (Bache and Gregory 2007).

Coagulation of COM Peptides and Proteins

The results of coagulation tests with peptides/proteins (DOC 8 mg l^{-1}) and ferric sulphate (7 mg l^{-1} = 0.125 mmol l^{-1} of Fe) are shown in Fig. 3. The lowest residual peptide/protein concentration (expressed as residual DOC concentration) was achieved within the pH range 4 to 6. The capability of COM peptides/proteins to be coagulated stems depended on the character and content of functional groups and also their molecular weight, which will be discussed later. Some of the functional groups, such as –OH, –COOH, –SH, $–NH_2$, $–CONH_2$ etc. may bear a charge under certain pH conditions, which allows coagulation by charge neutralization and/or adsorption (Bernhardt et al. 1985). If the isoelectric points of peptides/proteins are taken into consideration, it is obvious that at the pH of the highest coagulation efficiency (4–6), peptides/proteins bear both negatively and positively charged functional groups on their surface. They are therefore able to interact electrostatically with positively charged hydrolysis products of iron. At pH around 6, a noticeable increase in residual DOC and iron concentration was recorded, which means that a portion of the peptides/proteins and iron remained in solution owing to the formation of soluble Fe-peptide/protein surface complexes. Our previous study (Pivokonsky et al. 2012) showed that the mechanisms of Fe-peptide/protein complex formation are largely of electrostatic character and that the ability of peptides/proteins to form complexes with Fe (i.e. their binding capacity) is dependent on pH. It reaches its maximum at pH 6–7. Naturally, if Fe ions are bound to peptides/proteins, they cannot take part in the coagulation process (Bernhardt et al. 1985; Pivokonsky et al. 2006; Takaara et al. 2007). Moreover, iron bound to peptides/proteins blocks negatively charged sites on the peptide/protein surface, which prevents peptides/proteins from being coagulated by adsorption and charge neutralization mechanisms (Pivokonsky et al. 2012). As seen in Fig. 3, residual DOC and Fe concentrations sharply increased at pHs > 7. At this pH value, the negative charge of both hydrolysis products of iron and peptides/proteins prevails and thus, repulsive electrostatic interactions lead to inefficient coagulation.

In order to characterize peptides/proteins able to form soluble complexes with iron, affinity chromatography followed by HPSEC was performed. Fig. 4 shows that complex forming peptides/proteins have MWs of 1, 2.8, 6, 8, 8.5, 10 and 52 kDa. It is well known that cyanobacterial COM may contain several groups of metal binding compounds. The low-MW region probably includes iron-binding peptides of MW of 500–1500 Da called siderophores, which are secreted by cyanobacteria under conditions of iron stress and enable the transport of ferric ions into cells (Albrecht-Gary and Crumbliss 1998). In addition, low-MW compounds might be cyanobacteri-

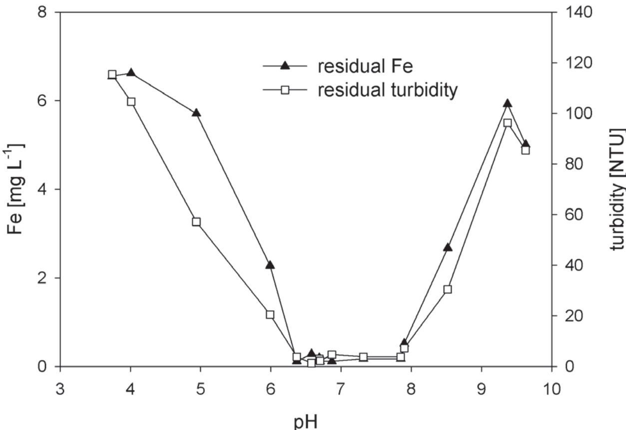

Fig. 2 Jar tests with kaolinite - dependence of residual Fe and turbidity on pH value (D_{Fe} = 7 mg l^{-1}).

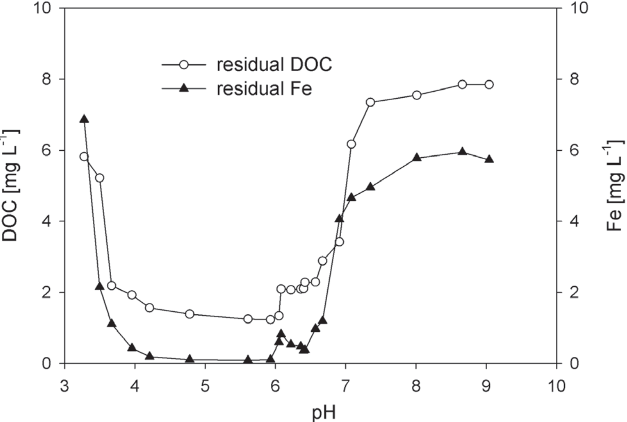

Fig. 3 Jar tests with peptides/proteins - dependence of residual DOC and Fe on pH value (D_{Fe} = 7 mg l^{-1}).

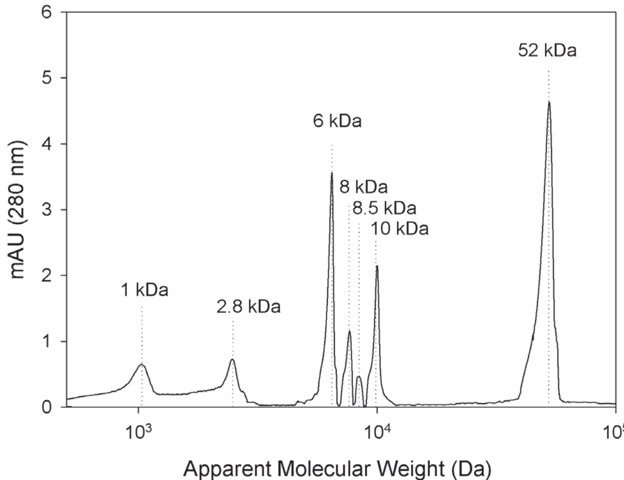

Fig. 4 HPSEC profile of complex forming peptides/proteins.

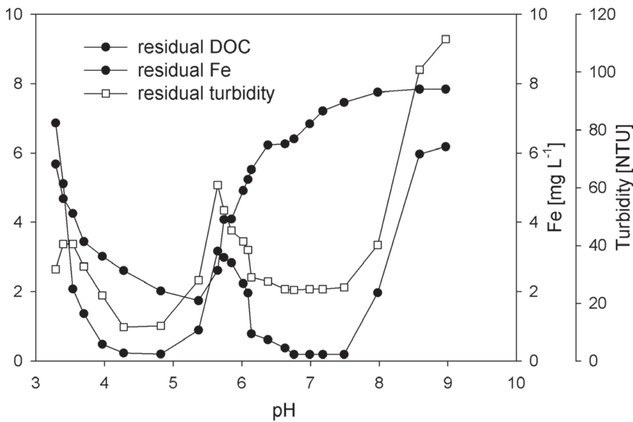

Fig. 5 Jar tests with kaolinite + peptides/proteins-dependence of residual DOC, Fe and turbidity on pH value (D_{Fe} = 7 mg l^{-1}).

al metallothioneins, cysteine-rich peptides/proteins that bind, sequester and buffer the excess intracellular metal cations through the thiol group of its cysteine residues (Turner and Robinson 1995). The isolated iron-binding protein of MW 52 kDa probably is the cyanobacterial metalloenzyme bidirectional hydrogenase, which has an affinity for iron and is present in *M. aeruginosa* (Tamagnini et al. 2007). Fe-binding protein of a similar molecular weight was also isolated in several other studies (Pivokonsky et al. 2006, 2012; Takaara et al. 2007).

Coagulation of Kaolinite Together with COM Peptides and Proteins

Coagulation tests with kaolinite (25 mg l^{-1}), peptides/proteins (DOC 8 mg l^{-1}) and ferric sulphate (7 mg l^{-1} = 0.125 mmol l^{-1} of Fe) revealed that the optimum pH conditions were within almost the same range as the above described coagulation tests with peptides and proteins. Kaolinite particles, peptides/proteins and iron were efficiently removed at pHs between 4 and 5.5 (Fig. 5). As the pH value rose, the DOC removal efficiency decreased and the peptide/protein coagulation ceased at pH about 7. Further, Fig. 5 also shows that the removability of kaolinite particles is closely connected with the coagulation of COM peptides/proteins. In the presence of these organic substances, kaolinite was removed even at pHs < 5.5. However, as demonstrated before, kaolinite particles were not removed within this pH range in the absence of peptides/proteins. The probable mechanisms involved in the coagulation of peptides/proteins and kaolinite are again charge neutralization and adsorption. At pHs < 5.5, not only electrostatic interactions between positively charged iron hydroxocomplexes and negatively charged ionized functional groups of peptides/proteins occur (as described for coagulation of peptides/proteins), but there are also electrostatic interactions between positively charged peptide/protein functional groups (e.g. $-NH_3^+$) and negatively charged kaolinite particles. These interactions lead to the gradual neutralization of the surface charge of both kaolinite particles and peptides/proteins and subsequently enable the formation of uncharged micro-aggregates (Fig. 6). Furthermore, kaolinite was also removed within the pH range 6.5–8 resulting in low residual turbidity and iron concentrations, but peptides/proteins obviously did not participate in the coagulation process and their residual content remained high. This pH optimum for removal of kaolinite corresponds to the optimum for coagulation in the test performed only with kaolinite and ferric sulphate (Fig. 2). Moreover, as in the case of coagulation tests with peptides/proteins (Fig. 3), there is a peak of residual iron at a pH of about 6 in Fig. 5. This peak is likely to be caused by two distinct features. Firstly, it can be attributed to the formation of soluble Fe-peptide/protein complexes, similar to the coagulation of peptides/proteins in the absence of kaolinite. Secondly, it may represent the transition between two different processes, i.e. the coagulation of peptides/proteins and kaolinite together at pH 4–5.5 and coagulation of kaolinite itself at pH 6.5–8. Finally, at pH > 8, no coagulation occurred due to the excess of the negative charge of all particles in the system – kaolinite, peptides/proteins and also iron constituents.

HPSEC analysis performed after coagulation tests with kaolinite, peptides/proteins and ferric sulphate demonstrated that high-MW proteins are removed under optimal reaction conditions (HPSEC profile at pH 5), whereas low-MW peptides/proteins remain in the solution (Fig. 7). These peptides/proteins were found to have MW 10, 8.4, 7.7, 6.5, 2.8 and 1 kDa. Interestingly, these peptides/proteins were shown by affinity chromatography to form soluble complexes with iron, which is consistent with findings of other studies (Pivokonsky et al. 2009a, 2015; Ma et al. 2012), in which high-MW COM compounds were removed with higher efficiency than low-MW compounds. In addition, only negligible amounts of peptides/proteins were removed during coagulation at pH 8.

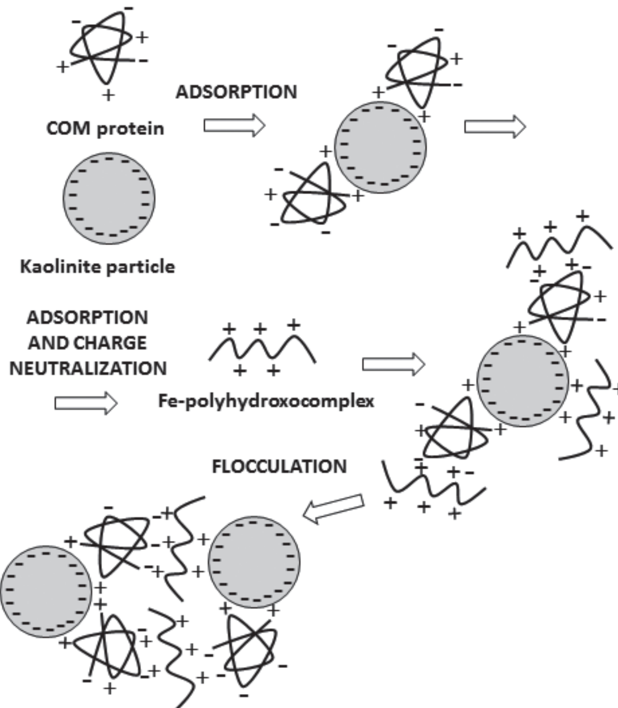

Fig. 6 Mechanism of coagulation of peptides/proteins and kaolinite.

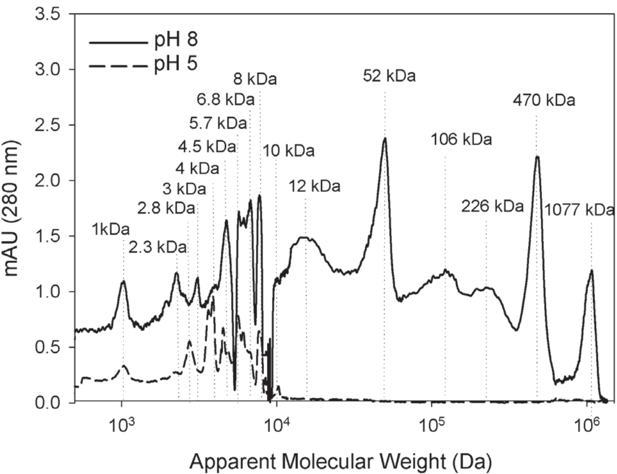

Fig. 7 HPSEC profile of peptides/proteins after coagulation tests at pH values 8 and 5.

Conclusions

This research demonstrates that the removal of COM peptides/proteins during water treatment is strongly pH-dependent and that COM peptides/proteins affect the removal of kaolinite particles. The coagulation process is commonly performed at neutral pH during water treatment. This pH value is suitable for treatment of highly turbid waters, which is supported by our finding that kaolinite particles are efficiently removed within a pH range of 6.5 to 8. Nevertheless, the results of coagulation tests showed that the optimum pH for COM peptides/proteins (DOC) removal by ferric sulphate is in the range of 4–6, when electrostatic interactions between positively charged iron constituents and negatively charged sites on peptide/protein molecules enable coagulation through charge neutralization and adsorption. Interestingly, at relatively low pHs (4–5.5), COM peptides/proteins contribute to the removal of kaolinite. The present findings suggest that during the seasonal growth of cyanobacteria, decrease in reaction pH is a prerequisite for the efficient removal of COM proteins and is also convenient for clearing turbid water. Regarding peptide/protein characteristics related to coagulation, HPSEC analysis showed that high-MW proteins are effectively removed at optimal reaction pHs (4–5.5), whereas low-MW proteins are poorly removed. Moreover, it was found that several peptides/proteins are able to form soluble complexes with iron used as a coagulant at a pH of about 6. This feature deteriorates the coagulation of COM peptides/proteins and of kaolinite particles.

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PUBLICATION 6

The impact of algogenic organic matter on water treatment plant operation and water quality: A review

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The impact of algogenic organic matter on water treatment plant operation and water quality: A review

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ABSTRACT

The proliferation of algal and cyanobacterial blooms globally has led to renewed interest in understanding the impact of cell populations on water treatment plant (WTP) performance and the resultant water quality, particularly the role of algogenic organic matter (AOM). This review discusses current knowledge on the composition of AOM, how it interferes with water treatment process technologies applied for the treatment of algal blooms, and how it affects water quality. Specifically, it was shown that AOM can lead to increased coagulant demand, increased propensity of membrane fouling, and contribute to disinfection by-products (DBPs). Identified knowledge gaps included a need for further research on the impact of cellular organic matter (COM) after an algal bloom collapses and the impact of interactions between AOM and natural organic matter (NOM).

KEYWORDS

Activated carbon; algae; coagulation and flocculation; cyanobacteria; disinfection by-products; membrane filtration

1. Introduction

With increasing eutrophication of aquatic environments and the consequent phytoplankton population increases, organic matter (OM) originating from algal cells is believed to comprise a substantial proportion of natural organic matter (NOM) in surface waters, playing an important role in aquatic ecosystems (Ozawa et al., 2005; Dyble et al., 2008; Henderson et al., 2008b; Zhang et al., 2010b; Li et al., 2011). This algogenic organic matter (AOM) is released into water as a by-product of algal and cyanobacterial photosynthesis and secondary metabolism, forming extracellular organic matter (EOM), and by cell lysis as cellular organic matter (COM). This COM is released mainly during the

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stationary and decline growth phases and also when water treatment processes induce cell rupture, for example, during preoxidation (Ma and Liu, 2002; Ma et al., 2012b; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013).

The AOM comprises a spectrum of organic compounds including monosaccharides and polysaccharides, nitrogen-containing compounds (amino acids, peptides, proteins, nucleic acids), lipids and various organic acids. The well-researched cyanobacterial toxins and taste and odor compounds are also components of AOM (Chorus and Bartram, 1999; Harada, 2004; Ozawa et al., 2005; Dyble et al., 2008). The composition of AOM strongly depends on the species, its growth phase, the age of the culture, and on the culture conditions (Hoyer et al., 1985; Pivokonsky et al., 2006, 2014; Henderson et al., 2008b; Leloup et al., 2013) as well as on external influences such as nutrient status (Myklestad, 1995; Wilhelm, 1995; Huang et al., 2007b, 2012; Kong et al., 2014) or the presence of toxic substances (Mohamed, 2008). It is understood from laboratory experiments that AOM can be produced in significant concentrations, for example, Henderson et al. (2008b) observed stationary-phase AOM concentrations from lab cultures in the range of approximately 4–27 mg L^{-1} as C dependent on the species. However, monitoring during algal and cyanobacterial blooms has focused mainly on the toxic and taste and odor fractions of the AOM. Consequently, there now exist management guidelines with associated cell population alert levels due to toxin concentrations, such as that advised by the World Health Organization of 2000 cells mL^{-1} and 100,000 cells mL^{-1} (Chorus and Bartram, 1999) and a provisional guideline value for the total microcystin-LR of 1 $\mu g\ L^{-1}$ (WHO, 2011). Yet there remains a lack of understanding on the typical total AOM concentrations experienced during blooms and, as a result, on the AOM concentrations above which water treatment performance issues are experienced.

When algal or cyanobacterial populations increase in reservoirs supplying drinking water treatment plants (WTPs), water treatment technologies have to cope not only with increased cell numbers but also with AOM. The sudden release of high concentrations of COM into the WTP source water may result in serious problems in drinking water treatment processes, especially during the decline phase of an algal bloom, and may even lead to the collapse of a WTP and/or to the production of drinking water of unsatisfactory quality (Zhang et al., 2010b). Among the most pronounced adverse effects of AOM on drinking water production are the reduction in coagulation efficiency (Bernhardt et al., 1985; Takaara et al., 2007; Ma et al., 2012b; Pivokonsky et al., 2012), membrane fouling (Amy, 2008; Qu et al., 2012b; Zhang et al., 2013a), decreased adsorption efficiency for micropollutants and low molecular weight (MW) compounds onto activated carbon (AC) (Hnatukova et al., 2011; Kopecka et al., 2014), occurrence of cyanobacterial toxins (Harada, 2004) and odor and taste compounds (Huang et al., 2007b; Li et al., 2012), and disinfection by-product (DBP) formation (Nguyen et al., 2005; Fang et al., 2010; Yang et al., 2011; Li et al., 2012; Wert and Rosario-Ortiz, 2013).

In recent years, a number of studies on AOM and its impact on water treatment have emerged. Hence, this review aims to consolidate current knowledge in this research area and identify knowledge gaps in order to guide future research endeavors. Specifically, this review aims to:

(1) Identify the key composition of AOM from a water treatment perspective.
(2) Evaluate the performance of water treatment technologies in the presence of AOM.
(3) Address the main factors influencing the effectiveness of constituent treatment processes and examine the removal mechanisms.
(4) Discuss AOM treatability with respect to its properties as well as its potential to form DBPs.

2. AOM properties from the perspective of water treatment

The concentration of AOM has been observed to reach up to approximately 27 mg L^{-1} in laboratory cultures (Henderson et al., 2008b) and therefore is considered to have a significant impact on the NOM composition in natural water, where typical humic substances and peptide/protein content are both present in concentrations of approximately 5 mg L^{-1} (Knauer and Buffle, 2001; Matilainen et al., 2008; Pivokonska et al., 2008). Previous studies characterizing AOM have found that it differs from NOM of humic character in several aspects that are important from the perspective of water treatment, such as hydrophobicity, specific UV absorbance (SUVA) value, or MW distribution (Hoyer et al., 1985; Nguyen et al., 2005; Pivokonsky et al., 2006, 2014; Henderson et al., 2008b; Fang et al., 2010; Huang et al., 2012; Li et al., 2012; Chon et al., 2013; Leloup et al., 2013; Zhou et al., 2014a). Actually, AOM more closely resembles such as secondary treated wastewater effluent, termed effluent organic matter (EfOM), in these respects (Her et al., 2003; Henderson et al., 2008b) Hydrophobicity and MW of the organic molecules influence their tendency to foul membranes and to be removed by coagulation and adsorption onto AC. It was found that hydrophilic (HPI), high-MW compounds are the main membrane foulants (Amy, 2008) and that high-MW fractions are more amenable to coagulation than low-MW ones (Pivokonsky et al., 2012). Moreover, AC was reported to be ineffective in adsorbing the HPI high-MW molecules (Zhang et al., 2011a). Specifically, it was shown that EOM and COM predominantly comprise more than 60% and 80% HPI compounds, respectively, including carbohydrates, hydroxy acids, low-MW carboxylic acids, amino acids, amino sugars, peptides, low-MW alkyl alcohols, aldehydes, and ketones. This is as opposed to hydrophobic (HPO) compounds, such as hydrocarbons, high-MW alkyl amines, high-MW alkyl carboxylic (fatty) acids and aromatic acids, phenols and humic substances (Edzwald, 1993; Penru et al., 2013), and transphilic (TPI) compounds, which are assumed to be weakly HPO compounds, such as fulvic acids (Carroll et al., 2000). Li et al. (2011) used an anion-exchange resin to separate the HPI fraction

of cyanobacterial EOM (species not reported) into neutral hydrophilic (NHPI) and charged hydrophilic (CHPI) fractions that accounted for 57% and 22%, respectively. Similar results were obtained by Li et al. (2012) who ascertained that EOM of cyanobacterium *Microcystis aeruginosa* comprised 53% of NHPI and 10% of CHPI compounds. The COM contains an even larger portion of HPI compounds than does EOM. For example, Pivokonsky et al. (2014) reported that HPI fractions of COM accounted for 87% for cyanobacterium *Microcystis aeruginosa*, 90% for diatom *Fragilaria crotonensis*, and 89% for green alga *Chlamydomonas geitleri*, which compares well with the findings of Li et al. (2012) that COM of *M. aeruginosa* contains 86% of the HPI fraction (76% of NHPI and 10% of CHPI). Accordingly, the portion of HPO fraction in COM was found to be significantly lower than in EOM, that is about 10% (Li et al., 2012; Pivokonsky et al., 2014; Zhou et al., 2014a) (Figure 1).

In accordance with the low hydrophobicity of AOM, both EOM and COM exhibit very low values of SUVA, reflecting the content of aromatic structures and conjugated double bonds in OM (Leenheer and Croue, 2003). In the case of EOM, SUVA decreases with the age of algal culture, which is related to the increasing content of largely HPI high-MW and/or cellular organics, whose concentration rises throughout the growth of algae due to increasing cell mortality rate. For example, EOM obtained during the exponential growth phase was reported to have a SUVA of about 1–2 m^{-1} mg^{-1} L, whereas the SUVA of stationary-phase EOM ranged between 0.3 and 1 m^{-1} mg^{-1} L depending on species and culture conditions (Henderson et al., 2008b; Huang et al., 2012; Pivokonsky et al., 2014). Huang et al. (2012) showed that the SUVA of EOM changes with different nutrient conditions (nitrogen:phosphorus ratio). The studies of Fang et al. (2010) and Pivokonsky et al. (2014) ascertained that COM

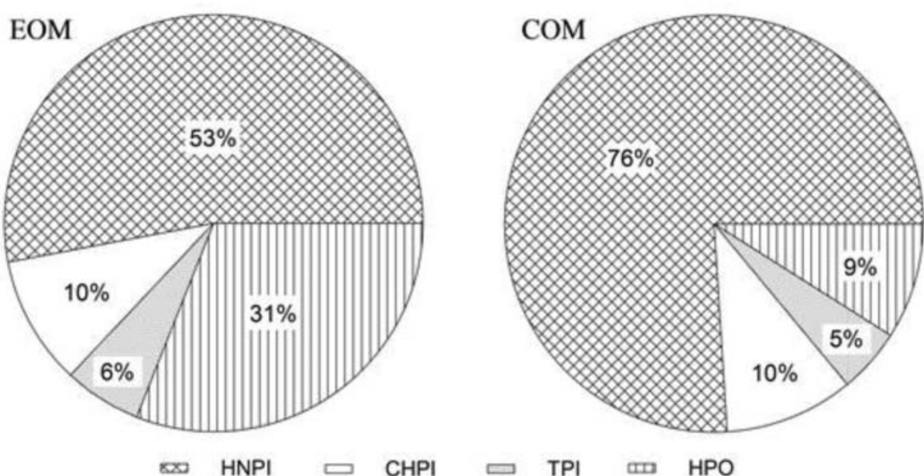

Figure 1. Comparison of the relative proportions of hydrophobic, hydrophilic (charged and neutral), and transphilic fractions in EOM and COM.

had a lower SUVA than EOM, congruent with its very low COM hydrophobicity. However, contradictorily, Li et al. (2012) reported that EOM of *M. aeruginosa* had a lower SUVA (0.72 m^{-1} mg^{-1} L) than COM (0.91 m^{-1} mg^{-1} L). This difference can be attributed to the fact that Li et al. (2012) determined the SUVA in EOM/COM samples derived from cells harvested during the exponential growth phase whereas Fang et al. (2010) and Pivokonsky et al. (2014) used samples from the stationary growth phase, highlighting the complexity associated with AOM characterization.

The MW distribution of AOM was shown to range from several hundred daltons to hundreds of kilodaltons and to be noticeably wider than the MW distribution of humic matter (Chrost and Faust, 1983; Pivokonsky et al., 2006, 2014; Henderson et al., 2008b; Fang et al., 2010; Huang et al., 2012; Li et al., 2012; Wang et al., 2013; Zhang et al., 2013a). Low-MW fractions include intermediate products of metabolism, that is aldehydes, hydrocarbons, amines, glycolic acids, amino acids, and peptides as well as mono- and oligosaccharides (Nguyen et al., 2005; Huang et al., 2007a). Higher-MW fractions (>10 kDa) are formed mostly by polypeptide molecules, such as enzymes and their components (Chróst et al., 1989; Pivokonsky et al., 2014). High-MW biopolymers (>100 kDa) such as proteins and polysaccharides were found to comprise a significant portion of AOM (Lewin, 1956; Chrost and Faust, 1983; Hoyer et al., 1985; Myklestad, 1995; Maksimova et al., 2004; Henderson et al., 2008b; Qu et al., 2012b; Pivokonsky et al., 2014). The concentration of these biopolymers in EOM rises throughout the culture growth and is the highest in COM (Hoyer et al., 1985; Lusse et al., 1985; Pivokonsky et al., 2014). The chemical structure of AOM biopolymers is species-specific and is the most investigated for polysaccharides. Maksimova et al. (2004) reported that extracellular polysaccharides of the green alga *Chlorella pyrenoidosa* S-39 comprised 20–40% of its EOM, whereas Lewin (1956) stated that 25% of EOM produced by *Chlamydomonas mexicana* comprised polysaccharides. Similarly, the recent study of Pivokonsky et al. (2014) showed that the portions of nonpeptide compounds larger than 3 kDa (considered to be mostly oligo- and polysaccharides) in *M. aeruginosa, C. geitleri*, and *F. crotonensis* were about 20–25% in EOM and 17–23% in COM. Algal polysaccharides were found to be composed of rhamnose, fucose, galactose, mannose, arabinose, xylose, ribose, and uronic acids (Lewin, 1956; Wang and Tischer, 1973; Hoyer et al., 1985; Myklestad, 1995; Maksimova et al., 2004; Huang et al., 2007b). Protein-like substances comprise up to 42% of EOM, with the quantity of peptides and proteins, as well as their diversity, increasing with culture age (Pivokonsky et al., 2006, 2014; Henderson et al., 2008b; Fang et al., 2010; Huang et al., 2012). In the case of some species (e.g. *M. aeruginosa, F. crotonensis*), proteinaceous DOC can even exceed 50% of the total DOC in COM (Pivokonsky et al., 2014).

Another important AOM property from the perspective of water treatment is its charge. Charge of particles/molecules governs their predisposition to be removed by the charge neutralization mechanism during coagulation. Charge

also plays an important role in the adsorption of impurities onto coagulant precipitates, onto AC or membranes, or in interactions with other particles/molecules present in source water. By electrophoretic mobility measurements, it was demonstrated that EOM as a whole is negatively charged throughout a wide range of pH values (Bernhardt et al., 1985, 1986; Bernhardt and Clasen, 1991; Henderson et al., 2008b), attributed to the presence of various functional groups in EOM molecules. The negative charge density of EOM changes with the species and the growth phase (Bernhardt et al., 1985; Paralkar and Edzwald, 1996; Henderson et al., 2008b). For instance, charge density of *M. aeruginosa* EOM at pH 7 decreased from 0.2 meq g^{-1} as C at the exponential growth phase to 0.1 meq g^{-1} as C at the stationary growth phase, while that of *Chlorella vulgaris* EOM at pH 7 increased from 0.9 meq g^{-1} as C at the exponential growth phase to 3.2 meq g^{-1} as C at the stationary growth phase (Henderson et al., 2008b). Henderson et al. (2008b) also observed a rapid shift from negative to positive electrophoretic mobility of EOM between pH 1 and 4, which can be attributed to the ionization of carboxylic groups (-COOH) present in charged polysaccharides and proteins. Decrease in electrophoretic mobility at alkaline pH values is caused by the dissociation of peptide/protein amino groups ($-NH_3^+$ and $=NH_2^+$ with pK_a values of 10.28 and 12.48, respectively) (Henderson et al., 2008b). The negative charge of polysaccharides stems from the presence of uronic acids, compounds containing weakly acidic -COOH groups. Their content is dependent on the species, growth conditions, and also the detection method used (Wang and Tischer, 1973; Hoyer et al., 1985). The presence of negatively charged extracellular polysaccharides was ascertained for an array of algal species (Lewin, 1956; Wang and Tischer, 1973; Strycek et al., 1992). Unlike polysaccharides, peptides and proteins are amphoteric due to their functional groups, such as $-OH$, $-COOH$, $-SH$, $-NH_3^+$, $=NH_2^+$, which can release or accept proton depending on the pH conditions (Creighton, 1993). The presence of both positively and negatively charged functional groups in proteins within a relatively wide pH range enables the proteins to electrostatically interact with both positively and negatively charged particles in water.

3. Coagulation of AOM

The coagulation of algal and cyanobacterial cells is a well-described process, the current knowledge of which is summarized in reviews of Henderson et al. (2008a) and Ghernaout et al. (2010). Since the surface of the cells is slightly negatively charged (Henderson et al., 2008a), the cells are easily coagulated in the pH range where coagulants (most commonly Al and Fe hydrolyzing salts or prepolymerized Al/Fe coagulants) bear positive charge and are, therefore, able to neutralize the opposite charge of cells. However, coagulation of algal and cyanobacterial cells may be hindered by the presence of AOM (Henderson et al., 2010; Ma et al., 2012b; Vandamme et al., 2012; Garzon-Sanabria et al., 2013; Wang

et al., 2013), which comprises a wide range of organic compounds with various properties, as discussed in Section 2. The coagulation effectiveness and mechanisms of both cell and AOM removal are dependent mainly on coagulant dose and reaction pH, and thus on the charge conditions in the system, as coagulants and organic substances usually carry a charge that is dependent on the pH value. The ratio of negative and positive charge in the system predetermines the effect of electrostatic interactions on coagulation. Electrostatic interactions between particles/molecules may lead either to charge neutralization giving rise to their efficient aggregation or to charge and/or steric stabilization of particles/molecules that prevents aggregation (Bach and Gregory, 2007). The studies of Bernhardt and Clasen (1991) and Henderson et al. (2008a, 2010) revealed a strong stoichiometric relationship between cell surface area and coagulant demand for spherical cells. In the presence of EOM, the coagulant dose needs to be increased (Henderson et al., 2010; Edzwald and Haarhoff, 2011; Ma et al., 2012b) as a result of both cell surface area and EOM influences (Henderson et al., 2010). Coagulant demand is then highly dependent on charge density, which appears to have a stronger influence than the cell surface area. Henderson et al. (2010) pointed out that, in the case of some algal species (e.g. *Chlorella vulgaris*), charge density is predominantly associated with the EOM component.

Contrary to coagulation of algal cells, the studies on coagulation of AOM itself are quite rare. Bernhardt and coworkers (Bernhardt et al., 1985, 1986, 1991) investigated the coagulation of EOM of MW >2 kDa excreted by several algal species and its influence on the coagulation on quartz particles. They concluded that the EOM contained a large concentration of nonionic and anionic polyelectrolytes, which can be characterized as neutral and acidic polysaccharides (polyuronic acids) and nonsaccharide macromolecular compounds, such as proteins. Since EOM bears a negative charge (as described in Section 2), it is removable by ferric coagulants in the pH range, where Fe forms hydrolytic products bearing a positive charge, that is at pH of about 5−6.5 (the exact pH values differ in species). At pH above 6.5, where Fe products lose their positive charge and then form anion complexes with increasing pH, the lack of positive charge in the system can be compensated by the addition of calcium ions, which enables efficient coagulation (Bernhardt et al., 1986). In the study of Henderson et al. (2010), EOM of *C. vulgaris, M. aeruginosa*, and *Asterionella formosa* was relatively treatable by coagulation using aluminum sulfate at pH 7 with removal efficiencies of 71%, 55%, and 46%, respectively. Some studies (Widrig et al., 1996; Safarikova et al., 2013) demonstrated that during the coagulation of AOM-laden waters, ferric and aluminum coagulants perform in a similar way. Only a difference in optimum pH ranges was observed (optimum for Al is at higher pH values than for Fe) (Safarikova et al., 2013), which results from the different hydrolysis product distributions of Al and Fe (Bach and Gregory, 2007). This does not apply to seawater, in which aluminum-based coagulants are too soluble and hence ferric ones are preferred (Edzwald and Haarhoff, 2011).

The coagulation of charged polysaccharides contained in AOM was described using alginic acid as a model compound. Alginic acid is composed of two uronic acids (poly-D-mannuronic acid and L-guluronic acid) containing -COOH groups with dissociation constants of 3.38 and 3.65, respectively (Kohn, 1973), and it could be coagulated by ferric salt at pH values below 6 (Bernhardt et al., 1986). Pivokonsky and coworkers (Pivokonsky et al., 2009b, 2012; Safarikova et al., 2013) used peptides and proteins contained in COM of the cyanobacterium *M. aeruginosa* and found that their coagulation was highly dependent on the ratio of positive and negative charges in the system, with the highest efficiency (between 60 and 85% depending on the initial concentration of peptides and proteins) at weakly acidic pH values. In the study of Pivokonsky et al. (2012), peptides and proteins were removed by ferric coagulant over pH 4−6 owing to the electrostatic interactions between negatively charged acidic functional groups (especially $-COO^{-}$ groups) of peptides/proteins and positively charged Fe-hydroxopolymers. The attractive electrostatic interactions led to the gradual neutralization of peptide/protein surface charge and subsequent aggregation (Figure 2a). At higher pH values (6−8), where Fe forms colloidal oxide-hydroxides (precipitates of FeO(OH)), the adsorption of peptides/proteins onto the surface of Fe-oxide-hydroxides may also play an important role. However, this process is highly dependent on the coagulant/DOC concentration ratio (Bernhardt et al., 1985, 1991; Pivokonsky et al., 2012). When the ratio is high, peptides/proteins attach to the surface of Fe-oxide-hydroxide particles, resulting in the development of negatively charged areas on the surface of iron colloidal particles, which interact with the surface of other Fe-oxide-hydroxide particles leading to particle aggregation (Figure 2b). On the other hand, when the coagulant/DOC ratio is low, the surface of Fe-oxide-hydroxide particles becomes completely covered with peptides/proteins, causing an increase in the negative charge density and, correspondingly, a charge stabilization of particles (Figure 2c) (Pivokonsky et al., 2012). In addition, steric stabilization of the particles may occur due to the protein polymer chains that project from the particles into the surrounding solution (Bernhardt et al., 1985; Bach and Gregory, 2007). Bernhardt et al. (1991) found that a critical mass Fe/DOC ratio for EOM removal was within the range of 2−3 mg (0.036−0.054 mmol) Fe per 1 mg DOC depending on species. Below this ratio, effective coagulation did not occur. Pivokonsky et al. (2014) observed that the coagulation of peptides and proteins of *M. aerugiosa* becomes ineffective at pH 6−8 when the Fe dose was 2.3 mg (0.041 mmol) Fe per mg DOC and lower. Henderson et al. (2010) reported that the doses of Al required to achieve maximum removal of EOM at pH 7 were 0.8, 1.2, and 1.5 mg (0.03, 0.044, and 0.056 mmol) Al per mg DOC for *C. vulgaris, M. aeruginosa*, and *A. formosa*, respectively. EOM of *M. aeruginosa* required a larger dose than *C. vulgaris* probably due to the formation of complexes between proteins of *M. aeruginosa* and the coagulant (see Section 3.4). EOM of *A. formosa* contains more

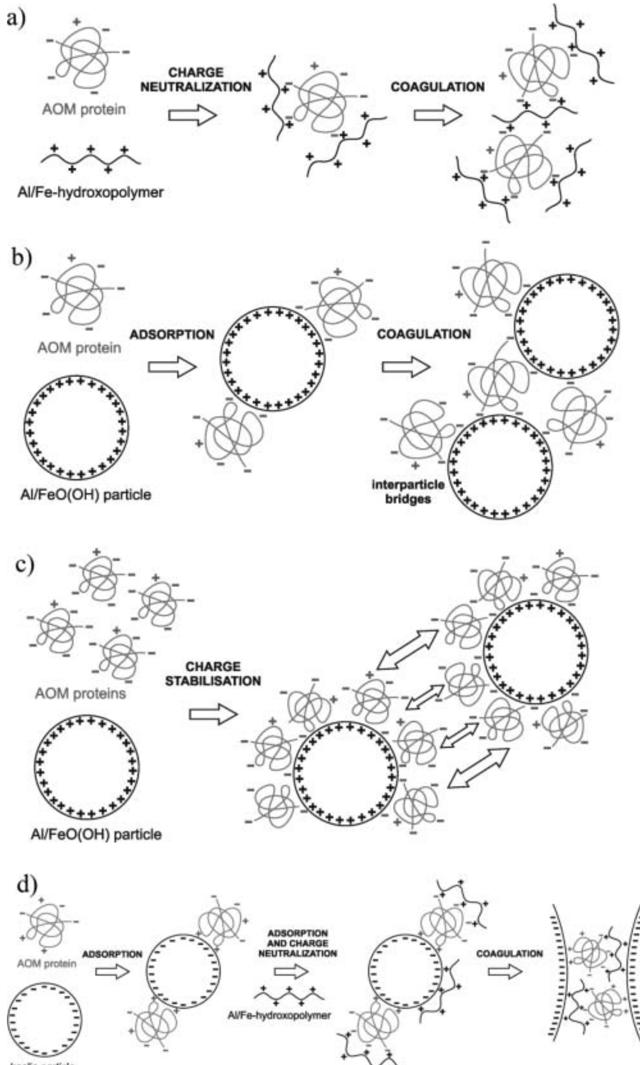

Figure 2. Mechanisms of coagulation: (a) electrostatic interactions between negatively charged acid functional groups ($-COO^-$) of peptides/proteins and positively charged Al/Fe-hydroxopolymers, leading to the coagulation (pH 4–6); (b) adsorption of peptides/proteins onto Al/Fe-oxide-hydroxide particles (pH 6–8), leading to the coagulation through the interparticle bridges at high coagulant/DOC ratio; (c) the coagulation inhibition by charge stabilization at low coagulant/DOC ratio; and (d) interactions of positively charged Al/Fe-hydroxopolymers with $-COO^-$ groups of peptides/proteins bound to kaolin particles, and with kaolin particles, leading to coagulation (pH 5–6.5 for aluminum and 4–6 for ferric coagulant).

low-MW material than the EOM of the other two species, resulting in the lowest removal efficiency and the highest coagulant demand.

High performance size exclusion chromatography (HPSEC) analyses performed after coagulation showed that high-MW protein compounds are much more effectively removed by coagulation than low-MW ones (Pivokonsky et al., 2012; Safarikova et al., 2013). This is consistent with the findings of Hoyer et al.

(1985), who demonstrated that coagulation of the EOM sample, which had undergone aeration and subsequent decomposition of the polysaccharides by bacteria, provided lower EOM removals. Furthermore, the study of Pivokonsky et al. (2009b) showed that protein-like substances (of MW >1 kDa) of *M. aeruginosa* COM were coagulated more easily than the nonprotein ones. This was probably due to the high portion of low-MW organics (<3 kDa) in nonprotein matter, which comprises 18% of *M. aeruginosa* COM.

3.1. *Impact of AOM on the coagulation of other substances*

Several studies dealt with the influence of AOM onto the coagulation of particles present in raw water (Bernhardt et al., 1985, 1986; Henderson et al., 2010; Ma et al., 2012a, 2012b; Zhang et al., 2012a; Pranowo et al., 2013; Safarikova et al., 2013; Wang et al., 2013). Some of them observed a positive influence of high-MW EOM as well as COM that was released on the coagulation of algae cells (Henderson et al., 2010; Ma et al., 2012a; Pranowo et al., 2013; Wang et al., 2013). Wang et al. (2013) found that the preoxidation of *M. aeruginosa* cells by low concentrations of $KMnO_4$ led to the secretion of high-MW compounds with MW above 200 kDa. These molecules easily combine with polyaluminum chloride (coagulant), aiding coagulation and improving the efficiency of algae removal, presumably through adsorptive bridging. On the other hand, when higher concentrations of preoxidant were applied, the significant release of COM accompanied by its degradation into low-MW compounds caused a reduction in the AOM removal efficiency and a concurrent increase in coagulant demand (Ma et al., 2012a, 2012b; Wang et al., 2013).

Furthermore, Bernhardt et al. (1985, 1986) and Safarikova et al. (2013) stated that AOM can serve as a coagulant aid in the coagulation of inorganic particles such as quartz or kaolin, which are negatively charged over a wide pH range. Bernhardt et al. (1985) concluded that EOM polysaccharides can attach to the negatively charged surface of quartz particles by means of hydrogen and covalent bonds already in the raw water before addition of the coagulant. Similarly, a study by Safarikova et al. (2013) showed that COM peptides/proteins of *M. aeruginosa* attach to kaolin particles, which was the result of electrostatic interactions between the negatively charged kaolin surface and the positively charged peptide/protein functional groups. This interaction is further discussed in Section 3.2. After the addition of the coagulant (Al or Fe), positively charged Al/Fe hydroxopolymers can interact with both $-COO^-$ groups of peptides/proteins bound to kaolin particles and also with kaolin particles (Figure 2d). This process is effective at pH values below neutral, that is 5−6.5 for aluminum and 4−6 for ferric coagulant (Safarikova et al., 2013). Bernhardt et al. (1985) pointed out that AOM behaves as a coagulation aid only at low concentrations (<2 mg L^{-1} DOC). However, it seems that achieving effective coagulation is not a matter of absolute concentration of interacting molecules/particles, but of the ratio of

charges in the system, and that higher AOM concentrations can be simply compensated by higher coagulant doses (Henderson et al., 2010; Ma et al., 2012a; Pivokonsky et al., 2012; Safarikova et al., 2013).

Moreover, Pivokonsky et al. (2015) showed that the molecular interactions between COM proteins and humic substances substantially impacted the coagulation process, as these interactions lead to a noticeable decrease in the coagulant dose and an increase in the coagulation effectiveness when compared with the coagulation of humic substances alone. The optimum pH for coagulation of a mixture of COM proteins and humic substances by alum did not differ from the optimum pH for single humic substances coagulation and ranged between pH 5.5 and 6.2. The study showed that HPO, dipole–dipole, and electrostatic interactions present the main mechanisms of proteins and humic substances interactions and the charge neutralization of proteins/humic substance mixture was detected as the main coagulation mechanism by Al hydroxopolymers (Pivokonsky et al., 2015).

3.2. *AOM as a coagulant*

Natural organic compounds, such as mono- and polysaccharides, amino acids, proteins, nucleic acids, and humic substances, are known to be able to adsorb on clay surfaces (Goring and Bartholomew, 1952; Greenland, 1956; De and Rastogi, 1962; Pinck, 1962; Blanton, 1969; Greaves and Wilson, 1969; Parfit and Greenland, 1970; Guidi et al., 1977; Labille et al., 2005) by electrostatic interactions and/or hydrogen bonds (Labille et al., 2005). It is therefore perhaps unsurprising that Devrimci et al. (2012) were able to demonstrate that calcium alginate was a successful coagulant for turbid waters containing clay (predominantly smectite). The coagulation experiments were conducted at pH 7.3 $\pm$ 0.1, at which both clay particles (colloids) and alginate (polymer) bear negative charge. A positively charged ion (calcium) was, therefore, required for the polymer to approach and bind to the clay surface. This indicates that compounds contained in AOM are likely to interact with other particles in water and may even coagulate them.

Bernhardt et al. (1985, 1986) demonstrated that EOM can serve as a coagulant aid in the coagulation of quartz particles (see Section 3.1), but they used ferric salt as the main coagulant and did not investigate the ability of EOM to coagulate quartz particles without any additional coagulant. A study by Paralkar and Edzwald (1996) showed that EOM from diatom *Cyclotella* sp., green algae *Scenedesmus quadricauda*, and *Chlorella vulgaris* can interact with positively charged latex particles (mean diameter 3.98 μm and concentration of 5×10^5 particles mL^{-1}) during experiments conducted at pH 7. It was shown that EOM from *Cyclotella* sp., which contained the highest percentage of high-MW material (90% of its EOM was larger than 30 kDa compared to 65% for *S. quadricauda* and 40% for *C. vulgaris*), adsorbed onto latex particles and neutralized its charge at a concentration

of 0.01 mg L^{-1} DOC. Reductions in the optical density and particles numbers were observed after settling. Higher concentrations of EOM from *Cyclotella* sp. resulted in charge reversal, as determined by electrophoretic mobility measurements, and restabilization. The EOM from other algae showed charge neutralization at concentrations of about 0.1 mg L^{-1} as C and charge reversal with increasing DOC concentrations, but the particles were not removable by settling. However, it should be noted that these neutralized EOM-latex particles might be removable by other techniques that can remove less-heavy particles unsuitable for sedimentation, such as sand filtration. A study by Safarikova et al. (2013) demonstrated that peptides and proteins contained in COM of *M. aeruginosa* can coagulate kaolin particles in the absence of an additional coagulant. Interestingly, the highest removal efficiencies of both impurities, that is COM peptides/proteins and kaolin particles, were accomplished at pH <4.5 as a result of electrostatic interactions between the negatively charged kaolin surface and positively charged peptide/protein functional groups (e. g. $-NH_3^+$ and $=NH_2^+$). The removal rates for DOC were about 45%, which was about 15−40% less than in the experiments in the presence of aluminum or ferric coagulant (see Section 3). The turbidity removal rates were quite similar to those in the presence of aluminum or ferric coagulant, that is about 75−80%. At pH <4.5, that is at pH values close to or lower than the dissociation constants of peptide/protein carboxyl groups (β-COOH of aspartic acid with pKa $= 3.86$ and γ-COOH of glutamic acid with pKa $= 4.25$) (Creighton, 1993), the amount of negative charge of peptides/proteins is reduced by the protonation of $-COO^-$ to $-COOH$ groups. Therefore, the repulsion between peptide/protein molecules occurs to a lesser extent and can be overcome by attractive forces that lead to coagulation. At higher pH values, the peptides/proteins attached to the kaolin surface bear more negatively charged $-COO^-$ groups, which results in the charge stabilization of kaolin particles by peptides/proteins. Similar results were obtained by Pivokonsky et al. (2015), who ascertained that COM proteins of cyanobacterium *M. aeruginosa* coagulate humic substances in the absence of an additional coagulant at low pH values (pH <4), where the negative charge of organic molecules was suppressed. A DOC reduction of 55%, 63%, and 69% was achieved for initial COM protein/humic substance concentrations of 3/5, 5/5, and 8/5 mg L^{-1}, respectively. The differences in removal rates are because the proteins were again more amenable to coagulation than humic substances. However, studies on the effect of AOM as a coagulant in a water treatment context are rare, with the majority of the research focusing on autoflocculation in algal biomass harvesting for biofuel as reviewed by González-Fernández and Ballesteros (2013). Further research is required to determine the potential for exploiting this mechanism at WTPs.

3.3. *Influence of AOM on floc properties*

Floc structural quality plays a significant role in the removal process. One of the factors that affect the properties of aggregates (size, structure, shape, density, etc.) formed during coagulation is the type of coagulated particles/substances. While the effect of inorganic particles and also allochtonous NOM on the floc properties has been investigated widely (Jarvis et al., 2005), there is a paucity of studies on the effect of AOM on algal floc properties. Since AOM contains a certain portion of polymeric substances and can also behave as polymeric aid during coagulation, it can be presumed that the influence of AOM on floc properties would be similar to that of polymers. It is generally accepted that the addition of polymer leads to an increase in floc size and strength, although it was found that this does not apply to some types of biological flocs; for example, sewage flocs showed a decline in floc strength in response to increased shear rate (Lee and Liu, 2001).

The study by Pivokonsky et al. (2009a) showed that the size of the flocs formed by AOM of cyanobacterium *Microcystis aeruginosa* and ferric sulfate is highly dependent on the initial concentration of AOM. The floc size was evaluated by the tests of aggregation after 1 minute of high-intensity mixing ($G = 300\ s^{-1}$) and 15 minutes of low-intensity mixing ($G = 80\ s^{-1}$). For initial DOC concentration of 3 mg L^{-1}, micro-aggregates prevailed over a wide range of Fe doses (0.05–0.20 mmol L^{-1} = 5.6–22.3 mg L^{-1}), whereas for initial DOC = 7 mg L^{-1}, macro-aggregates form the majority (40–60%) of particles. Moreover, Pivokonsky et al. (2009b) demonstrated that when aluminum sulfate was used as a coagulant, the portion of macro-aggregates substantially decreased (to 10–40%) at Al doses of 0.02–0.20 mmol L^{-1} (equivalent to 1.1–10.8 mg L^{-1}). This is consistent with the fact that, in general, aluminum coagulants produce smaller aggregates than ferric ones (Gregory and Yukselen, 2002; Jarvis et al., 2012; Bubakova et al., 2013).

Overall, research suggests that algal floc properties will be dependent on the character of the AOM present. For example, Henderson et al. (2006) compared the growth profiles of flocs formed by kaolin, allochtonous NOM, and cells of the green alga *Chlorella vulgaris* harvested at the exponential growth phase when using aluminum sulfate as a coagulant. Algal flocs were also bigger in the initial size than the other two types of flocs and on increasing shear rate the floc size of the algal flocs was altered by the largest proportion; these observations were attributed to the presence of AOM acting as a polymer flocculant whereby algal cells and hydroxide precipitate are bridged together by polymeric substances forming large flocs at low shear. More recent research has shown that different floc properties to those for *C. vulgaris* cells (Henderson et al., 2006) were observed for *M. aeruginosa* cells despite their similar morphological appearance (Gonzalez-Torres et al., 2014), again indicating that AOM, which was known to

be of very different character, was influential, although the degree to which it was involved is not clear as different pH values were applied in the two studies and thus further research is required to be undertaken.

3.4. *Formation of complexes between AOM and coagulants*

It was found that AOM coagulation can be deteriorated by the formation of dissolved complexes between AOM substances and coagulant metals (Bernhardt et al., 1985; Takaara et al., 2005, 2007, 2010; Pivokonsky et al., 2006, 2009b, 2012; Safarikova et al., 2013). A number of studies demonstrated that algal polysaccharides, especially those that are anionic-containing uronic acids, are able to bind metals (Bernhardt et al., 1985; Kratochvil and Volesky, 1998; Hamdy, 2000; De Philippis et al., 2007). It was shown that carboxyl groups (−COOH) of uronic acids play a major role in metal complexing. Metal uptake is typically dependent on the pH values because the binding of metal cations is determined primarily by the state of dissociation of the carboxyl groups. Sulfonate groups ($-OSO_3^-$) can also contribute, to a lesser extent, to heavy metal binding, particularly at low pH (Kratochvil and Volesky, 1998). It can be assumed that algal polysaccharides can bind coagulant metals (Al, Fe) since Al^{3+} was confirmed to form complexes with alginate (alginic acid), a model compound for polyuronic acids (Seely and Hart, 1976; Gregor et al., 1996). In addition, Takaara et al. (2010) suggested that coagulation inhibition can be attributed to the formation of complexes between anionic lipopolysaccharides bound to the cell surface of cyanobacterium *M. aeruginosa* and polyaluminum chloride (PACl) constituents.

Algal peptides and proteins were also found to bind Al and Fe through their dissociated carboxyl ($-COO^-$) groups, and their metal-complexing capacity was pH-dependent (Takaara et al., 2005, 2007; Pivokonsky et al., 2006, 2012; Sano et al., 2011; Safarikova et al., 2013). In the case of peptides and proteins of *M. aeruginosa*, their metal-complexing capacity reached a maximum at pH about 6.8 and 6 for Al and Fe, respectively (Pivokonsky et al., 2012; Safarikova et al., 2013). By affinity chromatography, Pivokonsky et al. (2012) identified peptides and proteins of MWs of approximately 1, 2.8, 6, 8, 8.5, 10, and 52 kDa to be those responsible for metal complexation. On the other hand, Takaara et al. (2007) isolated proteins of MW between 30 and 70 kDa. This difference may be due to using different isolation/detection techniques (HPSEC vs. SDS-PAGE), different elution strategy during affinity chromatography, and also by difference in strains and cultures of *M. aeruginosa.*

The formation of coagulant metal−AOM complexes has two consequences. First, Al− or Fe−AOM complexes do not undergo further hydrolysis and cannot take part in the coagulation process (Bernhardt et al., 1985; Gyurcsik and Nagy, 2000; Pivokonsky et al., 2006; Takaara et al., 2007). This feature results in a rise in coagulant demand. Second, metals bound to the AOM block negatively charged functional groups present on the organic molecule surface, and in this

way prevent the molecule from being coagulated through adsorption and charge neutralization mechanisms. Hence, metal–organic complexes tend to remain in solution after the coagulation process (Pivokonsky et al., 2006, 2012; Takaara et al., 2007). Pivokonsky et al. (2012), for example, demonstrated that at pH 6, 1 mg of proteinaceous DOC bound 0.077 mg of dissolved Fe, which was detected as residual Fe after coagulation.

It can be concluded that the coagulation of algae-derived organics is strongly dependent on the pH value, with the highest efficiencies at acidic pH values. Charge neutralization between negatively charged functional groups of AOM and positively charged coagulant species was reported to be the main coagulation mechanism. The formation of dissolved complexes between AOM and coagulant may deteriorate coagulation, but can be eliminated by consistent optimization of pH conditions as this feature occurs at quite narrow pH ranges. Since coagulation has been proved to be remarkably efficient in removing AOM (Bernhardt et al., 1985; Pivokonsky et al., 2012), especially high-MW organics, it is an essential pretreatment process for other removal techniques such as membrane separation, adsorption onto AC, and reverse osmosis (RO) in AOM-containing waters (Edzwald and Haarhoff, 2011; Myat et al., 2012). Moreover, the coagulation process offers advantages of low operating cost and relatively simple operation. Further research is required into the flocculating properties of AOM.

4. Impact of AOM on membrane separation

Membrane processes are now used internationally for water supply from freshwater, brackish water, and seawater (Nicolaisen, 2003) and also in recycled water systems (Wintgens et al., 2005). These water sources can all be subject to algal blooms; for example, algal blooms have been observed to disrupt desalination plants that use RO processes (Caron et al., 2010; Edzwald and Haarhoff, 2011) and have also been observed in waste stabilization ponds that supply water to recycled water plants that use membranes (Nguyen et al., 2014). Hence, research investigating the use of MF, ultrafiltration (UF), and nano-filtration (NF) for the treatment of water containing algae or cyanobacteria and therefore AOM has increased (Liang et al., 2008; Zhang et al., 2012b; Zhou et al., 2014b). This, combined with the interest in the use of membranes for algae harvesting for biofuel generation, has led to a number of recent publications in the area of membrane separation of algae and cyanobacteria. It is well understood that the major membrane operational issue experienced during algal blooms due to the presence of AOM is not related to water quality as such, as other water treatment processes such as powdered activated carbon (PAC) application are used for toxin and taste and odor treatment, but rather that of severe membrane fouling, with numerous studies confirming that transmembrane pressure increases, or flux significantly declines(Pongpairoj et al., 2011). However, understanding of the underlying, highly complex mechanisms by which AOM causes fouling is more

contentious. This is in part because fouling is caused by both free, dissolved AOM present in the system and by living cells. On attachment to the membrane surface, these living cells then produce additional AOM, which further accumulates on the surface (Babel et al., 2002; Liang et al., 2008).

4.1. *Relationship between AOM character and membrane fouling*

One of the first major studies to investigate the impact of AOM (combined EOM and COM) concentration and character on membrane fouling was that by Her et al. (2004) who compared the fouling propensity of AOM relative to Suwannee River humic acid (SRHA). When the proportion of AOM relative to SRHA increased, a higher flux decline was observed. Specifically, it was determined that poor rejection and fouling were caused by fractions with greater HPI character, more neutral charge, and low SUVA. Specifically, protein- and polysaccharide-like substances, respectively, were found to be linked to the initial stages of fouling, which has been subsequently confirmed across a range of species (Chiou et al., 2010; Zhang et al., 2010a; Qu et al., 2012a; Villacorte et al., 2013). For example, Chiou et al. (2010) suggested that the proportion of polysaccharides in AOM influenced the relative fouling rate between different species with decreasing fouling rates observed by species as: *Microcystis* sp. > *C. vulgaris* > *Chotadella* sp. Similar observations have also been made with EfOM; for example, Jarusutthirak et al. (2002) observed that polysaccharides from EfOM were a major contributor to fouling, which is consistent with the observation that AOM is similar in character to EfOM. In addition, this is consistent with work on transparent exopolymer particles (TEPs), which have been shown to spread across a membrane surface relatively evenly, eventually blocking pores or forming a cake/gel layer with high hydraulic resistance (Discart et al., 2013). It is of note that most studies have dealt with EOM, as opposed to COM, the latter comprising a higher proportion of peptides and proteins and this remains a gap in the knowledge (Section 2), particularly given recent research that demonstrates that protein−polysaccharide interactions to form biopolymer clusters can significantly influence membrane fouling (Neemann et al., 2013), and needs further investigation.

A number of studies have been undertaken to further characterize these foulants by fractionation of the EOM using either XAD® resin to indicate hydrophobicity: HPO, TPI, CHPI, or NHPI (as discussed in Section 2) or size fractionation. Two popular methods in this regard include: (A) direct membrane filtration of the resin fractionated sample and examination of the associated flux decline (Zhou et al., 2014b); or (B) recovery and fractionation of the foulant layers that have been retrieved via rinsing, backwashing, or chemical cleaning (Zhang et al., 2013b). Given the multitude of parameters that can be varied, large variations can be observed when attempting to relate fouling to hydrophobicity using XAD-resin fractionation (Table 1); however, while all fractions have

TABLE 1. Relating hydrophobicity fractionation of EOM to membrane fouling potential.

EOM Source	Method Relating fraction to foulant (A or B)[†]	Specific experimental conditions	Contribution of XAD-resin Fraction* to Fouling (Method A: **total**/irreversible specific fouling; Method B : % of **reversible**/irreversible foulant layer)					Conclusion: Major foulant(s)/ irreversible foulant	References
						HPI			
			Overall EOM	HPO	TPI	CHPI	NHPI		
Microcystis aeruginosa (stationary phase)	A	Constant pressure; pH 7; 4.5 mg L^{-1} as C; 50 mL; UF-Hydrophobic; UF-Hydrophilic	**52**/13 (hydrophobic; **55**/6 (hydrophilic)	**46**/21; **16**/2	**28**/21;**7**/3	**46**/16; **46**/2	**50**/8; **42**/3	UF-Hydrophobic: NHPI > HPO = CHPI > TPI/HPO = TPI > CHPI > NHPI UF-Hydrophilic: CHPI > NHPI > TPI > HPO/Very little	Zhou et al. (2014b)
Cyanobacteria	A	Constant pressure; pH 7; 5 mg L^{-1} as C; 800 mL	**90**	**85**	**80**	**60**	**90**	NHPI > HPO > TPI > CHPI	Li et al. (2011)
Microcystis aeruginosa (stationary phase)	A	Constant pressure; pH 7; 300 mL; UF-Hydrophilic	**89**/25		**63**/18 (TPI + HPI)	**60**/15		HPI > HPO > TPI/ HPI > HPO > TPI	Qu et al. (2012b)
Chlorella zofingiensis	A	Constant pressure; 50 mL, UF-Hydrophilic	–	**8**		**8**	**52**	NHPI >> HPO = CHPI	Zhang et al. (2013a)
Microcystis aeruginosa (stationary phase)	B	Constant pressure; pH 8; 8 mg L^{-1} as C; ceramic MF-Hydrophilic	–	**85**/5	**21**/15	**34**/33		HPO > HPI > TPI/ HPI > TPI > HPO	Zhang et al. (2013a)

*HPO: hydrophobic; TPI: transphilic; CHPI: charged hydrophilic; NHPI: neutral hydrophilic.
[†]Method A: direct membrane filtration of the resin fractionated sample and examination of the associated flux decline (%).
Method B: resin fractionation of solutions of the recovered reversible and irreversible foulant layers.

been observed to contribute to an extent to membrane fouling, broadly, it is the HPI fraction, and in particular the NHPI fraction, that was typically observed to be the major foulant (Qu et al., 2012b; Zhang et al., 2013a, 2013b). For example, Zhou et al. (2014b) reported a decrease in flux to 50% and 40% for an HPO and HPI membrane, respectively, when filtering 50 mL of 4.5 mg L^{-1} of NHPI fraction of AOM. While filtering a similar concentrated volume of the HPO fraction, a very similar flux profile was observed for the HPO membrane, whereas the flux of the HPI membrane remained relatively stable. Consistent observations between the different studies suggest that TPI does not play a major role in fouling, congruent with its representing a relatively small portion of the EOM (Section 2).

Major foulants have also been determined to be very large molecules, such as biopolymers; for example, Qu et al. (2012b) determined (using Method A) that specific total and irreversible fouling of 0.45 μm filtered EOM was approximately 80% and 15%, respectively, which decreased to 20% and less than 5% on filtration through a 100 kDa membrane. Similarly, using Method B, Zhang et al. (2013b) showed that the reversible foulant layer comprised a large proportion (>90%) of biopolymers $\gg$20,000 Da, which were not observed in the irreversible foulant layer that was dominated by a combination of high-MW substances ($\sim$1000 Da) and substances of 500 Da or less. Another study showed for a suspension of *Chlamydomonas* that the material with the highest fouling potential was able to pass through a 5 μm MF filter, but was then retained by a 0.22 μm membrane (Rickman et al., 2012).

The various studies reviewed have led to a number of fouling mechanisms being proposed for both reversible and irreversible fouling of AOM, including: (1) cake layer formation and pore plugging by HPI organics contributing to reversible fouling, with the adhesion of HPO organics onto the membrane and the cake layer causing irreversible fouling (Qu et al., 2012b); (2) reversible fouling caused by very high-MW substances ($\gg$20,000 Da), considered to be proteins and polysaccharides, with irreversible fouling caused by mostly high (1000 Da) and low (<350 Da) MW HPI AOM restricting the pores via adsorptive fouling (Zhang et al., 2013b); (3) polysaccharides attaching to the membrane via intermolecular bridges leading to the formation of a cake-gel layer (Zhang et al., 2013b); and (4) deposition of smaller colloidal species rather than cake-layer formation (Rickman et al., 2012). Such suggestions and their diversity are analogous to earlier developments concerning the mechanisms of fouling in a membrane bioreactor that consider wastewater bacterial exudates, termed extracellular polymeric substances (EPS) and soluble microbial products (SMPs) (Drews, 2010). A major review undertaken by Le-Clech et al. (2006) identified four key fouling mechanisms, all of which AOM will be involved with: cake filtration, the buildup of a layer of particles larger than membrane pore size; complete pore blocking, where pores are blocked by particles; intermediate blocking, pore blockage with superimposition of additional

particles, considered to be a long-term process; and direct adsorption of small particles and colloids onto the pore walls, thus reducing the overall pore size. The exact balance of each mechanism will depend on the concentration and character of the AOM and this remains an area for the future development of understanding.

4.2. *Impact of cell growth conditions and associated water quality on fouling by AOM*

While the debate surrounding the fundamental mechanisms of fouling caused by membrane filtration of EOM ensues, research is also being undertaken at a more practical level to investigate the relationships between growth conditions and water quality and membrane fouling by EOM. Babel et al. (2002) investigated the impact of temperature and radiation on membrane resistance when filtering *Chlorella vulgaris*. At optimal growth temperatures of between 28 and 35°C, specific cake resistance was reported at a minimum at approximately 2×10^{11} m g^{-1} for fresh culture as cells deposited on the membrane were isolated and void spaces were present; however, for temperature ranges outside the optimal range or high solar radiation, higher specific cake resistances of up to around 1×10^{12} m g^{-1} were observed, attributed to a layer of mucilaginous, cell-bound EOM that minimized the presence of void spaces. Control of nutrient conditions can also impact membrane fouling; for example, Huang et al. (2012) found that membrane fouling caused by EOM extracted from *M. aeruginosa* cells grown under various nutrient conditions decreased in the following order: 1N:10P > 1N:2P > 1N:1P > 2N:1P, with 1N:10P generated EOM rich in neutral HPIs. Further research is required in this area to more clearly elucidate the relationship between growth conditions and EOM production such that it can be linked more conclusively to membrane fouling. However, these initial studies indicate that it is not only algal species and growth phase that need to be closely controlled when extracting EOM for characterization and testing, but also temperature, radiation, and nutrient concentration.

In addition to nutrient availability, the presence of divalent calcium and magnesium ions have also been shown to exacerbate the resultant fouling potential of EOM and algae (Zou et al., 2011, 2014b), not by impacting the production of EOM by algae as with nutrients, but by complexing with carboxylate groups in the EOM after production to form large gel complexes with a high fouling potential, according to the "egg-box" model (Katsoufidou et al., 2007; Ahn et al., 2008). For example, flux further decreased from 50% to 60% on addition of 10 mM of Ca^{2+} in UF tests of EOM extracted from *M. aeruginosa* (Zhou et al., 2014b), which is in accordance with observations by Zou et al. (2011) on filtration of *Chlorella sorokiana* in the presence of Mg^{2+}. On the other hand, no influence of the Ca^{2+} ion was observed by Lee et al. (2006a), although they suggest that this might be due to the focus of previous studies on high-pressure

membranes while their study investigated low-pressure systems, while Zhang et al. (2013a) in fact observed a reduced flux decline from approx. 70% to 55% when adding 2.5 mM Ca^{2+}, attributed to the formation of a more porous "pre-layer." Another important water-quality parameter is that of pH. Zhou et al. (2014b) observed only minor changes in flux decline when examining EOM fouling from *M. aeruginosa* at three pH values: 5.0, 7.2, and 9.5; however, irreversible fouling was increased at pH 5 with a specific fouling of 0.15 compared with 0.05 at pH 9.5, attributed to a weakening in electrostatic repulsion.

Finally, the presence of NOM other than that produced by algae should be considered. Only a few research studies have been undertaken to examine the interactions between allochtonous (terrestrially derived) OM and autochtonous (produced in situ, i.e. of microbial origin) OM and how these may impact membrane fouling. Lee et al. (2006a) compared fouling by COM extracted from a eutrophic lake compared with allochtonous HPO fractions for four different membrane types. For the HPI UF membrane, flux decline was similar at 12−13%, whereas for the HPI MF membrane, COM flux decline was 55% compared with 18% for HPO. In another study, similar flux decline of around 30% was also observed for an OM sample extracted from a reservoir and an *M. aeruginosa* sample (note this sample comprised cells and EOM) (Kwon et al., 2005) when filtered independently. Interestingly, the flux decline increased significantly by 70% when the NOM and *M. aeruginosa* were introduced to the membrane simultaneously. Myat et al. (2012) showed via molecular dynamic simulations that interactions in an alginate−humic system were instigated by Ca^{2+} bridging. These studies suggest that interactions between terrestrial NOM and AOM could form a more aggressive foulant, particularly in the presence of divalent cations, but further research would be required in this regard.

4.3. *Amelioration of membrane fouling by AOM*

Mitigation of the impact of membrane fouling is critical if membrane separation processes are to be used during algal blooms. This is typically achieved through (a) pretreatment to remove AOM foulants prior to membrane filtration, (b) optimizing membrane operation, and/or (c) optimizing membrane cleaning procedures. Pretreatment measures to remove AOM prior to membrane filtration include coagulation, either alone or with separation (Babel and Takizawa, 2011; Goh et al., 2011) (Section 3), ozonation (Hung and Liu, 2006; Babel and Takizawa, 2011), adsorption (Zhang et al., 2011b) (Section 5) and ion-exchange (Liu et al., 2011a), and potentially combinations of these, resulting in a decreased concentration of AOM introduced to the membrane, and therefore reduced overall fouling potential. For example, on coagulation using alum coagulant prior to membrane filtration of a recycled water sample spiked with *M. aeruginosa*, the flux profile was improved and the permeate volume increased from 400 mL with no coagulant to over 1.5 L with a dose of 5 mg Al L^{-1}. Similarly,

Babel and Takizawa (2011) observed a 70% reduction in cake resistance for *Chlorella* sp. after coagulation whereas Zhang et al. (2014) observed normalized flux improving from 0.35 to 0.7 with 5 mg Al L^{-1} when using alum (at pH 8) and to 0.85 with 5 mg Al L^{-1} when using aluminum chlorohydrate and also 10 mg Fe L^{-1} as both ferric chloride and ferric sulfate. Furthermore, the irreversible fouling significantly decreased at optimized coagulant doses (Goh et al., 2011; Zhang et al., 2014); for example, Zhang et al. (2014) observed a decrease in irreversible fouling resistance from 1×10^{12} m^{-1} to approx. 2×10^{11} m^{-1} after coagulation. However, when Goh et al. (2011) removed coagulated flocs through a 1.5 μm membrane, there was a low flux recovery as the flocs provided a protective cake layer that filtered out residual, low-MW organics that would otherwise adsorb at the surface of the membrane via direct adsorption processes causing internal fouling. Interestingly, Zhang et al. (2014) further determined that the noncoagulated and coagulated AOM were best modeled by the cake filtration and intermediate blocking models, respectively, as previously described, suggesting to be a result of removing high-MW AOM during coagulation.

Use of preozonation prior to UF treatment of *Chlorella* showed an increase in total membrane resistance for contact times of 3–10 minutes from approx. 1.3×10^{11} m^{-1} to 1.7×10^{11} m^{-1} after 3 hours of filtration (Hung and Liu, 2006). A subsequent decrease to 1.45×10^{11} m^{-1} was observed on increasing the contact time to 30 minutes. Interestingly, while total resistance was increased, resistance associated with the cake layer actually decreased, a phenomenon also observed by Babel and Takizawa (2011), attributed to the removal of EOM from the cell surface and reduced biomass load. The concurrent increase in fouling resistance was attributed to the adsorption of the released polysaccharides. It may also be a result of the release of COM on cell lysis as described by Plummer and Edzwald (2001) and Henderson et al. (2008a). No studies have investigated the impact of preozonation on AOM without cells. PAC addition was observed to reduce the severity of fouling by *M. aeruginosa* in a hybrid submerged UF system over a two-week period, whereby the resultant transmembrane pressure was decreased from approx. 45 kPa to 30 kPa, thereby enabling approximately 10% DOC removal and 40% microcystin-LR removal (Zhang et al., 2011b); however, it had no impact on the rejection of high-MW, HPI AOM. PAC dosing is discussed in more detail in Section 5. Magnetic ion-exchange (MIEX®) resin has been investigated as a pretreatment to coagulation where it was shown to remove both low- and high-MW, anionic OM, amounting to between about 25 and 60% of OM without coagulation and 60–80% removal when combined with coagulation (Mergen et al., 2008; Liu et al., 2011a). The degree of OM removal depends on effective coagulation optimization and AOM character. Liu et al. (2011b) evaluated the impact of including MIEX treatment on membrane fouling by AOM, determining that a reduction in fouling rate was the result of the removal of high-MW, HPO AOM. Although few studies have investigated combinations of pretreatment methods for

membranes subject to AOM fouling, the outcomes of the reviewed studies suggest that PAC and/or MIEX treatment in combination with coagulation may extend the range of organic molecules targeted. Treatment processes such as PAC and MIEX are, however, expensive and economic assessments would have to be conducted regarding the overall effectiveness of one over another in combination with coagulation and this remains a knowledge gap.

Another strategy to control fouling is the careful operation of the membrane separation process. It is important to avoid shear prior to membrane filtration, for example, by pumping, to reduce stress on the algae (Ladner et al., 2010). Selection of membrane type should also be considered. Studies are in agreement that HPO membranes are more susceptible to fouling by AOM, including faster flux decline and more adsorptive and irreversible fouling than HPI membranes (Sun et al., 2013; Qu et al., 2014). Zhou et al. (2014b) found little difference with respect to flux decline between HPO and HPI membranes, but observed that irreversible fouling was slightly greater for the HPO membrane as previously discussed. Babel and Takizawa (2010) tested two HPI membranes with different protein-binding capacities, including polyvinylidene difluoride (PVDF) with a protein-binding capacity of only 4 μg cm^{-2}, and found no difference in fouling. Flux and crossflow velocity can also be carefully controlled to mitigate fouling when employing cross-flow membrane systems (Pongpairoj et al., 2011; Wicaksana et al., 2012). For example, it was noted that higher crossflow velocity resulted in less deposition of *Chlorella sorokiniana* cells on the membrane surface (Pongpairoj et al., 2011). As flux increased, greater amounts of polysaccharides penetrated the membrane; however, after reaching a maximum, at greater fluxes polysaccharide in the permeate decreased, possibly due to fouling in the membrane pores by the AOM (Pongpairoj et al., 2011). Use of novel membrane systems including vibrating membranes (Bilad et al., 2013) has also been examined with promising results, but further investigations are required in this regard.

Irrespective of which of the previous mitigation strategies are applied, a degree of fouling will occur and cleaning methods will need to be applied to ensure membrane longevity. In one study of *S. quadricauda*, the flux declines of the supernatant (AOM), algae suspension, and algae in deionized water was compared and found to be approx. 5%, 70% and 70%, respectively (Zhang et al., 2010a). Although only a small amount of fouling was observed for the AOM, this fouling was irreversible. It was determined that the fouled membrane could be cleaned with NaClO. Similarly, Liang et al. (2008) determined that while hydraulic cleaning could achieve 50–80% flux recovery for raw water containing 710–1100 $\times$ 10^4 cells L^{-1} of *Microcystis* and *Chlorella*, NaOCl provided more effective chemical cleaning than NaOH or citric acid. Furthermore, it was observed that combining NaOH and NaOCl was the best chemical cleaning strategy.

5. Adsorption of AOM onto AC

The adsorption process using AC, usually in the form of granular (GAC) or PAC, is frequently integrated in the drinking water treatment chain for removing undesirable organic impurities of both natural and anthropogenic origin (Moreno-Castilla, 2004; Delgado et al., 2012). Most adsorption studies dealing with the removal of NOM focused on humic and fulvic material (Matilainen et al., 2006; Kristiana et al., 2011; Velten et al., 2011; Matsui et al., 2012; Gibert et al., 2013) or generally on dissolved organic matter (DOM) passing through a 0.45 μm filter (Schreiber et al., 2005; Gur-Reznik et al., 2008; Wei et al., 2008). Only a limited number of studies have addressed the specific adsorption of AOM although both groups of these compounds, humic matter and AOM, are together classified as NOM (Table 2).

AC is often used in combination with other treatment techniques. The study of Zhang et al. (2011b) evaluated the effect of PAC addition on an immersed UF of algal-rich waters. The UF membrane alone could achieve an absolute removal of *M. aeruginosa* cells, but it was less effective for the removal of AOM. The addition of PAC alleviated the development of transmembrane pressure and enhanced the removal of AOM (DOC by 10.9 $\pm$ 1.7%) and microcystins (by 40.8 $\pm$ 4.2%). However, PAC had little effect on the rejection of proteins and carbohydrates, indicating that PAC could not effectively adsorb the HPI high-MW AOM. Comparable results were obtained in the study of Campinas and Rosa (2010) on the removal of different types of NOM by the PAC/UF system. PAC enhanced the rejection of AOM (consisting of EOM and COM) of *M. aeruginosa* from 35 to 55%, which was similar to the rejection of tannic and humic acid, but had no significant effect on the rejection of EOM. This may be explained by the different composition of AOM versus EOM and by the higher salt contents in EOM solutions. Moreover, PAC controlled the irreversible membrane fouling, minimizing the chemical cleaning frequency. Dixon et al. (2011) investigated the removal of cyanobacterial cells, toxins, and other AOM during blooms of *Anabaena circinalis* and *Microcystis flos-aquae* by coagulation-PAC-UF system. They showed that, in the case of *Anabaena circinalis* bloom, coagulation and coagulation-PAC increased the removal rates of AOM from 35 to 69 and 71%, respectively, compared to UF alone. Coagulation and coagulation-PAC also significantly reduced flux decline and improved toxin removal. On the other hand, removal rates for DOC in the case of *Microcystis flos-aquae* bloom were about 65% with or even without the addition of coagulant and PAC. This might be due to the formation of a denser cake layer by *M. flos-aquae* cells on the UF surface enhancing AOM removal and higher water turbidity. However, coagulation and coagulation-PAC substantially improved flux again.

A technology that combines coagulation and PAC adsorption with microfiltration (MF) membrane is already being employed in some experimental

Table 2. Adsorption studies dealing with AOM.

Cyanobacterial	Adsorbate	Treatment process/experiment	Activated carbon	References
Anabaena planktonica, Anabaena smithii, Anabaena spp.	cyanobacteria cells, intra- and extracellular geosmin	laboratory-scale ceramic MF experiments and pilot-scale AC adsorption/coagulation/ ceramic MF experiments	N-PAC ($d_{50} = 7.6\ \mu m$) and S-PAC ($0.65\ \mu m$) (Futurama Chemical Industries Co., Ltd., Japan)	Matsuchita et al. (2008)
Microcystis aeruginosa (exponential phase)	EOM and AOM (total EOM and intracellular matter released from destroyed cells)	laboratory-scale experiments with hollow-fiber cellulose acetate UF membrane/PAC	PAC Norit SA-UF (Cabot Norit Americas Inc., USA)	Campinas and Rosa (2010)
Microcystis aeruginosa (stationary phase)	COM peptides $<$10 kDa	laboratory-scale batch adsorption experiments	GAC Norit 1240 (Cabot Norit Americas Inc., USA), GAC Filtrasorb 400 (Chemviron Carbon, Belgium)	Hnatukova et al. (2011)
Microcystis aeruginosa (stationary phase)	cyanobacterial cells, AOM, MC-LR, MC-RR, MC-YR	laboratory-scale experiments with a hollow-fiber polyvinyl chloride UF membrane and AC	coconut shell PAC unspecified (China)	Zhang et al. (2011b)
Microcystis aeruginosa	dimethyl trisulfide (98%) and β-cyclocitral (90%) (Sigma Aldrich) in natural water to simulate products of *M. aeruginosa*	laboratory-scale batch and kinetic experiments	GAC unspecified (Calgon Carbon Corp., USA)	Zhang et al. (2011a)
Microcystis aeruginosa (stationary phase)	COM peptides $<$10 kDa	laboratory batch and kinetic adsorption experiments	GAC Filtrasorb TL830 (Chemviron Carbon, Belgium), GAC Picabiol 12×40 (Jacobi Carbons AB, Sweden)	Kopecka et al. (2014)

plants (Matsuchita et al., 2008) for the removal of cyanobacteria *Anabaena planktonica, Anabaena smithii*, and *Anabaena spp.* and associated odor compound geosmin. This system can simultaneously and effectively remove both intracellular and extracellular geosmin and the cyanobacteria cells from the water.

A number of studies focused on the adsorption of cyanobacterial toxins, especially microcystins and saxitoxins (Pendleton et al., 2001; Huang et al., 2007b; Campinas and Rosa, 2010; Dixon et al., 2011; Ho et al., 2011). Although these pollutants are not targeted in this review, the results evaluating their adsorption could be useful for further research on AOM.

5.1. *Character of adsorbed AOM*

Investigations into the properties of adsorbed AOM are rare and focus mostly on the MW of adsorbed compounds. As mentioned in the previous section, Zhang et al. (2011b) found that the addition of PAC to UF reactor had negligible effect on the removal of high-MW carbohydrates and proteins contained in AOM of *M. aeruginosa*, but significantly improved the removal of lower-MW microcystins (MW about 1 kDa). The study by Kopecka et al. (2014) focused on the adsorption of COM peptides of *M. aeruginosa* with MW <10 kDa, which were previously found to be hardly removable by coagulation (Pivokonsky et al., 2012; Safarikova et al., 2013). Peptides with lower MW of 1.0−4.5 kDa were adsorbed more effectively than those with higher MWs of 8.3 and 9.5 kDa. This is likely to be given by the relatively microporous character of the used GACs as compounds are preferentially adsorbed into pores of similar size. Similarly, Hnatukova et al. (2011) who also used COM peptides of *M. aeruginosa* with MW <10 kDa in their experiments showed that low-MW peptides of 700−1700 Da were adsorbed preferentially. Furthermore, these low-MW peptides were identified as the most relevant components in the restriction of GAC adsorption capacity for herbicides alachlor and terbuthylazine of MW 269.8 and 229.7 Da, respectively. This is in accordance with studies on NOM, which showed that low-MW NOM fractions are adsorbed substantially more than higher-MW ones, compete with organic pollutants for the active sites on the AC, and may consequently inhibit the adsorption of these pollutants (Zhang et al., 2011a; Newcombe et al., 1997). The study by Dixon et al. (2011) showed that during *Anabaena circinalis* bloom, coagulation in combination with PAC adsorption and UF removed a greater amount of compounds in the region between 1500 and 3000 Da than the UF membrane alone.

It can be concluded that MW is one of the most important factors that influences the adsorption of AOM. High-MW HPI AOM compounds are apparently difficult to remove by adsorption onto AC. However, these substances may be effectively removed by coagulation pretreatment, as described in Section 3. Moreover, AOM adsorption is also strongly influenced by the functional groups (their polarity, degree of ionization)

of AOM substances as was found out by studies that focused on adsorption factors and are discussed in the following section.

5.2. *Adsorption factors and mechanisms*

Adsorption onto AC is a complex process resulting from a contribution of solution properties (pH, ionic strength, chemical composition), and both AC (pore size distribution, surface chemistry) and adsorbate characteristics (MW, conformation, functional groups, solubility) (Moreno-Castilla, 2004; Newcombe, 2006). Zhang et al. (2011a) found that the GAC adsorption capacities for two algal odorants, dimethyl trisulfide and β-cyclocitral, were related to the micropore volume rather than to the GAC surface charge. Considering the very low solubility of dimethyl trisulfide and β-cyclocitral and the weak solute−solvent interactions, the authors attributed the relatively high adsorption capacities (dozens of mg g^{-1}) to the irreversible chemisorption on the GAC, rather than to the formation of the adsorption multilayer. The dimethyl trisulfide adsorption was identical at pH 2−10 and slightly increased at pH >10, while the capacity for β-cyclocitral was unchanging at pH 2−13. This relative independence of pH can be due to the existence of these compounds in the form of neutral molecules in a wide pH range.

On the other hand, Hnatukova et al. (2011) and Kopecka et al. (2014) observed a significant effect of pH on the adsorption of COM peptides of *M. aeruginosa*. Their adsorption on different types of GACs was noticeably higher at pH 5 than at pH 8.5. High adsorption capacity at pH 5 was explained by the formation of hydrogen bonds between protonated functional groups of the peptides and protonated surface groups of GACs and/or by electrostatic attraction between oppositely charged surfaces of GACs and peptide functional groups depending on points of zero charge of different GACs. At pH 8.5, where both peptides and all GACs were negatively charged, the repulsive electrostatic forces between them reduced the adsorption. Furthermore, the study by Kopecka et al. (2014) found that ionic strength is another key factor that controls the adsorption of COM peptides. Increase in ionic strength was shown to screen both the electrostatic repulsive and attractive forces in the system and to consequently change the adsorption regime.

It is difficult to conclude from such a low number of adsorption studies conducted on AOM. Nevertheless, the adsorption mechanisms seem to be influenced by the MW, degree of ionization (surface charge), content of polar functional groups, and solubility of AOM substances as well as surface characteristics of ACs.

6. AOM and DBP formation potential

Disinfectants, or oxidants, are used extensively in water treatment as a final process prior to the distribution of treated water to supply. The extent to which

disinfectants are applied can increase during algal blooms for two reasons: (1) preoxidation can be implemented for algal cell inactivation (Coral et al., 2013; Xie et al., 2013) and (2) increased disinfectant dose due to increased DOC content (Henderson et al., 2008a). The first scenario is not normally significantly impacted by AOM per se, but can result in additional AOM content being released to the system causing problems downstream (Coral et al., 2013). In addition to the impacts discussed in the previous sections, it is well-understood that oxidants, or disinfectants, can react with OM present in the system to produce potentially harmful DBPs, which is equally true for AOM. However, the types of DBPs that are formed will be driven to an extent by the molecular structure of the AOM, which differs quite significantly from that of allochtonous OM, as discussed in Section 2. This section will examine AOM (EOM and COM) as a precursor material for DBPs in chlor(am)ination processes.

6.1. *AOM as a DBP precursor*

6.1.1. *Carbonaceous disinfection by products (C-DBPs)*

Research on reactivity of AOM during disinfection has focused on carbonaceous DBP (C-DBPs) formation, in particular, the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) on the chlorination of EOM (and COM where stated) extracted from various species of cyanobacteria, green algae, and diatoms during either the exponential or stationary phases of growth (Table 3). From examination of Table 3, it is apparent that the specific C-DBP formation potential is not dependent on the phase of growth; for example, Nguyen et al. (2005) examined change in specific THM and HAA formation potential at numerous time points over the exponential and stationary phase of growth and found no significant difference, and hence reported only the average specific DBP. This suggests that while overall DOC concentration changes with the growth phase, the reactivity remains similar. On comparing the specific C-DBP reactivity obtained at different growth phases in other studies (Table 3), this outcome by Nguyen et al. (2005) appears to be supported more broadly. Rather, it appears that it is the length of disinfection time that impacts C-DBP formation rather than the growth phase or species. To illustrate, over a 3-day chlorination period, specific total THM reactivity for cyanobacteria and green algae species similarly varied in the range 12–17 $\mu g\ mg^{-1}$ as C (Fang et al., 2010; Yang et al., 2011) and 13–18 $\mu g\ mg^{-1}$ as C (Yang et al., 2011), respectively, irrespective of the growth phase, increasing to 26–32 $\mu g\ mg^{-1}$ as C (Nguyen et al., 2005; Huang et al., 2009; Li et al., 2012) and 15–63 $\mu g\ mg^{-1}$ as C, respectively, for a disinfection period of 7 days. Specific total HAA reactivity has only been investigated with a 3-day disinfection time in one study, where it was determined to be 11 $\mu g\ mg^{-1}$ as C for *M. aeruginosa* (Fang et al., 2010); at 7 days, this is again significantly increased to 48–66 $\mu g\ mg^{-1}$ as C for cyanobacteria (Huang et al., 2009; Li et al., 2012) and 54–62 $\mu g\ mg^{-1}$ as C for green algae (Nguyen et al.,

Table 3. Specific carbonaceous disinfection by product formation potential for reaction of chlorine with EOM extracted from a number of different algal and cyanobacterial species at various growth phases (N.M.: not measured; DCAA: Dichloroacetic Acid; TCAA: Trichloroacetic Acid; TCM: Trichloromethane)

Cyanobacteria	Chlorination Conditions	Specific C-DBP Reactivity (μg mg^{-1} as C)		References
		THM	HAA	
Microcystis aeruginosa (exponential phase)	7 d, 25 ± 1°C, pH = 6.8, Cl_2/DOC (w/w) = 5	32.4 (COM: 21.5)	54.6 (COM: 68.3)	Li et al. (2012)
	3 d, 22 ± 1°C, pH = 7.2 Residual Cl_2 >0.5 mg/L	12.9	N.M.	Yang et al. (2011)
Microcystis aeruginosa (stationary phase)	3 d, 22 ± 1°C, pH = 7.2 Residual Cl_2 >0.5 mg/L	12.0	N.M.	Yang et al. (2011)
	7 d, 21°C, pH = 7, Residual Cl_2 >0.5 mg/L	28	42 (DCAA) 24 (TCAA)	Huang et al. (2009)
	3 d, 22 ± 1°C, pH = 7 Cl_2/DOC (w/w) = 5 (EOM), 3 (COM)	*17 (TCM) (*COM: 28 (TCM))	*11 (DCAA) *(COM: 14 (DCAA))	Fang et al. (2010)
Anabaena flos-aquae (stationary phase)	7 d, 21°C, pH = 7 Residual Cl_2 >0.5 mg/L	26	26 (DCAA) 22 (TCAA)	Huang et al. (2009)
Oscillatoria prolifera (average: exp-stationary)	7 d, 20°C, pH = 7.0 Cl_2/DOC (w/w) = 5	30±4	N.M.	Nguyen et al. (2005)
Green algae				
Scenedesmus quadricauda (late exponential phase)	7 d, 20°C, pH = 7 Cl_2/DOC (w/w) = 4.5 Residual Cl_2 >0.5 mg/L	14.9	N.M.	Plummer and Edzwald (2001)
Scenedesmus quadricauda (average: exp-stationary)	7 d, 20°C, pH = 7.0 Cl_2/DOC (w/w) = 5	48 ± 12 (TCM − 0.9 L reactor) 63±14 (TCM − 20 L reactor)	N.M. 34.8 (DCAA) 22.9 (TCAA) 2.3 (MCAA)	Nguyen et al. (2005)
Chlorella vulgaris (exponential phase)	3 d, 22 ± 1°C, pH = 7.2 Residual Cl_2 >0.5 mg/L	14.9	N.M.	Yang et al. (2011)
Chlorella vulgaris (stationary phase)	3 d, 22 ± 1°C, pH = 7.2 Residual Cl_2 >0.5 mg/L	13.0	N.M.	Yang et al. (2011)
Chlamydomonas sp. (exponential phase)	4 d, 20°C, pH = 7 Cl_2/DOC (w/w) = 10	33.9±1.08	28.9±0.92 (DCAA) 32.9±0.17 (TCAA)	Hong et al. (2008)

(Continued)

Table 3. (*Continued*)

Cyanobacteria	Chlorination Conditions	Specific C-DBP Reactivity (μg mg^{-1} as C)		References
		THM	HAA	
Diatoms				
Cyclotella sp. (exponential phase)	7 d, 20°C, pH = 7; Cl_2/DOC (w/w) = 14 Residual Cl_2 >0.5 mg/L	15 (TCM)	27.1	Plummer and Edzwald (2001)
Chaetoceros muelleri (average: exp-stationary)	7 d, 20°C, pH = 7 Cl_2/DOC (w/w) = 5	29±6	N.M.	Nguyen et al. (2005)
Nitzschia sp. (exponential phase)	4 d, 20°C, pH = 7 Cl_2/DOC (w/w) = 10	47.8±1.23 (TCM)	24.5±3.68 (DCAA) 18.5±0.69 (TCAA)	Hong et al. (2008)

* Actual values not reported, rather data has been calculated from graph presented in referenced article.

2005). No study was found that examined diatom AOM reactivity at 3 days disinfection time; for 4–7 days disinfection time, specific total THM and HAA reactivity was in the range 15–48 μg mg^{-1} as C and 27–43 μg mg^{-1} as C, respectively (Plummer and Edzwald, 2001; Nguyen et al., 2005; Hong et al., 2008). Overall, cyanobacteria and green algae formed more HAAs than THMs for a given DOC concentration. Interestingly, the presence of bromide in source water has been shown to enhance THM formation, such that THM and HAA formation become approximately equivalent (Huang et al., 2009). For diatoms, total THM and HAA formation was equivalent in the absence of bromide (Table 3). Only a couple of studies investigating AOM as a DBP precursor have investigated COM (Fang et al., 2010; Li et al., 2012). As for EOM, THM formation was lower than that of HAA and the specific reactivity was similar; for example, for a 7-day disinfection time, specific THM reactivity was 21 μg mg C^{-1} whereas specific HAA reactivity was 68 μg mg^{-1} as C. Fang et al. (2010) also investigated the haloalkenes (dichloro- and trichloro-propanone) and chloral hydrate finding trace levels of reactivity of 0.54 μg mg^{-1} as C and 7 μg mg^{-1} as C, respectively.

Guideline values for THMs and HAAs range globally. As an example, the US Environment Protection Agency drinking water standards for THMs and HAAs are 80 μg L^{-1} and 60 μg L^{-1}, respectively (USEPA, 2006). If an AOM concentration of 5 mg L^{-1} is assumed, values from Table 3 indicate that AOM could produce THMs and HAAs in an approximate range of 50–315 μg L^{-1} and 135–275 μg L^{-1} and thus should be considered a serious contributor to C-DBPs during an algal bloom. Despite this, AOM has been found to have a lower specific C-DBP reactivity on chlorination than allochtonous NOM. For example, Fang et al. (2010) found that the specific THM reactivity of Suwanee River NOM was 72 μg mg^{-1} as C at a 3-day reaction time, approximately four times that of AOM. Similarly, Reckhow et al. (1990), reported HAA reactivity (DCAA and TCAA) for humic acid of 113 μg mg^{-1} as C after 3 days of chlorination; interestingly, fulvic acid was more similar to AOM in this respect with specific HAA reactivity of 65 μg mg^{-1} as C. However, while C-DBP formation is more likely to be governed by NOM concentration in source water, there are concerns that the presence of AOM may lead to the formation of more harmful nitrogenous-DBPs (N-DBPs) (Krasner et al., 2008).

6.1.2. *Nitrogenous DBP formation*

While N-DBPs including nitrosamines, haloacetonitriles (HANs), halonitromethanes (HNMs), haloacetamides (HAcAms), and cyanogen halides (CNX) are generally much lower in concentration than their C-DBP counterparts, they are of particular concern due to their increased cyto- and geno-toxicity (Muellner et al., 2007). Dissolved organic nitrogen (DON) concentration has been observed to increase from 186 μg N L^{-1} to 290 μg N L^{-1} during algal blooms and even up to 1 mg L^{-1} (Pocernich and Litke, 1997; Westerhoff and Mash,

Table 4. Specific nitrogenous disinfection by product formation potential for reaction of chlorine with EOM and COM extracted from cyanobacterium *Microcystis aeruginosa* at various growth phases and with NOM of humic character.

Cyanobacteria	Disinfection Conditions	Specific N-DBP formation (μg mg^{-1} as N)				References
		Nitrosamines	HANs	HNMs	CNCl	
Microcystis aeruginosa (exponential phase)	7 d, 25 $\pm$ 1°C, pH = 6.8, Cl_2/DOC (w/w) = 5	0.0189 COM: 0.0096	N.M.	N.M.	N.M.	Li et al. (2012)
Microcystis aeruginosa (stationary phase)	3 d, 22 $\pm$ 1°C, pH = 7 Cl_2/DOC (w/w) = 5 (EOM), 3 (COM)	N.M.	1.5 (DCAN) COM: 4.8	0.16 (TCNM) COM: 0.06	0.40 COM: 0.7	Fang et al. (2010)
NOM (for comparison)						
NOM	3 d, 22 $\pm$ 1°C, pH = 7 Cl_2/DOC (w/w) = 3	N.M.	1.6 (DCAN)	0.06	0.12	Fang et al. (2010)

2002; Lee et al., 2006b; Dotson et al., 2009). With DON a key precursor for N-DBPs, the expectation is therefore that algal-impacted water will have a higher potential for the formation of N-DBPs (Lee et al., 2007; Krasner et al., 2008). Using Table 4 and assuming a concentration of 0.5 mg L^{-1} as N, approximate concentrations of 0.01 $\mu g\ L^{-1}$, 2.4 $\mu g\ L^{-1}$, 0.08 $\mu g\ L^{-1}$, and 0.2 $\mu g\ L^{-1}$ could be expected for nitrosamines, HANs, HNMs, and CNCl, respectively. This is comparable with the literature where a US drinking WTP survey detected a shift in median concentrations of HANs, HNMs, and CNX from 3 to 4 $\mu g\ L^{-1}$, 0.3 to 0.5 $\mu g\ L^{-1}$ and 2.2–2.6 $\mu g\ L^{-1}$ when algae (or wastewater) was present (Lee et al., 2006b).

Researchers have therefore initiated studies investigating the N-DBP formation potential of EOM and COM on chlor(am)ination; however, limited studies have been conducted to date (Table 4). Li et al. (2012) observed that the formation potential of nitrosodimethylamine (NDMA) of EOM was roughly double that of COM, whereas in the study by Fang et al. (2010) the type of N-DBP influenced whether more was in EOM or COM. The latter study also observed that while HAN formation decreased on switching from chlorination to chloramination, the specific HNM and CNCl formation potentials increased. Both studies measured the DOC/DON ratio as being in the order of around 4.5, approximately 4–20 times less than that found in surface water not impacted by algae and allochtonous NOM (18–90), respectively (Westerhoff and Mash, 2002; Nguyen et al., 2005; Lee et al., 2006b; Fang et al., 2010). Fang et al. (2010) also performed N-DBP formation potential analyses for NOM, finding that in general N-DBP formation was less than for EOM and COM on chlorination (Table 4). On chloramination, however, NOM reactivity was either greater than or equal to that of EOM and COM (Fang et al., 2010). Further research is required to establish a similar understanding of N-DBP formation potential to that which has been achieved for C-DBPs.

6.2. *Relating AOM character to DBP formation potential*

The reactivity of AOM to form DBPs has been investigated according to hydrophobicity, MW, and also according to structural classes including proteins, lipids, and fatty acids (FAs). For example, Zhou et al. (2014a) examined the reactivity of EOM and COM HPO, TPI, and HPI fractions extracted from *M. aeruginosa* in the late exponential growth phase. It was found that the DOC/DON ratio was increased for COM fractions, and that within the HPO and TPI DOC/DON ratio was similar for EOM and COM (5.3 and 7.0, respectively). The HPI DOC/DON ratio was decreased relative to HPO and TPI at 2.4 and 5.0 for EOM and COM, respectively. Generally, reactivity for THMs, HANs, and haloketones (HKs) was of the order HPO > TPI > HPI, except in the case of COM for HAN and HKs where HPI exhibited the highest concentration. Nguyen et al. (2005) identified that the HPO acid fraction isolated from green algae extracted

EOM was a heterogeneous mixture of aliphatic and aromatic compounds with a SUVA of 2.3 mg^{-1} m^{-1} L. In this case, only THM and HAAs were measured on chlorination and it was found that only trichloromethane (TCM) was produced (68 μg mg^{-1} as C) and that this was at greater concentrations than that formed by the bulk EOM (Table 3), again suggesting a high potential for THM formation from highly reactive compounds present in the HPO fraction.

Lui et al. (2012) performed MW fractionation of *Chlamydomonas* sp. COM to give solutions of: <3 kDa, 3−10 kDa, and >100 kDa. It was found that after a 5-day chlorination experiment, the high-MW fraction was more reactive with respect to THM and HAA formation (TCM: 27 μg mg^{-1} as C; TCAA: 51.7 μg mg^{-1} as C); however, the medium-MW fraction formed more HANs and HKs. The low-MW fraction was the least reactive. The high-MW fraction was characterized with a high SUVA (4.52 mg^{-1} m^{-1} L), similar to NOM. This study also examined the DBP formation potentials for algal proteins in the same size ranges and, interestingly, found that high-MW algal proteins had the lowest THM formation potential and that in fact it was medium-range algal proteins, with a SUVA value of 0.48 mg^{-1} m^{-1} L, that were most reactive (7.43 μg mg^{-1} as C). In a separate study, the same authors further fractioned extracted algal proteins to isolate HPO and HPI proteins (Lui et al., 2011). It was determined that HPI proteins had a specific THM reactivity of 85 μg mg^{-1} as C, much greater than HPO proteins, which only formed 2.45 μg mg^{-1} as C, whilst HPO proteins were found to be more likely to form HAAs. Another study investigated FAs and proteins extracted from COM of a diatom, *Navicula pelliculosa*, as precursors of DBPs after similar 5-day chlorination experiments (Liang et al., 2012). Purchased FAs standards were found to produce up to 7.6 μg mg^{-1} as C, 2.0 μg mg^{-1} as C, and 8.7 μg mg^{-1} as C of TCM, DCAA, and TCAA, respectively, and, interestingly, for TCM and DCAA the formation potential increased with an increasing number of double bonds in the FA, a phenomenon that was anticipated since double bonds can serve as reactive sites, leading to DBP formation (Fukayama et al., 1986; Liang et al., 2012). For algal-extracted FAs, the formation potential was found to be greater than that calculated for purchased FA standards, at 13.5 μg mg^{-1} as C. The same study found that HPI and HPO proteins had a THM formation potential of 93.5 μg mg^{-1} as C and 12 μg mg^{-1} as C, respectively. Notably, the reactivity of FAs with time did not change, whilst for both protein fractions, the formation potential increased significantly when the chlorination period was extended; this indicates that potentially the impact on reaction time as discussed in Section 6.1 (Table 3) may be the result of HPI proteins. Although it might be expected that FAs and HPO proteins may be removed to a large extent by coagulation-flocculation if performed and optimized, this is less likely for HPI proteins and suggests that the presence of AOM in water sources could impact a WTP's ability to meet THM guideline values. These studies also suggest that SUVA is not a good indicator to predict THM formation, especially in autochthonous sources.

7. Conclusions

Research clearly demonstrates that AOM can impact performance at every stage of the treatment process and therefore requires consideration when a drinking water source is impacted by an algal or cyanobacterial bloom. Generally, most studies to date have investigated the impact of EOM on WTP performance, with little emphasis on the COM that forms a major portion of AOM after the collapse of an algal bloom and brings the most serious problems to WTPs; this remains a knowledge gap. Observations indicate that AOM is most similar in character to EfOM, largely due to the microbial origin of the organic material, and quite different from humic-derived NOM. Specific research needs highlighted by the individual sections of this review have been identified as follows:

- The general composition of AOM in terms of 'bulk' characterization techniques (hydrophobicity, charge, MW) is now well understood. However, there is a need for greater investigation as to how these parameters vary when algal/cyanobacterial populations are subject to different growth conditions or stress. Better structural characterization of the proteinaceous and polysaccharidic components is required in order to tailor mitigation measures to optimize process performance during algal blooms, or indeed take advantage of naturally produced bioflocculants.
- During coagulation, coagulant metal–AOM complexes can form, leading to a rise in coagulant demand as for terrestrially derived NOM. However, additional influences of metal complexation include blockage of negatively charged functional groups that prevent the molecule from being coagulated through adsorption and charge neutralization mechanisms. As with NOM, pH optimization is essential for efficient AOM removal and the same organic characterization techniques (charge, hydrophobicity, SEC) can be applied to optimize removal. Further work on understanding the role of bioflocculants produced by algae/cyanobacteria during coagulation and investigation into the impact of AOM on floc properties is required.
- Membrane fouling by AOM can be controlled via pretreatment, avoidance of shear prior to membrane filtration, and selection of HPI membranes. The same membrane fouling mechanisms that have been identified for other systems, such as wastewater, will apply to AOM; the exact balance of each mechanism will be dependent on AOM concentration and character and further research is required to improve understanding in this area. Interactions between terrestrial NOM and AOM could form a more aggressive foulant, particularly in the presence of divalent cations, and further research is required in this regard.
- Investigations of AOM adsorption on AC has focused mainly on cyanotoxins and taste and odor compounds. However, it appears that AC adsorption could be a viable option for the removal of the low-MW AOM

fraction that is not removed by coagulation. Studies focused primarily on the adsorption of AOM are still limited and systematic evaluation of adsorption effectiveness is necessary.

- Specific C-DBP reactivity appears to be consistent with the growth phase, and hence, it is the length of disinfection time that impacts C-DBP formation. The risk associated with DBP formation due to the presence of AOM is increased in relation to the HAAs and emerging DBPs, such as the N-DBPs, rather than THMs. Further studies are required to fully understand the formation pathways and define the true hazard associated with the total range of DBPs formed in accordance with changing regulations globally.

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PUBLICATION 7

The impact of pre-oxidation with potassium permanganate on cyanobacterial organic matter removal by coagulation

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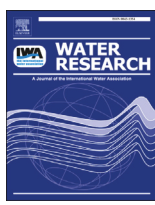

The impact of pre-oxidation with potassium permanganate on cyanobacterial organic matter removal by coagulation

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ABSTRACT

The study investigates the effect of permanganate pre-oxidation on the coagulation of peptides/proteins of *Microcystis aeruginosa* which comprise a major proportion of the organic matter during cyanobacterial bloom decay. Four different permanganate dosages (0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ mg^{-1} DOC) were applied prior to coagulation by ferric sulphate. Moreover, changes in sample characteristics, such as UV_{254}, DOC content and molecular weight distribution, after pre-oxidation were monitored. The results showed that permanganate pre-oxidation led to a reduction in coagulant dose, increased organic matter removals by coagulation (by 5–12% depending on permanganate dose), microcystin removal (with reductions of 91–96%) and a shift of the optimum pH range from 4.3 to 6 without to 5.5–7.3 with pre-oxidation. Degradation of organic matter into inorganic carbon and adsorption of organic matter onto hydrous MnO_2 are suggested as the main processes responsible for coagulation improvement. Moreover, permanganate prevented the formation of Fe-peptide/protein complexes that inhibit coagulation at pH about 6.2 without pre-oxidation. The study showed that carefully optimized dosing of permanganate improves cyanobacterial peptide/protein removal, with the benefit of microcystin elimination.
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1. Introduction

Cyanobacteria are ubiquitous in rivers and reservoirs supplying drinking water treatment facilities. When cyanobacterial populations rise, water treatment technology has to deal not only with increased cell concentration but also with dissolved algal organic matter (AOM) (Zhang et al., 2010). AOM can seriously impair water treatment process efficiency, especially during the decline phase of an algal bloom, when high concentrations of cellular organic matter (COM) are released into source water (Henderson et al., 2008; Zhang et al., 2010; Pivokonsky et al., 2016). The most pronounced adverse effects of AOM on drinking water production and quality are the reduction in coagulation efficiency, shortening filter runs, membrane fouling, decreased adsorption efficiency for micropollutants onto activated carbon, and disinfection by-product formation (Pramanik et al., 2015; Zamyadi et al., 2015; Pivokonsky et al., 2016).

Numerous studies have showed that pre-oxidants such as ozone, chlorine dioxide, chlorine, potassium ferrate (K_2FeO_4) or potassium permanganate ($KMnO_4$) can improve the removal of algal and cyanobacterial cells by coagulation/flocculation (Ma and Liu, 2002; Plummer and Edzwald, 2002; Chen and Yeh, 2005; Henderson et al., 2008; Chen et al., 2009; Ma et al., 2012; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013; Xie et al., 2013; Qi et al., 2016). The improvement in cell removal is attributed to cell inactivation, changes in external cell architecture and release of high-molecular weight (MW) extracellular organic matter (EOM) and/or cellular organic matter (COM) that easily combine with coagulants (Pranowo et al., 2013; Wang et al., 2013). However, many studies indicated that pre-oxidants may negatively affect coagulation. Pre-oxidation can lead to cell lysis, releasing undesirable toxins or taste and odour compounds, and to EOM/COM degradation, forming low-MW compounds which may be difficult to coagulate (Hoyer et al., 1987; Paralkar and Edzwald,

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1996; Henderson et al., 2008; Ma et al., 2012; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013; Xie et al., 2013). Furthermore, pre-oxidation by chlorine, chlorine dioxide and ozone forms undesirable disinfection by-products (DBPs) (Henderson et al., 2008; Coral et al., 2013; Xie et al., 2013). To avoid the formation of DBPs, permanganate may be used as an alternative pre-oxidant when treating eutrophic source water (Xie et al., 2016).

The effect of permanganate and ozone pre-oxidation on aqueous suspensions of *Microcystis aeruginosa*, subsequent coagulation by aluminium sulphate and the formation of DBPs during downstream chlorination was previously investigated (Xie et al., 2013). Both pre-oxidants increased the removal rate of cells during coagulation (from 56.7% without pre-oxidation) by 15–25% depending on the dose. Pre-ozonation destroyed cell walls and membranes to release COM, and produced more organic nitrogen and lower-MW precursors than permanganate oxidation. This resulted in a dramatic increase in DBP formation during downstream chlorination. Pre-oxidation with permanganate mainly released organic matter adsorbed on the cells' surface without causing any cell damage and led to much lower DBPs formation. Similarly, Chen et al. (2009) demonstrated that both ozone and permanganate, can improve the subsequent algae (*Chlorella* sp.) removal through alum coagulation and sedimentation, increasing algae removal efficiency from 84% to 93% and 98% for optimum doses of ozone and permanganate, respectively. While extensive cell destruction was observed after ozonation, permanganate had the potential to induce the release of EOM from cells. Permanganate also generates manganese dioxides (MnO_2), which may accelerate the flocculation kinetics by increasing particle concentration and promote flocculation by adsorption onto other particles (Petrusevski et al., 1996; Chen and Yeh, 2005; Chen et al., 2009; Xie et al., 2016). However, if $KMnO_4$ is overdosed, increases in residual manganese, colour, turbidity and cell damage can occur (Qi et al., 2016; Xie et al., 2016). Petrusevski et al. (1996) noted that $KMnO_4$ dose and residual manganese were positively correlated, although it was observed that the subsequent application of cationic polymer lowered residual manganese levels. Furthermore, Ma et al. (2012) and Qi et al. (2016) showed that the cellular organic matter (COM) release can be controlled by the subsequent introduction of Fe(II) to quench residual $KMnO_4$.

Studies on the oxidation of AOM in the absence of algal cells are rare and they focus only on the ozonation of algal EOM (Hoyer et al., 1987; Paralkar and Edzwald, 1996; Widrig et al., 1996). Widrig et al. (1996) showed that macromolecular proteins and fatty acids were degraded by ozone into smaller molecules. Similarly, Paralkar and Edzwald (1996) found that ozonation of the EOM showed a consistent trend of decreasing high-MW fractions and increasing medium- and low-MW fractions with increasing ozone dose. Moreover, the charge of the EOM samples was observed to become more negative with ozonation. No attention has been given to the pre-oxidation of COM which has been reported to form the majority of AOM in raw water during algal bloom decay (Zhang et al., 2010).

The aim of this study was to investigate the impact of pre-oxidation on the coagulation of dissolved COM. Potassium permanganate was used as the pre-oxidant to minimize the degradation of COM into smaller molecules which are resistant to coagulation (Pivokonsky et al., 2012). The specific objectives were: (1) to evaluate the changes in COM and MW distribution after permanganate oxidation, and (2) to assess the influence of permanganate oxidation on the coagulation of the COM derived from *Microcystis aeruginosa*. Hence, this paper presents for the first time novel data on the fate of cyanobacterial COM during oxidation using $KMnO_4$. These data demonstrate the operational benefits of selection of optimising the pre-oxidation agent and conditions for successful coagulation of cyanobacterial COM and removal of the associated toxins.

2. Material and methods

2.1. Cultivation of Microcystis aeruginosa and preparation of COM

Microcystis aeruginosa (strain Zap. 2006/2, Department of Culture Collection of Algal Laboratory, Institute of Botany, CAS, Czech Republic) was the species selected for this study, as it is the most abundant and common cyanobacterium occurring in natural water (Pivokonsky et al., 2016). It was cultivated and harvested during the stationary growth phase as described by Pivokonsky et al. (2014). The cells were separated from the culture media by filtering through a 0.22 μm membrane filter (Millipore, USA) and subsequently stirred with ultra-pure water and disrupted in ice bath using an ultrasonic homogeniser (UP400S, Hielscher Ultrasonics, Germany) at 60% amplitude of ultrasonication (240 W) in pulse mode for 5 min. The residual solids were removed by a 0.22 mm membrane filter, and filtrates were concentrated tenfold in a rotary evaporator (Laborota 4000 HB/G1, Germany) at 20 °C. The peptide and protein fraction of COM, which was reported to represent the major portion of *M. aeruginosa* COM (Pivokonsky et al., 2014), was isolated from the cells and used in all subsequent experiments. Peptides and proteins were isolated from the COM by precipitation using $(NH_4)_2SO_4$. The peptide/protein precipitate was separated from the dissolved organic matter by filtration through a 0.22 mm membrane filter (Millipore, USA) and then dissolved in ultra-pure water (Pivokonsky et al., 2012). Peptide/protein concentrations in the subsequent experiments are expressed as concentration of dissolved organic carbon (DOC). The isoelectric points of the isolated peptides and proteins are within a range of 4.8–8.1 (Pivokonsky et al., 2012). In addition, both COM and isolated peptide/protein samples were analyzed for microcystin content (the sum of MC–LR, –RR and –YR). It was found that more than 95% of microcystins contained in COM passed to peptide/protein fraction during its isolation. In the peptide/protein sample with 100 mg L^{-1} of DOC, total MCs concentration was 143.7 μg L^{-1}.

2.2. Pre-oxidation experiments

Solution of 0.02 M $KMnO_4$ (Sigma-Aldrich, USA) was used as pre-oxidant. Solutions of COM peptides/proteins were diluted to the concentration of 8 mg L^{-1} as DOC and adjusted to pH 7 and alkalinity 1.5 mmol L^{-1} (75 mg L^{-1} $CaCO_3$) using 0.1 M HCl and 0.1 M $NaHCO_3$. These were then oxidized with 0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ per 1 mg DOC. Samples were collected for residual $KMnO_4$ analysis after 1, 3, 5, 10, 20, 30, 40, 50 and 60 min of contact time under mixing at a shear rate of 100 s^{-1} with a magnetic stirrer. This was undertaken to monitor the side effects of extensive oxidation (increased Mn, colour and turbidity levels). In addition, the time needed for the oxidation was determined by measuring the variation of UV absorbance at 254 nm, absorbance at 620 nm, proportional to the amount of phycocyanin, and residual DOC concentrations at each time point, where the time, at which absorbance and DOC stabilised, was taken as the oxidation time required. The changes in the MW distribution of COM peptides/proteins and microcystin concentrations at the time required for complete oxidation, i.e. at 10 min time, were monitored by high-performance size-exclusion chromatography (HPSEC) and by solid phase extraction (SPE) followed by LC/MS/MS, respectively (see section 2.4). The samples were rotary evaporated prior to MW distribution and microcystin analyses (Laborota 4000 HB/G1, Germany) to reach concentrations of 50 mg L^{-1} DOC that are convenient for HPSEC performance and enable microcystin detection.

2.3. Coagulation tests

Jar tests with COM peptides/proteins of initial concentrations of 1, 3, 5 and 8 mg L^{-1} as DOC, both with and without $KMnO_4$ pre-oxidation, were performed. These DOC concentrations correspond with concentrations produced during laboratory experiments. For example, Henderson et al. (2008) observed stationary phase AOM concentrations from lab cultures of *M. aeruginosa* of approximately 18 mg L^{-1}, Nguyen et al. (2005) reported that cyanobacterium *Anabaena cylindrica* produced about 25 mg L^{-1} of DOC during stationary phase and Pivokonsky et al. (2014) measured between 50 and 70 mg L^{-1} of DOC during stationary phase growth of *M. aeruginosa*. Moreover, Pivokonsky et al. (2014) found that COM of *Microcystis aeruginosa* contains about 60% of proteinaceous matter. The model water was ultra-pure water with alkalinity adjusted to 1.5 mmol L^{-1} (75 mg L^{-1} $CaCO_3$) using 0.1 M $NaHCO_3$ and pH 8.5. In the experiments with $KMnO_4$ pre-oxidation, doses of 0.1, 0.2; 0.4 and 0.6 mg $KMnO_4$ per 1 mg DOC were applied. 1% solution of ferric sulphate ($Fe_2(SO_4)_3 \cdot 9H_2O$; Sigma-Aldrich, USA) was used as a coagulant. Firstly, coagulant doses were optimized for COM peptides/proteins of initial concentrations of 1, 3, 5 and 8 mg L^{-1} as DOC using doses ranging from 1 to 14 mg L^{-1} as Fe (0.018–0.251 mmol L^{-1} Fe). Secondly, jar tests with the coagulant doses optimized for four COM peptide/protein concentrations were performed in the pH range 3–9.5. 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 pre-oxidation and addition of the coagulant.

The experiments were carried out using a variable speed eight position paddle stirrer (LMK 8-03, IH CAS, Czech Republic) and 2 L jars. After pH adjustment, $KMnO_4$ was dosed and samples were mixed at a shear rate of 100 s^{-1} for 10 min, after which the ferric sulphate (1% solution) was added. The coagulation process included steps of agitation at a shear rate of 300 s^{-1} for 1 min, followed by a slow stir phase at a shear rate of 50 s^{-1} for 15 min before a 60 min settling period. Residual solids were then separated by centrifugation. The supernatants were analysed for pH, DOC, residual Mn and Fe, microcystins and MW distribution. Prior to MW distribution analyses, the samples were concentrated in a rotary evaporator (Laborota 4000 HB/G1, Germany) to reach concentrations of 50 mg L^{-1} DOC.

2.4. Analytical methods

The UV absorbance at 254 nm (UV_{254}), absorbance at 620 nm, proportional to the amount of phycocyanin, and the residual $KMnO_4$ as absorbance at 530 nm after pre-oxidation experiments were measured with a UV-VIS 8453A spectrophotometer (Agilent Technologies, USA) with 1 cm quartz cells (Ma et al., 2012). Potassium permanganate standards of concentrations of 0.1, 0.5, 1, 2, 3, 5, 7 and 10 mg L^{-1} were used to calibrate $KMnO_4$ measurements. All measurements were conducted in triplicate with measurement errors less than 3%.

An inductively coupled plasma optical emission spectrophotometer (ICP OES, Optima 2000 DV, Perkin-Elmer, USA) was used to determine the concentrations of residual Mn and Fe after coagulation tests. Measurements of Mn and Fe samples were carried out in triplicate and errors were less than 3%.

DOC was analysed using a Shimadzu TOC-V_{CPH} analyser (Shimadzu Corporation, Japan). Samples for DOC analysis were pretreated by filtering through a 0.45 μm membrane filter (Millipore, USA). All measurements of DOC concentrations were conducted in triplicate and errors of measurement were less than 2%.

Microcystin analyses, specifically the sum of MC–LR, –RR and –YR, were performed following the standard operation procedure adapted from EPA Method 1694 (EPA Method 1694, 2007). Microcystins were cleaned-up by solid phase extraction and analyzed by an Agilent 6410 Triple Quadrupole LC/MS/MS system (Agilent Technologies, USA) in the positive electrospray ionization (ESI+) mode and column Zorbax Eclipse XDB-C18 (4.6 × 100 mm × 3.5 lm) (Agilent Technologies, USA). Two mobile phases (A - water and B - methanol, both containing 0.1% (v/v) formic acid and 5 mM sodium formate) were used at a flow rate of 300 μl min L during the gradient run: 3–30% B for 0.3 min, 30–35% B for 6 min, 35–95% B for 3 min. Microcystin concentration was evaluated using a combination of isotope dilution (Isoproturon D6; Sigma Aldrich, USA) and internal standard quantitative techniques. The quantification limit of the method is 100 ng L^{-1}.

The MW distributions were accomplished by HPSEC with a diode array detector (DAD) (Agilent 1260 Infinity, Agilent Technologies, USA) operated at 280 nm. 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 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^{-1} and a 50 μL sample loop 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. Reproducibility of the MW fractionation of COM peptide/protein samples was very good, with MW deviations of less than 3% from repeated measurements.

3. Results and discussion

3.1. Influence of permanganate pre-oxidation on COM peptides/proteins

The optimal $KMnO_4$ dose for pre-oxidation during the experiments was selected based on variations in UV_{254}, DOC and residual $KMnO_4$. Fig. 1a shows that UV_{254} of peptide/protein solutions of 8 mg L^{-1} DOC sharply rose after pre-oxidation and then dropped to a relatively constant level after 10 min, with the exception of the lowest $KMnO_4$ dose, at which changes in UV_{254} were observed until 60th minute (an additional 8% drop). On the other hand, absorbance at 620 nm, which is typical for blue-coloured protein phycocyanin substantially decreased and stabilised after 5 min of pre-oxidation (Fig. 1b). After 10 min of pre-oxidation, residual $KMnO_4$ reached a plateau (Fig. 1c) following similar oxidant decay rate for all initial $KMnO_4$ doses. $KMnO_4$ decay rates were calculated and for the 0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ per 1 mg DOC dosages the first-order decay rate constants were 0.0048 s^{-1} (R^2 = 0.98), 0.0048 s^{-1} (R^2 = 0.96), 0.0041 s^{-1} (R^2 = 0.81), and 0.0032 s^{-1} (R^2 = 0.98), respectively. No significant difference was observed between $KMnO_4$ decay rates for the 2 low dosages. With increase in $KMnO_4$ dose the decay rate decreased, however, the rate values were all within the same order of magnitude. Residual $KMnO_4$ concentrations of <0.1, 0.2, 0.4 and 0.6 mg L^{-1} were recorded for initial doses of 0.8, 1.6, 3.2 and 4.8 mg L^{-1} (~0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ mg^{-1} DOC), respectively. Furthermore, DOC concentrations also stabilised after 10 min of pre-oxidation and were reduced by 7.5%, 21%, 24% and 26% for doses of ~0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ mg^{-1} DOC, respectively (Fig. 1d). Thus, for subsequent experiments, a 10 min pre-oxidation time was applied. The doses of 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC are presented in the following text, since application of the dose of 0.1 mg $KMnO_4$ mg^{-1} DOC did not enhance coagulation compared with experiments without pre-oxidation, while the dose of 0.6 mg $KMnO_4$ mg^{-1} DOC provided high residual manganese levels (data not shown). These were about 0.05 mg L^{-1} Mn, which represent EC drinking water directive limit for manganese (Directive 98/83/EC). Permanganate doses used in this study are not expected to cause cell damage in case that *M. aeruginosa* cells are also present in raw water. According to the study by Xie et al. (2013),

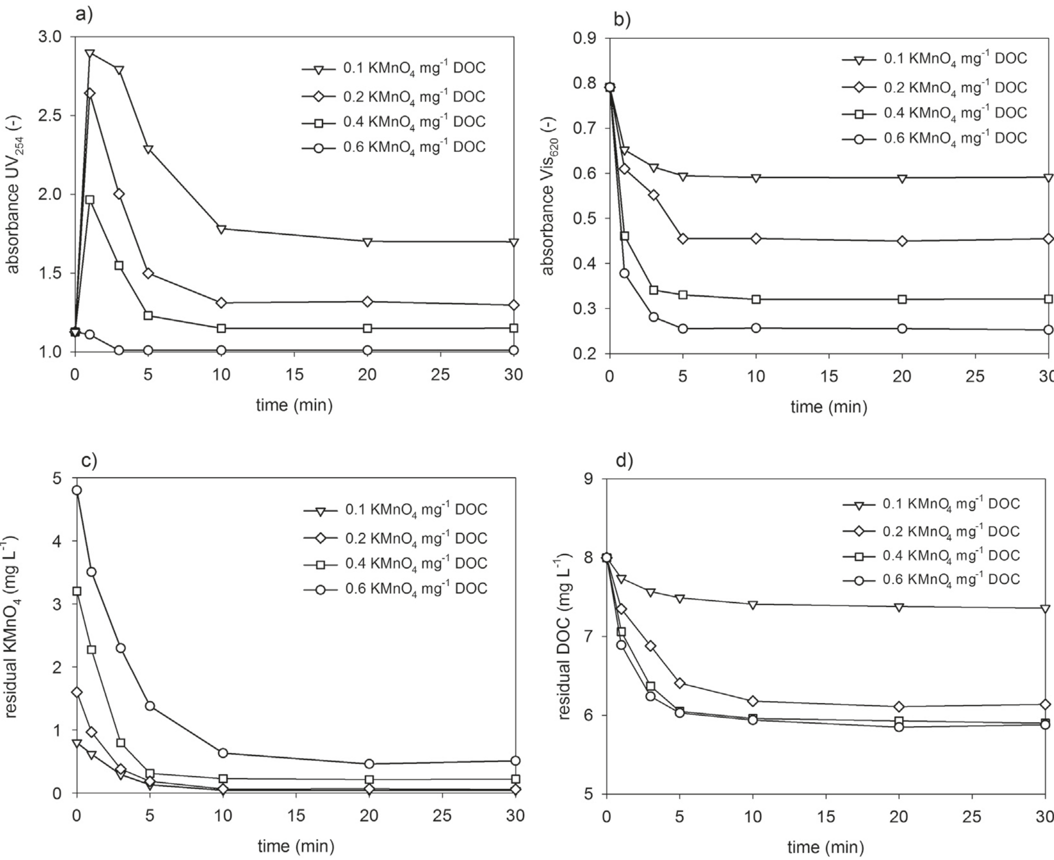

Fig. 1. Dependence of absorbance of UV_{254} (a), absorbance of Vis_{620} (b), residual $KMnO_4$ (c) and residual DOC (d) on pre-oxidation time and $KMnO_4$ dose (initial DOC concentration 8 mg L^{-1}, pH = 7).

$KMnO_4$ doses up to 2 mg L^{-1} did not damaged *M. aeruginosa* cells at concentration of 1.01 ± 0.06 × 10^6 cells/mL. Our doses of 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC are equivalent to 0.2–3.2 mg L^{-1} $KMnO_4$ for DOC concentrations 1–8 mg L^{-1}.

Fig. 2 compares HPSEC analyses of COM peptides/proteins without and with $KMnO_4$ pre-oxidation. For the $KMnO_4$ dose of 0.2 mg per 1 mg of DOC, peaks of high-MW proteins (9–1300 kDa) became significantly smaller, while at 0.4 mg $KMnO_4$ per 1 mg of DOC almost all peaks were reduced. The reduction in UV (280 nm) absorbance may be due to the decomposition of organic matter to inorganic carbon, which is apparent from Fig. 1d, changes in amino acid compositions and also due to changes in UV chromophores in the peptide/protein molecules. Protein absorbance at 280 nm is caused by amino acids with aromatic rings. Oxidation of the proteins can lead to the formation of molecules of different amino acid compositions, the absorbances of which are lower. The changes in UV chromophores could be observed even by the naked eye in case of the blue-coloured protein phycocyanin with tetrapyrrole chromophoric groups, which was contained in the peptide/protein samples. Phycocyanin exhibits the absorption maximum at 620 nm, which is characteristic of tetrapyrrole-based chromophore. Phycocyanin lost its blue colour with pre-oxidation (see Fig. S1 in Supplementary Data). Changes in the chromophoric groups are consistent with the significant decrease in absorbance at 620 nm (shown in Fig. 1b). Similarly, large reductions in aromaticity were observed for ozonation of natural organic matter (Reckhow et al., 1986). That mechanism may occur during permanganate oxidation of protein substances, resulting in their decreased absorbance of protein substances. In addition, the reduction in high-MW peaks may be caused by the transformation of high-MW proteins into low-MW peptides that are difficult to coagulate (Pivokonsky et al., 2012).

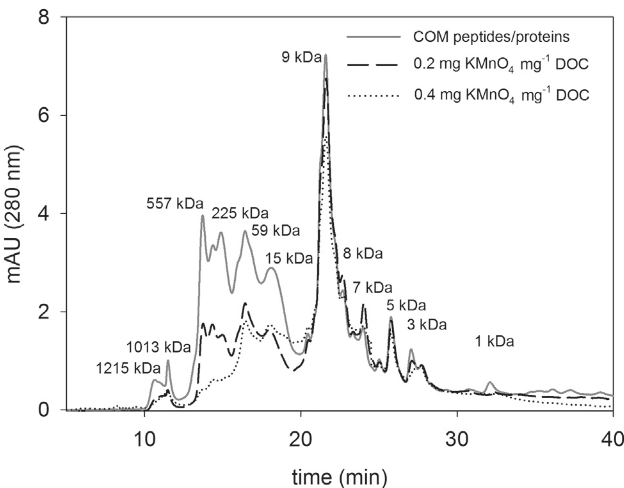

Fig. 2. HPSEC analyses of COM peptides/proteins before and after pre-oxidation by 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC (sample DOC = 50 mg L^{-1}, pH = 7, reaction time 10 min).

Other studies that performed HPSEC analyses after $KMnO_4$ pre-oxidation applied $KMnO_4$ onto cell suspensions. Therefore, they monitored not only changes in MW distribution due to oxidation, but also the release of organics from cells after oxidation (Ma et al., 2012; Wang et al., 2013; Xie et al., 2013). In general, they observed an increase in high-MW fractions due to release of organic matter

adsorbed on the cells' surface and also decomposition to lower-MW compounds or to inorganic carbon. Nevertheless, these effects were considerably less pronounced than in the case of ozonation (Xie et al., 2013).

In Fig. 2, the peak of MW of 1 kDa can probably be assigned to toxic heptapeptides microcystins (Kopecka et al., 2014). This peak is substantially reduced at both $KMnO_4$ doses. The measurements of total MC (the sum of MC–LR, –RR and –YR) concentration showed that most of the MCs were oxidized by all four doses of $KMnO_4$ (0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ mg^{-1} DOC). Prior to MC analyses, the pre-oxidized samples had to be concentrated in order to reach detectable MC concentrations. After an oxidation time of 10 min, MC concentrations (initial MC concentration of 11.5 μg L^{-1}, DOC = 8 mg L^{-1}) were reduced by 91, 93, 95 and 96% for $KMnO_4$ doses of 0.1, 0.2, 0.4 and 0.6 mg $KMnO_4$ mg^{-1} DOC, respectively. This is consistent with other studies which indicated that pure microcystins are quickly oxidized by $KMnO_4$ (Chen et al., 2005; Rodriguez et al., 2007), while in the presence of cyanobacterial cells degradation was slower, probably due to the competing reactions with other molecules from the cyanobacterial cells (Fan et al., 2013; Shao et al., 2014).

3.2. Coagulation

The optimum coagulant ($Fe_2(SO_4)_3 \cdot 9H_2O$) doses for the removal of all initial concentrations of COM peptides/proteins were set to 7 mg L^{-1} as Fe (0.125 mmol L^{-1} Fe) for experiments without pre-oxidation and 6.4 mg L^{-1} as Fe (0.115 mmol L^{-1} Fe) for samples pre-oxidized with 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC (see Fig. S2 in Supplementary Data). A previous study by Pivokonsky et al. (2012) showed that the removal of peptides/proteins of *M. aeruginosa* was strongly dependent on pH value. Therefore, jar tests with the optimized coagulant doses and four DOC concentrations (1, 3, 5 and 8 mg L^{-1}) were performed in the pH range 3–9.5. Fig. 3 compares residual Fe and DOC concentrations as a function of pH of jar tests without pre-oxidation (Fig. 3a and b) and with pre-oxidation by 0.2 (Fig. 3c and d) and 0.4 mg $KMnO_4$ mg^{-1} DOC (Fig. 3e and f). Similarly to our previous study (Pivokonsky et al., 2012), optimum pH for coagulation without pre-oxidation was in the pH range of 4.3–6 for initial DOC concentrations of 3, 5 and 8 mg L^{-1} and 5.5–6.5 for initial DOC concentration of 1 mg L^{-1}. Charge neutralization between peptide/protein deionized functional groups and positively charged Fe hydrolysis species is the most probably the predominant removal mechanism (Matilainen et al., 2010; Pivokonsky et al., 2016). Adsorption is also known to play an important role in the coagulation. At higher coagulant/DOC concentration ratio and pH about 6–8, peptides/proteins attach to the surface of Fe-oxide-hydroxide particles leading to particle aggregation. On the other hand, when the coagulant/DOC ratio is low, the surface of Fe-oxide-hydroxide particles becomes completely covered with peptides/proteins, which leads to an increase in the negative charge density to the charge stabilization of particles that hinders their aggregation (Pivokonsky et al., 2016).

Coagulation of peptides and proteins without pre-oxidation was disturbed by the formation of dissolved complexes between Fe and peptides/proteins at pH about 6.2, as also described in Pivokonsky et al. (2012). By contrast, no such interference was observed in the experiments with pre-oxidation (Fig. 3c–f). The break-up of organo-metal complexes and subsequent release of oxidized metals, such as Fe(III) and Mn(IV), was also observed after ozonation of natural organic matter (Reckhow et al., 1986). Furthermore, coagulation tests with pre-oxidation provided higher DOC removal rates than without pre-oxidation (see Table 1). This is probably given by the degradation of organic matter to inorganic carbon, which was ascertained in the pre-oxidation experiments (Fig. 1d). These showed that the DOC reductions by $KMnO_4$ doses of 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC were 21% and 24%, while DOC removal rate increases after coagulation tests are 5–12% compared to coagulation alone. Moreover, some authors hypothesized that pre-oxidation increases the concentration of oxygenated functional groups, especially the carboxylic acids (Reckhow et al., 1986; Shao et al., 2014), which are responsible for organic matter-coagulant surface associations. In addition, the potential adsorption of organic matter onto hydrous MnO_2 may also contribute to coagulation (Chen and Yeh, 2005; Ma et al., 2012). The latter is evident on examination of Fig. 3a,c,e, which shows that at pH > 7.5, residual DOC concentration decreases with the increased $KMnO_4$ dose. At the same time, residual manganese levels were observed to be quite low at pH > 7.5 due to the formation of hydrous MnO_2 (Fig. 4). However, residual coagulant (Fe) rose sharply (Fig. 3d,f), which indicates that it is not likely to be responsible for reductions in DOC levels at these pH values. It should be noted that the difference in DOC removals after single pre-oxidation (21% and 24%) and pre-oxidation-coagulation (increased by 5–12% compared to coagulation alone) is probably given by the different fates of peptides/proteins after pre-oxidation. For example, as mentioned in section 3.1. high-MW proteins may be degraded by permanganate in two ways: 1) to inorganic carbon, which means they are no more available for subsequent coagulation, or 2) to low-MW peptides that are resistant to coagulation (Pivokonsky et al., 2012), which may decrease coagulation efficacy.

Besides improved DOC removal rates, pre-oxidation also leads to widening optimum pH range for DOC removal up to pH 7.3 (see Table 1). This can be attributed firstly to permanganate-induced rupture of Fe-peptide/protein complexes and secondly to adsorption activity of hydrous MnO_2 toward peptides/proteins. As mentioned above, adsorption plays its role in coagulation at higher pH values, but its contribution depends on the ratio of peptide/protein molecules and Fe-oxide-hydroxide particles. When formation of hydrous MnO_2, which is promoted by higher pH values and by rising concentration of MnO_2 (Stumm and Morgan, 1996), increases the number of the particles in the system, the adsorption of peptides/proteins onto these particles results in particle aggregation instead of stabilization. This enables the shift of pH optimum to higher pH values. In addition, hydrous MnO_2 particles also promote the formation of primary insoluble flocs during early phases of Fe^{3+} hydrolysis, where they serve as initial cores of precipitation.

Fig. 4 further compares residual DOC and manganese concentrations for different initial peptide/protein concentrations. Evaluation of Fig. 4 indicates that higher $KMnO_4$ doses enhance DOC removals, but provide higher residual Mn concentrations. Moreover, it shows that acceptable manganese levels are achieved at pH 5.5–8.5 for both $KMnO_4$ doses and all initial DOC concentrations. Manganese levels rise with lower pH above the EC drinking water directive limit (Directive 98/83/EC), because the formation of hydrous MnO_2 is governed by pH and is very slow at pH < 5.5 (Stumm and Morgan, 1996). Therefore, even if high DOC and Fe removals were observed at pH 4.3–5.5 (Fig. 3), the water produced at this pH would not be acceptable for drinking purposes. The increase in Mn residuals due to pre-oxidation with $KMnO_4$ could cause excessive Mn issues for treatment plants, particularly for sludge and spent water recovery streams. In these plants, the recovered water is returned to the head of the plant and added to the raw water, leading to increasing concentration of compounds to be removed during the treatment (Zamyadi et al., 2016). The cumulative impact of these recycling streams could lead to Mn concentrations higher than plants design removal capacity. On the other hand, oxidation could help to prevent accumulation and recycling of harmful cellular metabolites, including toxins and taste and odour compounds, within the water treatment and recovery systems.

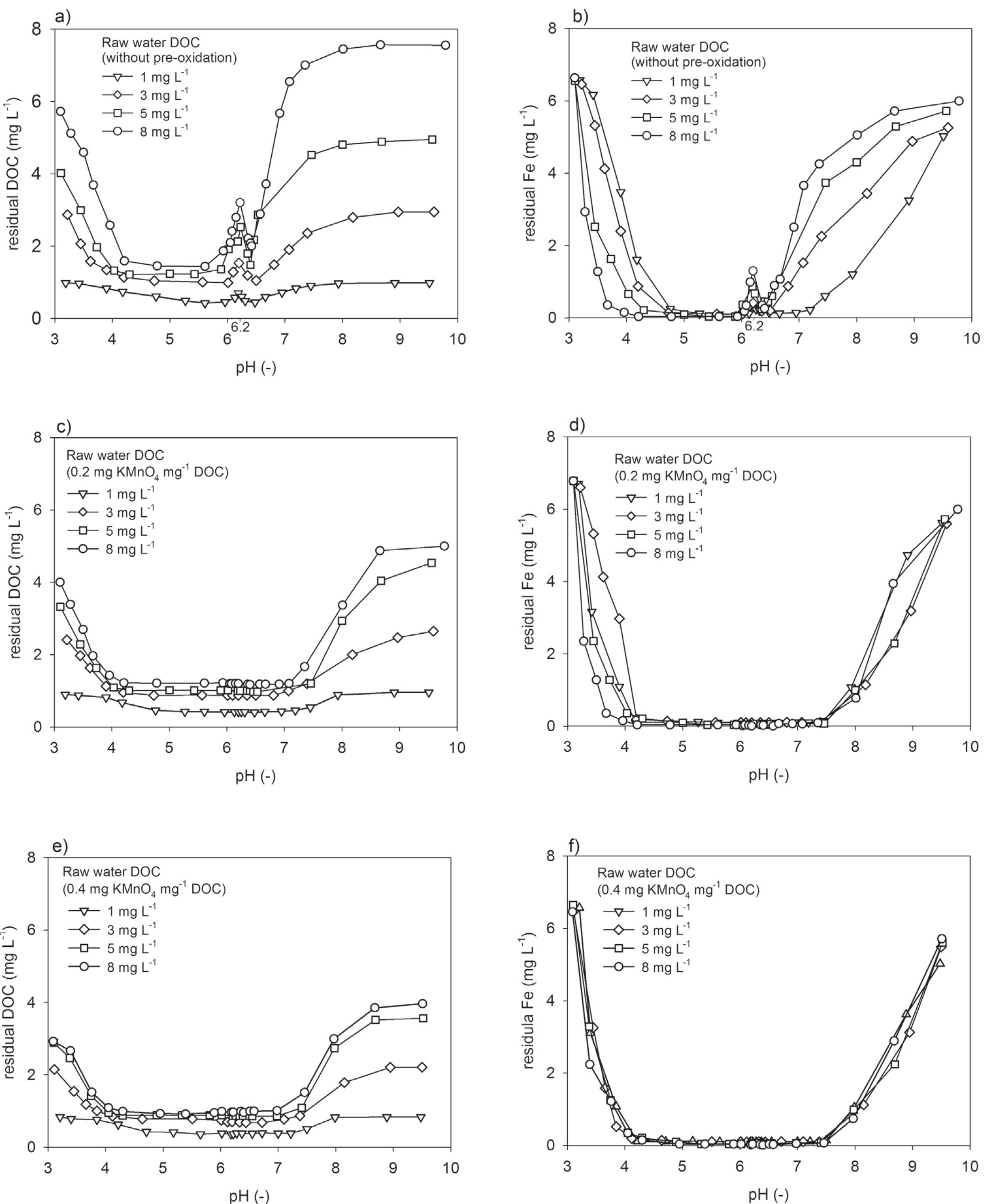

Fig. 3. Dependence of residual DOC and residual Fe on pH value for initial DOC concentrations of 1, 3, 5 and 8 mg L^{-1} without pre-oxidation (a, b) and after pre-oxidation (reaction time 10 min) by 0.2 mg $KMnO_4$ mg^{-1} DOC (c, d) and 0.4 mg $KMnO_4$ mg^{-1} DOC (e, f).

Table 1
Optimum coagulation conditions and removal rates for COM peptides/proteins with and without pre-oxidation.

	Without $KMnO_4$	0.2 mg $KMnO_4$ mg^{-1} DOC	0.4 mg $KMnO_4$ mg^{-1} DOC
DOC removal rate (%)	55, 66, 75, 82[a]	60, 71, 80, 87[a]	64, 78, 83, 90[a]
optimum pH for DOC, Fe (-)	4.3–6.0	4.3–7.3	4.3–7.3
optimum pH for Mn (-)	–	5.5–8.5	5.5–8.5
residual Mn (mg L^{-1})	–	0.01	0.03
residual microcystin (μg L^{-1})	1.4–11.2[a]	<0.1	<0.1

[a] For initial DOC 1, 3, 5, 8 mg L^{-1}.

HPSEC analyses (Fig. 5) in pre-concentrated samples after pre-oxidation-coagulation showed that high-MW proteins were removed, while low-MW peptides (<9 kDa) remain in treated water. This is consistent with our previous study that showed coagulation as ineffective in removing low-MW peptides (Pivokonsky et al., 2012) and these need to be removed by other

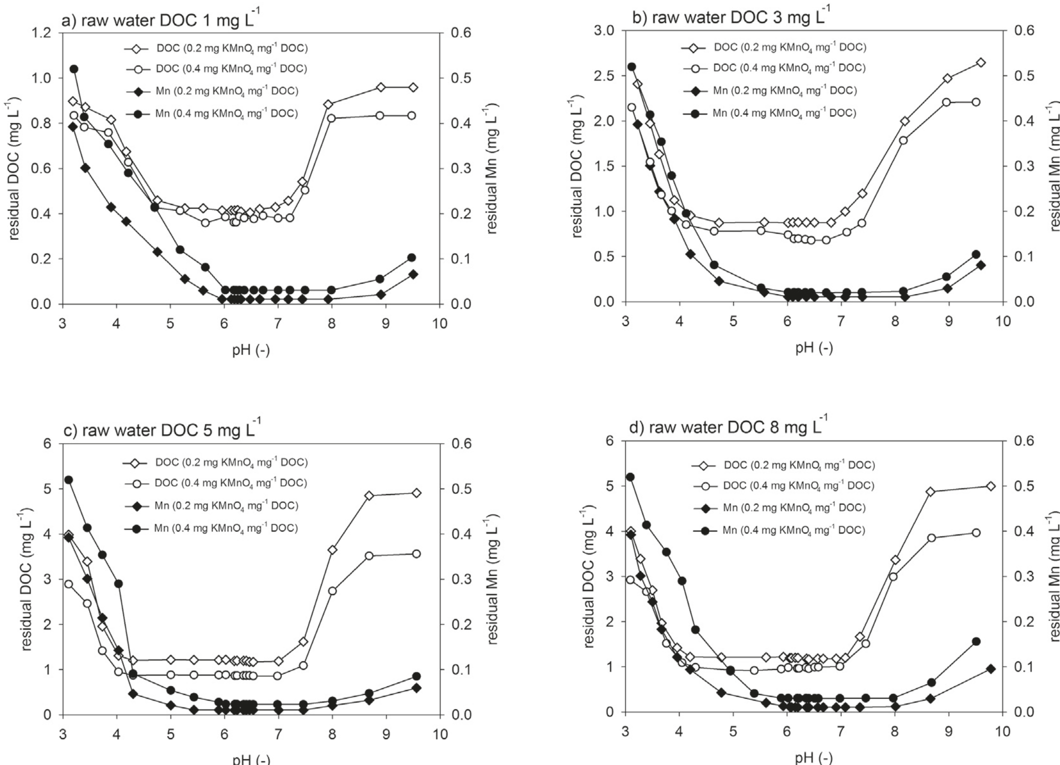

Fig. 4. Dependence of residual DOC and residual Mn on pH value for initial DOC concentrations of 1, 3, 5 and 8 mg L^{-1} after pre-oxidation (reaction time 10 min) by 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC.

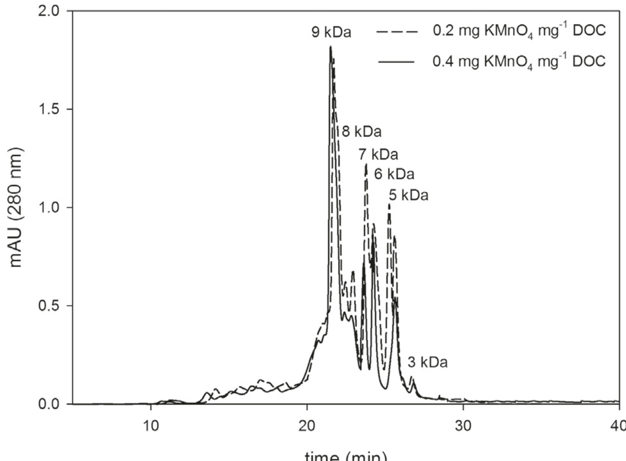

Fig. 5. HPSEC analyses of COM peptides/proteins after coagulation and pre-oxidation by 0.2 and 0.4 mg $KMnO_4$ mg^{-1} DOC (sample DOC = 50 mg L^{-1}, pH = 7, reaction time of pre-oxidation 10 min).

treatment techniques. For example, peptides (MW < 10 kDa) of *M. aeruginosa* were efficiently removed by adsorption onto granular activated carbon (Kopecka et al., 2014). Peptides are expected to contribute to nitrogenous DBP formation. Goslan et al. (2017) reported that AOM of *M. aeruginosa* generates 1.32 ± 0.01 μg mg^{-1} C of dichloroacetonitrile (DCAN) and 0.13 ± 0.01 μg mg^{-1} C of trichloromethane after chlorination. It should be noted that DBP formation potential was shown to be highly species specific even among different cyanobacteria (Goslan et al., 2017). Lui et al. (2012) found that the AOM fraction of 3–10 kDa of green alga *Chlamydomonas* sp. was potent DCAN precursor and showed high mutagenicity. However, findings about protein mutagenicity after chlorination substantially differ (Lui et al., 2012).

Microcystin (peak of 1 kDa) was not observed after pre-oxidation-coagulation (Fig. 5) compared to coagulation alone (Pivokonsky et al., 2012) and the concentration of microcystins was below the WHO guideline value of 1 μg L^{-1} and even below the quantification limit of 0.1 μg L^{-1} (Table 1).

4. Conclusions

The results showed that pre-oxidation with potassium permanganate followed by coagulation with ferric sulphate enhanced the removal of peptides/proteins of *M. aeruginosa*. Beside higher DOC removals, permanganate pre-oxidation lowered coagulant dosage, shifted the optimum pH range and obviated formation of dissolved organo-metal complexes, which interfere with coagulation. The results indicate that the improvement in DOC removals (by 5–12%) after pre-oxidation mainly stems from the degradation of organic matter to inorganic carbon by pre-oxidation. Permanganate was highly effective in removing toxic microcystins and higher microcystin removals were achieved with increasing permanganate dosage. However, HPSEC analyses showed that there is a variety of low-MW compounds that cannot be either coagulated or degraded by $KMnO_4$ and other treatment techniques need to be employed for their removal. The optimum pH for peptide/protein and coagulant removal widened from 4.3 to 6 without to 4.3–7.3 with pre-oxidation, attributable to the involvement of hydrous MnO_2 particles into adsorption processes. Nevertheless, pH range 5.5–7.3 is recommended, because at pH < 5.5 residual manganese levels rise.

The beneficial effect of permanganate pre-oxidation on coagulation of COM peptides/proteins removal lies not only in the improvement in DOC removal, but also in the shift of optimum pH

range to values commonly applied in water treatment practice and in the simultaneous removal of microcystins. However, the oxidant dose must be carefully optimized in order to avoid high residual manganese concentrations in treated water and to maintain integrity of algae cells in case that they are present in raw water.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.02.029.

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Supplementary Data for

The impact of pre-oxidation with potassium permanganate on cyanobacterial organic matter removal by coagulation

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This supplementary data contains the following figures:
Fig.S1 Samples of COM peptides/proteins before (a) and after pre-oxidation by 0.1 (b), 0.2 (c), 0.4 (d) and 0.6 (e) mg $KMnO_4$ mg^{-1} DOC (sample DOC = 50 mg L^{-1}, pH = 7, reaction time 10 min)

Fig. S2 Dependence of residual DOC and residual Fe on Fe dose for initial DOC concentrations of 1, 3, 5 and 8 mg L^{-1} without pre-oxidation (a, b) and after pre-oxidation (reaction time 10 min) by 0.2 mg $KMnO_4$ mg^{-1} DOC (c, d) and 0.4 mg $KMnO_4$ mg^{-1} DOC (e, f)

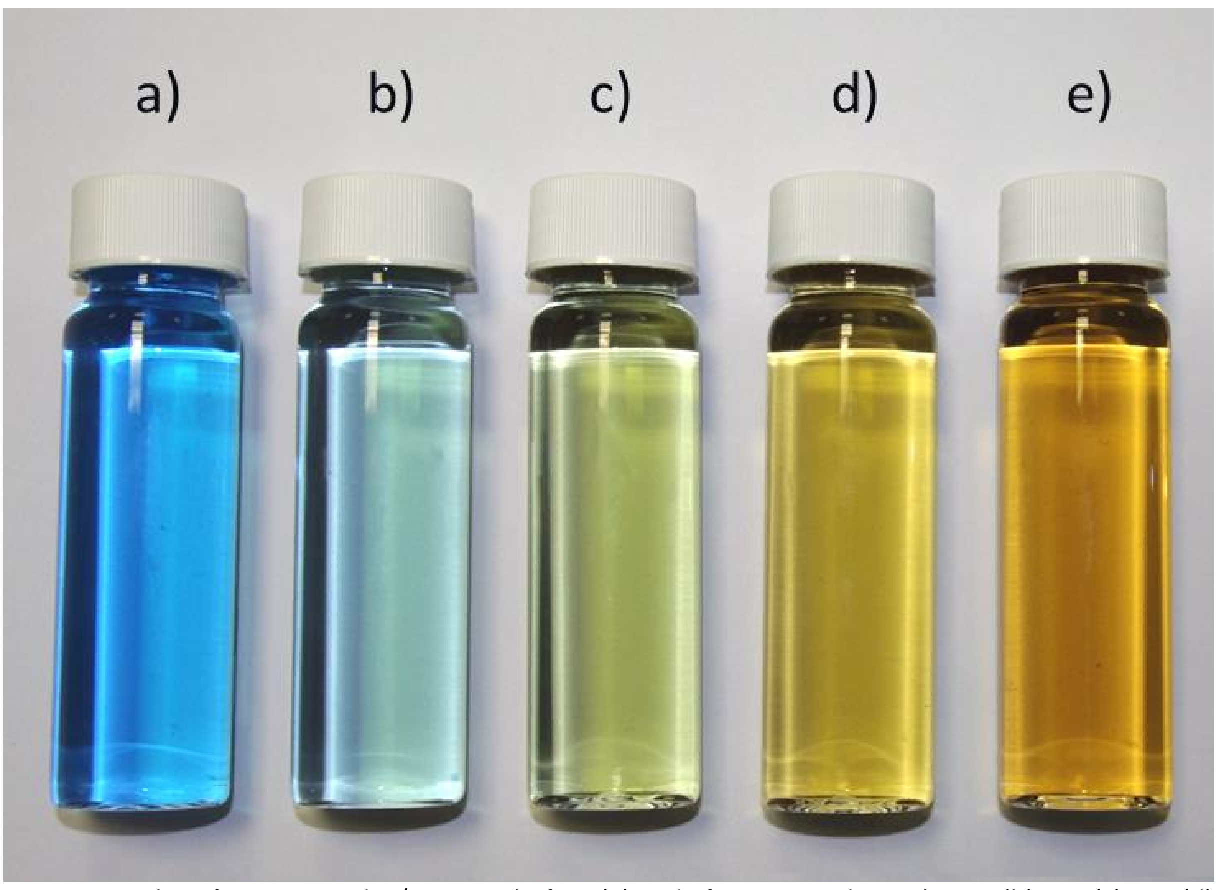

Fig. S1 Samples of COM peptides/proteins before (a) and after pre-oxidation by 0.1 (b), 0.2 (c), 0.4 (d) and 0.6 (e) mg $KMnO_4$ mg^{-1} DOC (sample DOC = 50 mg L^{-1}, pH = 7, reaction time 10 min)

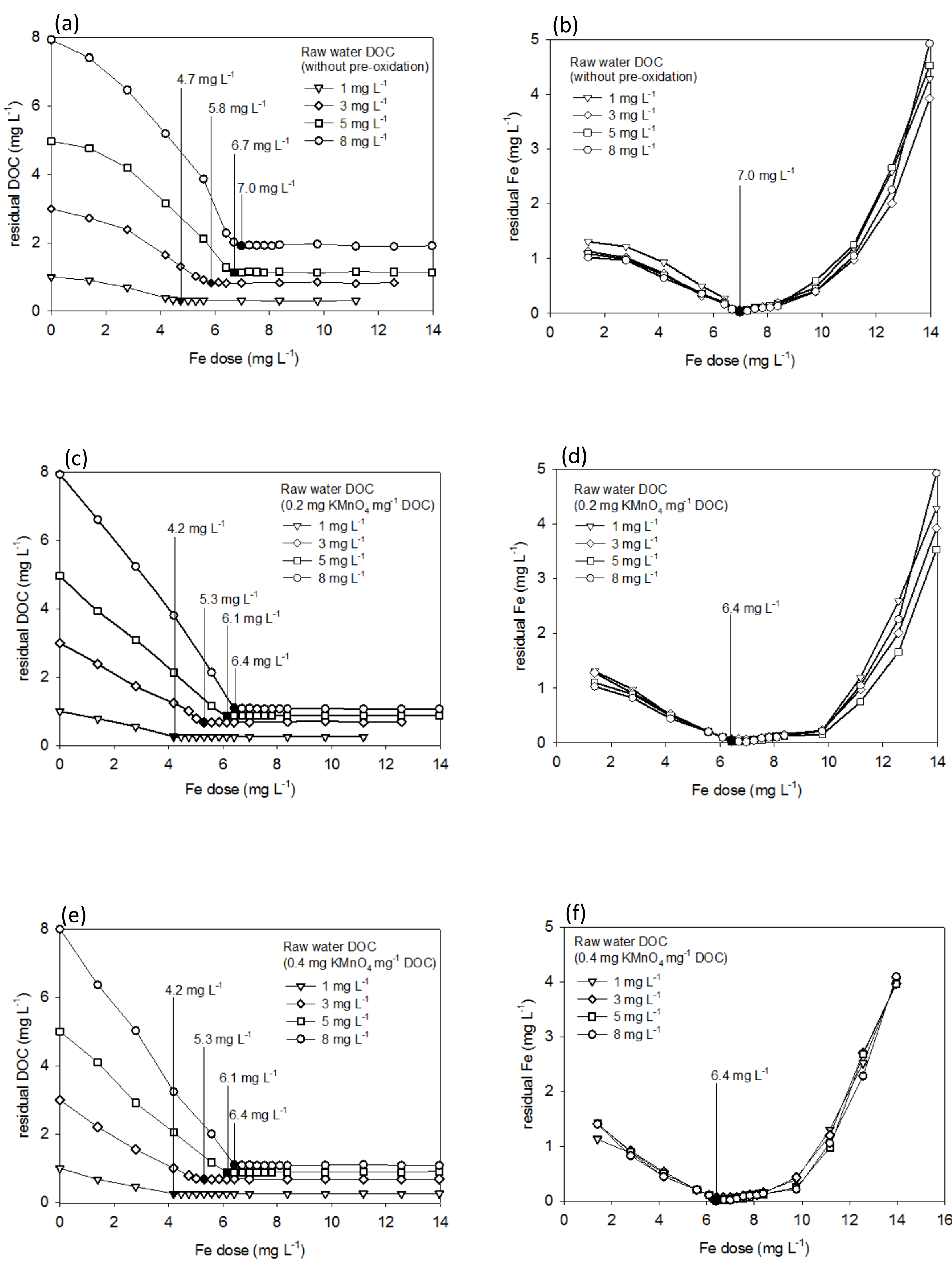

Fig. S2 Dependence of residual DOC and residual Fe on Fe dose for initial DOC concentrations of 1, 3, 5 and 8 mg L^{-1} without pre-oxidation (a, b) and after pre-oxidation (reaction time 10 min) by 0.2 mg $KMnO_4$ mg^{-1} DOC (c, d) and 0.4 mg $KMnO_4$ mg^{-1} DOC (e, f)

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An application of cellular organic matter to coagulation of cyanobacterial cells (*Merismopedia tenuissima*)

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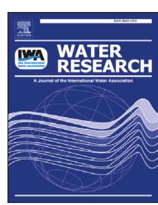

An application of cellular organic matter to coagulation of cyanobacterial cells (*Merismopedia tenuissima*)

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ABSTRACT

Algae affect the performance of drinking water treatment significantly when they decay and release considerable amounts of cellular organic matter (COM). The study describes the cyanobacterium *Merismopedia tenuissima* and its COM and investigates the effect of their simultaneous coagulation. As COM is highly complex mixture, we characterised it in terms of hydrophobicity, protein content and molecular weights (MWs). To describe the coagulation mechanisms and molecular interactions in the system, we determined both COM and cell surface charge by means of potentiometric titration and zeta potential analysis, respectively, and performed the jar tests with single components and their mixtures with and without a coagulant (ferric sulphate).

The coagulation tests performed with the individual components or with their mixtures proved efficient cell removals (up to 99%) but relatively low COM removals (37 ÷ 57%). This disproportion can be attributed to the prevalence of hydrophilic compounds and to the high portion of low-MW organics in COM. Coagulation of COM/cell mixtures achieved comparable efficacy with single component tests, using even lower coagulant doses. Furthermore, COM presence substantially deviated the pH optimum for cell removal and thus altered coagulation mechanisms. While single cells interacted prevailingly through adsorption onto Fe-oxide-hydroxides at about neutral pH (6.0–7.7), the COM/cell mixtures succumbed to charge neutralisation by Fe-hydroxopolymers within moderately acidic pH range (5.0–6.5). Moreover, COM initiated cell flocculation also at acidic pH in both the presence (pH 3.4–3.9) and the absence of a coagulant (pH 3.6–4.6). This supportive effect is ascribed to relatively high-MW COM (>10 kDa), serving as a natural flocculant through inter-particle bridging mechanism and exhibiting nearly the same COM/cell removals as ferric sulphate.

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1. Introduction

The quality of surface water resources is remarkably deteriorating in the presence of algae and cyanobacteria. These produce a variety of organic compounds denoted as algal organic matter (AOM). AOM can seasonally form a prevalent part of natural organic matter (NOM) present in water reservoirs (Henderson et al., 2008). Algae release AOM as a by-product of photosynthesis and secondary metabolism and as a consequence of cell lysis, and thus creating extracellular (EOM) and cellular organic matter (COM), respectively (Takaara et al., 2007). Algae and associated AOM cause severe problems for conventional treatment processes (i.e. coagulation/flocculation - (sedimentation) - filtration), especially when algal blooms decay and both algal cells and high AOM concentrations are present in water (Henderson et al., 2008).

AOM may either improve or hinder coagulation/flocculation of algae depending on its concentration, composition and characteristics (Bernhardt et al., 1985; Vandamme et al., 2012). Research addressing the impact of AOM on algal cell removal has so far mostly focused on EOM released from pre-oxidation (e.g. Plummer and Edzwald, 2002; Ma et al., 2012) or during the harvest of algal biomass (e.g. Vandamme et al., 2012; Garzon-Sanabria et al., 2013).

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Since EOM differs from COM in their overall amount, protein content, degree of hydrophobicity or molecular weight (MW) distribution (Henderson et al., 2008; Pivokonsky et al., 2014), they may exert a different impact on cell removal. Takaara et al. (2007) reported that COM produced by the cyanobacterium *Microcystis aeruginosa* completely hindered coagulation of kaolin particles, whereas EOM only caused a weak inhibition in even 10-times higher concentrations. So far, little attention has been given to the simultaneous removal of both cells and COM. Most studies have focused on the aforementioned compounds separately (Pivokonsky et al., 2009, 2012; Gonzalez-Torres et al., 2014) or dealt with the influence of COM on coagulation of other impurities. Under the appropriate conditions (pH value, coagulant dose), COM was found to induce coagulation of both inorganic kaolin particles (Safarikova et al., 2013) and organic compounds – humic substances (Pivokonsky et al., 2015).

Algal and cyanobacterial cells make the conventional treatment process vulnerable because of their diverse morphology and physiology, particularly their physical dimensions, high motility and low specific density (Ghernaout et al., 2010; Henderson et al., 2010). This is exactly the case of the coccoid cyanobacterium *Merismopedia tenuissima* which may disrupt water treatment due to its remarkably tiny cell size (not exceeding 2 μm in diameter (McGregor, 2013)) which enables the cells breakage through the water treatment plant (WTP) facilities. *M. tenuissima* is characterised by spherical solitary cells, prevailingly organized into flat, rectangular colonies (McGregor, 2013). This cyanobacterium exists in a range of environments covering both oligotrophic lakes with low alkalinity (a predominant species in late summer phytoplankton in Scandinavia, Hörnström, 1999) and eutrophic water reservoirs found in Brazilian semi-arid region (Dantas et al., 2011). *M. tenuissima* tends to occupy nearly the entire water column (Dantas et al., 2011; Koza and Rederer, 2014), and differs from the typical cyanobacterial upper bloom found near the water surface. WTPs cannot prevent *M. tenuissima* from its input by adjusting the depth of the sampled profile. In 2011, a local outbreak of *Merismopedia* sp. caused a collapse in the water treatment plant Bedrichov, supplied by the water reservoir of Josefuv Dul (the Jizera Mountains, Czechia). The abundance of this cyanobacterium had attained more than 800,000 cells mL^{-1} over the past decade (Koza and Rederer, 2014).

This paper aims to address the challenges that *M. tenuissima* poses for water treatment by examining its traits and the potential for its removal. COM and cell characterisation are essential for understanding the treatability of *M. tenuissima*, and the subsequent optimization of the removal processes or even the evaluation of the level of coagulation interference. Furthermore, an influence of COM on coagulation of *M. tenuissima* cells was evaluated with the goal to test our hypothesis, that COM may enhance cell coagulation under appropriate conditions. Finally, based on the molecular interactions between cells, COM and the coagulant, we will indicate the optimum coagulation conditions and present novel data and insights into the impact of cyanobacterial COM on cell coagulation.

2. Material and methods

2.1. *Merismopedia tenuissima* cultivation

An inoculum of *M. tenuissima* (strain NIES-23) was obtained from the Microbial Culture Collection of the National Institute for Environmental Studies (NIES), Japan. As a culture medium, we used medium C buffered with Tris (hydroxymethyl)aminomethane (TRIS) at pH 7.5 (Ioki et al., 2012). The culture medium contained 210 mg L^{-1} of dissolved organic carbon (DOC), of which TRIS comprised consistently 95% during the cultivation, as proved by high-performance size exclusion chromatography (HPSEC) and UV-visible spectroscopy. The cultivation was performed in 500 mL Erlenmeyer flasks with 250 mL of culture volume (Palinska et al., 1996). The culture was shaken at 75 rpm in orbital apparatus (GFL Shaker 3020, Germany), maintained at 20 °C and exposed to a 12 h-light/12 h-dark cycle under light intensity of about 30 μmol m^{-2} s^{-1}, provided by a combination of cool-white (4200 K, 18 W, SunGlo, Hagen, Germany) and plant-colour fluorescent lamps (4900 K, 20 W, Sera, Germany). All materials and media were sterilised by autoclaving (a Tuttnauer autoclave-steam sterilizer 3850ELD, Israel); sterile conditions were maintained by air-filtration (air HEPA filter KS BESTFIL, KS Klima-Service, Czechia).

2.2. Growth monitoring

The *M. tenuissima* growth was monitored by chlorophyll-*a* (chl-*a*), optical density at 683 nm (OD_{683}), and concentration of extracellular organics released into culture media expressed as DOC_{EOM}. Chl-*a* was determined spectrophotometrically following the ISO 10260 standard procedure (ISO, 1992). OD_{683} was used as a surrogate parameter of cell counts, as it correlates well with the cell number (Knauer and Buffle, 2001; Pivokonsky et al., 2014) and was determined by a UV-vis 8453A spectrophotometer of high resolution (0.0001 AU, Agilent Technologies, USA) with a 1 cm quartz cuvette. The wavelength of 683 nm corresponded to the absorption maximum of *M. tenuissima* suspension matching maxima observed in Palinska et al. (1996). The OD_{683} response of the culture medium corresponded with the response of ultra-pure water and hence, was not taken into consideration. DOC_{EOM} was measured using a TOC-V_{CPH} analyser (Shimadzu, Japan), more details are explained by Safarikova et al. (2013). All the above-mentioned measurements performed in triplicate, mutually deviated by less than 5% and for DOC analyses by less than 2%. The growth curve was constructed based on two separate cultivating occasions and the presented data shows their arithmetic means.

Furthermore, by employing HPSEC, we determined the MW distributions of EOM in the exponential (EXP) and stationary (STAT) growth phases corresponding to the 11th and 23rd cultivation days, respectively (see Section 2.4.).

2.3. Cell harvesting and COM extraction

In the subsequent experiments, *M. tenuissima* cells were harvested at the beginning of the STAT phase (19th day) when the culture reached the values of chl-*a*~3 mg L^{-1} and OD_{683}~2.0. The cells were separated from the culture media by centrifugation (5200 rpm, 20 min) (a Centrifuge MPW-350, MED. Instruments, Poland) and were rinsed with ultra-pure. The cells were then centrifuged again, with the aim to remove a majority of EOM as well as medium residuals and finally were re-suspended in ultra-pure water. Cell concentrations for subsequent coagulation tests were determined by flow cytometry (see Section S3. in Supplementary Data – SD). COM was extracted using ultrasonication followed by filtration as described by Pivokonsky et al. (2014). The acquired COM sample was considered as a fraction comprising intracellular (IOM) and surface-retained organic matter (SOM) (Takaara et al., 2007), and possibly some tightly-bounded EOM as well.

2.4. Cell and COM characterisation

COM was characterised in terms of hydrophobicity, specific UV absorbance (SUVA), protein portion, apparent MW distribution, and charge properties by potentiometric titrations (see Section S2. in SD). Furthermore, cell surface charge was expressed by means of zeta potential (ZP) (see Section S2. in SD). The presented data

supports arithmetic means of the analyses carried out in triplicate. Overall measurement errors do not exceed 5%, for DOC analyses 2%.

The COM sample was divided into hydrophobic (HPO), transphilic (TPI), and hydrophilic (HPI) fractions using DAX-8 and XAD-4 resins in series, as described by Pivokonsky et al. (2014). Concentrations of the fractions were expressed as DOC portion. For the determination of SUVA, used as an indicator of aromaticity of the organics and expressed as a ratio of UV absorbance at 254 nm (UV_{254}) to DOC, UV_{254} was measured by the UV-vis 8453A spectrophotometer.

Proteinaceous DOC (DOC_P) was determined as the difference between total DOC (DOC_T) and DOC of non-proteinaceous compounds (DOC_{NP}) after precipitating the proteinaceous organic matter in COM by $(NH_4)_2SO_4$ as described by Safarikova et al. (2013).

The COM sample and its proteinaceous and non-proteinaceous components were fractionated using the Amicon Ultra-15 centrifugal filters of nominal molecular weight cut-offs of 100, 50, 30, 10, and 3 kDa (Millipore, USA) as described by Pivokonsky et al. (2014). The MW distribution was expressed as a proportional part of DOC.

Apparent MWs of proteinaceous COM (at unified DOC of 100 mg L^{-1}) were further determined by HPSEC (1260 Infinity, Agilent Technologies, USA) using the Agilent Bio SEC-5 100 Å and 300 Å columns connected in series and coupled with the diode array detector (DAD) operating at 280 nm. The system was calibrated with SEC protein standard mix (BEH450, Waters Corporation, USA) of MW range from 1355 Da to 1400 kDa. For more methodical details, we refer to Pivokonsky et al. (2015).

2.5. Application of jar tests

To investigate possible interactions between the *M. tenuissima* cells and related COM, and to clarify the probable effect of their simultaneous removal by coagulation, three types of standard jar tests were performed and compared:

1) Jar tests with individual components and a coagulant (ferric sulphate, $Fe_2(SO_4)_3 \cdot 9H_2O$, Sigma-Aldrich, USA), i.e. with COM concentrations of 3 (COM3), 5 (COM5) and 8 (COM8) mg L^{-1} as DOC or cell concentrations of 10^5 (cells10^5) and 10^6 (cells10^6) cells mL^{-1} (average for all tests: $4 \times 10^5 \pm 2 \times 10^4$ and $4 \times 10^6 \pm 3 \times 10^5$ cells mL^{-1}, OD_{683}~0.002 and 0.02, respectively);
2) Jar tests with COM/cell mixtures combining the above-mentioned concentrations and a coagulant;
3) Jar tests with the same COM/cell mixtures without a coagulant.

The selected DOC and cell concentrations are in compliance with the environmentally-relevant concentrations (Knauer and Buffle, 2001; Gonzalez-Torres et al., 2014).

First, to optimize coagulant doses, we performed jar tests with the individual components and coagulant doses ranging from 1 to 16 mg L^{-1} as Fe (~0.018–0.286 mmol L^{-1} Fe) at pH values 5.7 and 7.2. These pH values were previously testified as effective for COM (Pivokonsky et al., 2009) and cell removal (Gonzalez-Torres et al., 2014), respectively. To evaluate the effect of COM on cell removal and compare the tests with the individual components and COM/cell mixtures, the coagulant doses optimized for single COM were also used during the tests with the COM/cell mixtures.

Due to the dependence of the coagulation process on the reaction pH, coagulation performance was investigated by jar tests with the optimized coagulant doses in the pH range 3–8 and 2.5–6 for the tests with and without the coagulant, respectively. The target pH values were reached by the addition of predetermined amounts of HCl or NaOH to ultra-pure water with alkalinity pre-adjusted to 1.5 mmol L^{-1} by $NaHCO_3$.

The jar tests were carried out in 2 L jars using an eight-position variable-speed paddle stirrer (LMK 8-03, IH CAS, Czechia) at laboratory temperature. The operating conditions involved high-intensity homogenization (shear rate of 400 s^{-1}, 1 min) followed by low-intensity agitation (100 s^{-1}, 30 min) and centrifugation (3500 rpm, 20 min). The supernatants subject to measurements of pH, residual Fe, DOC and OD_{683}. Residual Fe was measured spectrophotometrically at 480 nm (UV-vis 8453A spectrophotometer) using thiocyanate colorimetric agent. DOC and OD_{683} were analysed as introduced in Section 2.2. Moreover, the samples derived under optimal reaction conditions were analysed in terms of apparent MW distribution (see Section 2.4. and Section S1. in SD) and cell counts by flow cytometry (see Section S3. in SD). Jar tests carried out in triplicate, exhibited very similar courses. Hence, for conciseness, we only report the results from one representative experiment.

3. Results and discussion

3.1. M. tenuissima growth

M. tenuissima growth expressed by chl-*a*, OD_{683} and DOC_{EOM} is depicted in Fig. 1. The growth curve over a 34-day cultivation period delineates four stages: lag, EXP, STAT, and decline phase, mutually distinguishable by changes in chl-*a* and OD_{683}. The lag phase lasted for only 2 days. A sharp increase in chl-*a* indicated the onset of the EXP phase that lasted until the 19th day of cultivation, when chl-*a* stopped increasing while OD_{683} continued to increase moderately until the 29th day. Based solely on chl-*a*, the STAT phase took about 9 days with the maximum of chl-*a* attaining 3 mg L^{-1}. The decline phase (chl-*a* decrease) started beyond the 28th day. Unlike chl-*a*, OD_{683} developed rather gradually and reached its maximum of 2.2 during the 29th day. In contrast to chl-*a* and OD_{683}, DOC_{EOM} grew almost linearly in the course of cultivation reaching its maximum of 273 mg L^{-1} on the 30th day (including initial 210 mg L^{-1} DOC of the culture medium). DOC_{EOM} course is in compliance with the gradual EOM release and cyanobacterial decay related to the excretion of COM (Henderson et al., 2008; Pivokonsky et al., 2014).

3.2. COM characterisation

M. tenuissima characteristics, such as hydrophilic character, protein portion, as well as MW diversity increasing with the culture age or prevailing negative ZP fully correspond with those of another cyanobacterial species *Microcystis aeruginosa*.

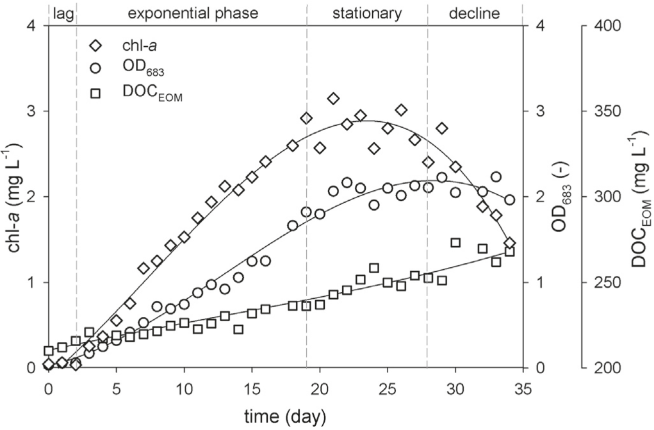

Fig. 1. Growth curve of *M. tenuissima* expressed as chl-*a*, OD_{683} and DOC_{EOM} in dependence on time.

As shown in Fig. 2a, COM of *M. tenuissima* contains predominantly HPI material (77% as DOC). The TPI fraction comprises 16% while the HPO fraction only 7%. The largely hydrophilic nature of COM is in agreement with the values reported for *M. aeruginosa*, where HPI fraction accounted for 87% (Pivokonsky et al., 2014). Moreover, the hydrophilic character of COM as analysed by the DAX/XAD fractionation is in accordance with the obtained low SUVA value (~0.5 L mg^{-1} m^{-1}). Similar SUVA values, i.e. 0.5 and 0.4 ± 0.2 L mg^{-1} m^{-1}, were also determined for COM of *M. aeruginosa* by Liao et al. (2015) and Pivokonsky et al. (2014), respectively.

In terms of proteinaceous content, COM of *M. tenuissima* comprised 43% proteinaceous (expressed as DOC_P) and 57% non-proteinaceous matter (DOC_{NP}) (Fig. 2b). Although cyanobacteria generally contain higher protein portion in comparison to green algae (Pivokonsky et al., 2014), the proteinaceous content of *M. tenuissima* COM is slightly lower compared to *M. aeruginosa* (DOC_P 63%) (Pivokonsky et al., 2014).

Both proteinaceous and non-proteinaceous cellular matter were divided into MW fractions of <3, 3–10, 10–30, 30–50, 50–100, and >100 kDa as illustrated in Fig. 2c. Proteinaceous COM includes mainly high-MWs (>100 kDa) accounting for 86% in terms of DOC. On the contrary, non-proteinaceous matter prevailed in low-MW fractions <3 kDa (41%) and 3–10 kDa (39%). The insignificant content of low-MW proteinaceous matter could be assigned to the limitations of the salting out method to isolate amino acids. This results in an overestimation of the non-proteinaceous low-MW portion (Pivokonsky et al., 2014).

Furthermore, the results of HPSEC analyses of COM and EOM samples at the EXP and STAT show that the MW diversity rose throughout the culture growth (Fig. 2d). While EOM consisted mainly of low-MWs <10 kDa, the MW distribution of COM ranged from less than 1.4 to more than 1400 kDa. These data are in accordance with Pivokonsky et al. (2014) who identified *M. aeruginosa* COM proteins of apparent MWs from approx. 1 to 1077 kDa.

3.3. Jar tests

3.3.1. Coagulation of individual components

In the test with coagulant doses ranging from 1 to 16 mg L^{-1} Fe, the highest removals were achieved in the ranges 2–16, 4–16 and 7–16 mg L^{-1} Fe for COM3/5/8, respectively, and 2–16 and 4–16 mg L^{-1} Fe for cells$10^5/10^6$, respectively. The obtained removals did not improve with increasing coagulant dose (up to 16 mg L^{-1} Fe). In the subsequent tests (at pH values ranging from 3 to 8), we used the coagulant doses of 3, 5 and 8 mg L^{-1} Fe for COM3/5/8, respectively, coincidently equal to 1 mg Fe mg^{-1} DOC, and of 3 and 5 mg L^{-1} Fe for cells $10^5/10^6$.

To clarify the effect of simultaneous coagulation on *M. tenuissima* cell and COM removal efficacy, we carried out jar tests starting with single components. In the tests with COM3/5/8, the maximum DOC removal rates increased from 43% to 53% with the increasing COM concentration. The optimum pH range extended slightly with the increasing COM concentration from pH 5.4–6.2 to 5.1–6.4 (Fig. 3a–b, Table S1 in SD). This pH range overlaps with the optimum for coagulation of *M. aeruginosa* COM determined by Pivokonsky et al. (2009).

In comparison to the single COM, the cells of *M. tenuissima* were coagulated much more effectively at higher pH values – within pH 6.0–7.7 and with maximum cell removal rates of 99% for both initial cell concentrations (Fig. 3c, Table S1). Comparably, high removal rates of algal or cyanobacterial cells were achieved in other studies. For instance, Gonzalez-Torres et al. (2014) reached even 99.7% removal of *M. aeruginosa* (initial $8 \times 10^5 \pm 4 \times 10^5$ cells mL^{-1}, at pH 6 and 7 using 1 and 7.3 mg L^{-1} Fe ($FeCl_3$), respectively). Similar rates were achieved in the studies which focused on harvesting algal biomass, in these cases at lower pH values and considerably higher coagulant doses. Garzon-Sanabria et al. (2013) reported nearly 100% flocculation efficacy of the marine microalga *Nannochloropsis salina* (initial 1×10^7 cells mL^{-1} ~ $OD_{750} = 3$, pH 5.3, 10 mg L^{-1} Al ($AlCl_3$)). Vandamme et al. (2012) then reached again nearly 100% yield of the green alga *Chlorella vulgaris* (0.25 g L^{-1} biomass concentration, pH 5.5, 20 mg L^{-1} Al ($Al_2(SO_4)_3 \cdot 18H_2O$)).

3.3.2. Coagulation of cells in presence of COM

Regarding the tests with COM/cell mixtures, the highest removals were achieved in the ranges 2–16, 3–16 and 4–16 mg L^{-1} Fe for both cell concentrations mixed with COM3/5/8, respectively. These doses were even lower than those for single COM coagulation. Nevertheless, we applied the same coagulant doses as for single COM3/5/8 (i.e. 3, 5 and 8 mg L^{-1} Fe) in order to investigate how COM affects the coagulation of cells.

As shown in Fig. 4, the highest removals of both cells and COM were reached at almost the same pH range as for single COM. This outcome suggests that COM contributed to the cell removal below pH 6, where single cells were not coagulated. Similarly to the single COM tests, the optimum pH range slightly widened with the

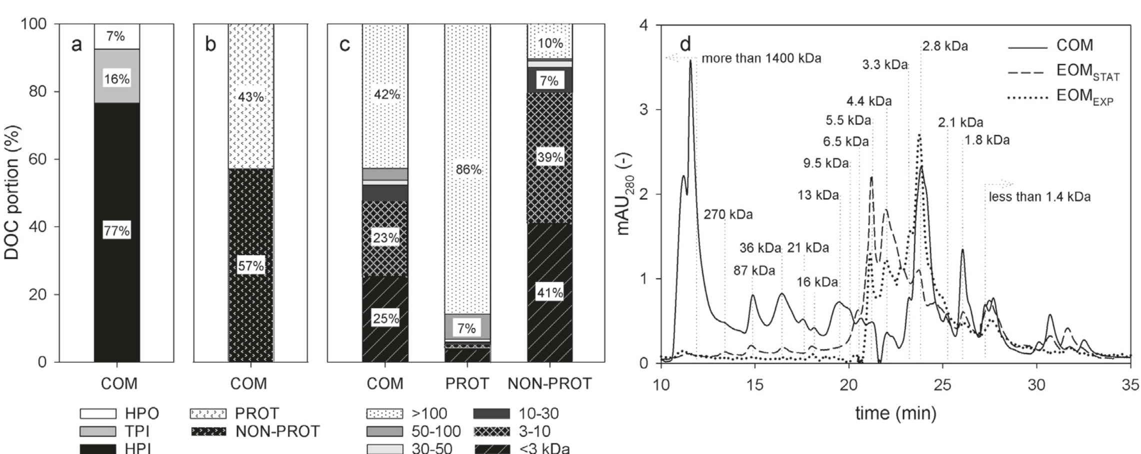

Fig. 2. Characterisation of *M. tenuissima* COM in terms of a) HPO/TPI/HPI fractions, b) (non)proteinaceous content, c) MW fractions expressed as DOC portion, d) apparent MW distribution of COM and EOM at EXP and STAT growth phases (sample DOC 100 mg L^{-1}).

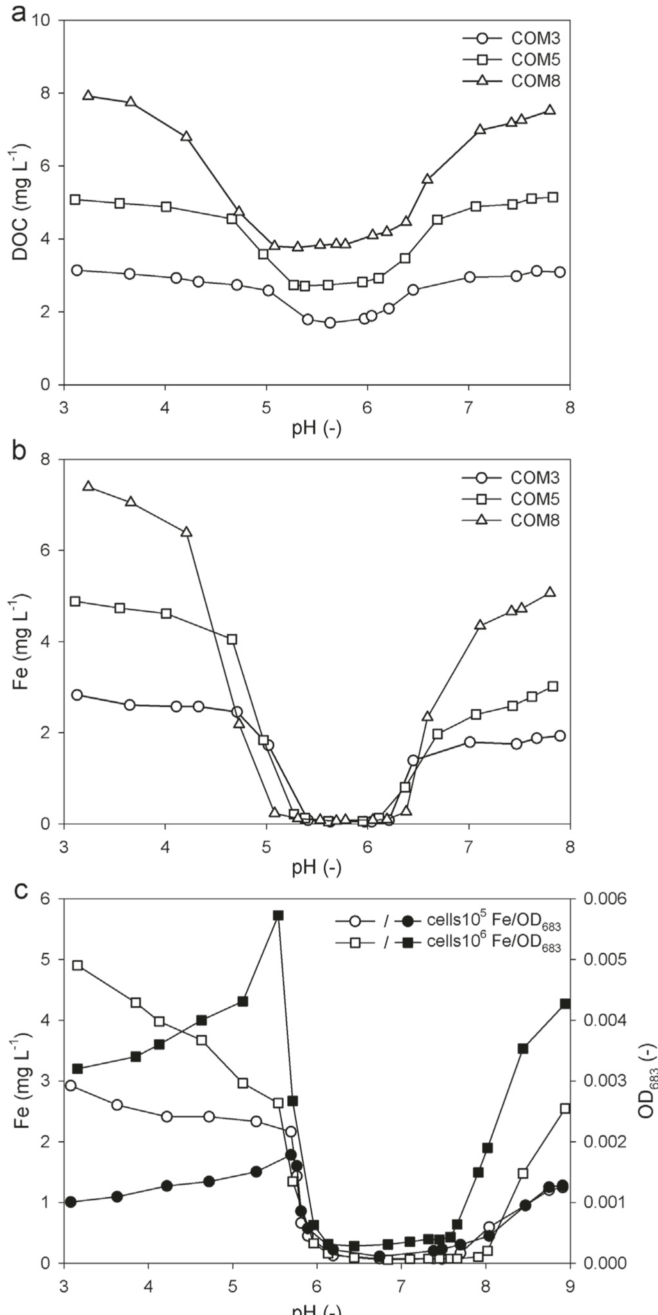

Fig. 3. Dependence of residual DOC, Fe and OD_{683} on pH value for coagulation of single COM of 3, 5 and 8 mg L^{-1} DOC (a, b) and single cells of 10^5 and 10^6 cells mL^{-1} (c).

increasing concentration of organics from pH 5.6–6.3 to 5.0–6.2 for COM3+cells10^5 and COM8+cells10^6, respectively. Furthermore, the achieved DOC removal rates were comparable to the single COM coagulation, i.e. 37–57% rising with the increasing initial COM concentration (Table S1). The high cell removal rates (up to 99%) remained unchanged. It should be noted that DOC concentrations slightly rose at acidic pH values, which may be attributed to the cell damage and COM release. This is supported by Zhang et al. (2012) who observed destroyed cells of *M. aeruginosa* already at pH 5.

Additionally, we observed a noticeable decrease of both DOC and OD_{683} at acidic pH for all COM/cell combinations. This phenomenon occurs at pH values 3.4–3.9, depending on the initial concentration of the organics (Fig. 4, Table S1). Up to 53% of DOC and 99% of cells was removed, which corresponds to the average efficiencies in the single components' tests. However, the residual Fe almost reached the initial coagulant concentrations, which indicates that the coagulant is probably not responsible for DOC/OD_{683}/cell reduction in the acidic pH region. This points to the existence of interactions between COM and cells that result in coagulation. This idea is also supported by the fact that a lower dosage of the coagulant was sufficient for coagulation of COM/cell mixtures compared to the single COM. To understand better these interactions, we also performed jar tests without a coagulant. These tests revealed that coagulation with no coagulant was effective at pH around 4 (Fig. 5, Table S1). Similarly to the experiments with the coagulant, COM removal rates rose with the initial DOC from 36% to 46% and cell removals again attained 99%. These results are in accordance with previously conducted studies by Safarikova et al. (2013) and Pivokonsky et al. (2015) which proved the ability of *M. aeruginosa* COM proteins to coagulate kaolin and humic substances, in the absence of a coagulant at similar pH values (<4.5 and < 4.0, respectively). A benefit of AOM release in consequence of pre-oxidation with chlorine was documented by Ma et al. (2012). Chlorine exposure of 1 and 4 mg L^{-1} increased DOC from 4.4 to 5.6 and 5.8 mg L^{-1} improving *M. aeruginosa* cell removal from 65% to 75% and to almost 95%, respectively (initial 2.2×10^6 cells mL^{-1}, pH 8.4, 4.1 mg L^{-1} Al ($AlCl_3 \cdot 6H_2O$)). In the case of ozone, the dose of 1.2 mg L^{-1} O_3 caused the DOC release of 0.8 mg L^{-1} reducing the green alga *Scenedesmus quadricauda* cell counts by 99% compared to 83% without pre-oxidation (initial 2×10^4 cells mL^{-1}, pH 6.5–6.8, 1 mg L^{-1} Al (PACl)) (Plummer and Edzwald, 2002).

3.4. DOC and cell removability

Cell stability is generally related to the electronegativity of the cell surface (Henderson et al., 2010; Ma et al., 2012). This explains the easy combination of algal cells with the commonly applied coagulants bearing a positive charge. *M. tenuissima* cells carry a negative charge within the entire studied pH range 3–9 with ZP values below −35 mV, for pH tending to 9 it continuously drops to nearly −60 mV. This decrease can be attributed to the gradual ionisation of surface-retained organic matter (SOM) bound onto cells surface (Takaara et al., 2007; Henderson et al., 2010).

On the contrary, COM comprises a wide spectrum of compounds exhibiting different removal efficiencies (Pivokonsky et al., 2009). The overall relatively low COM removal rates (less than 60% DOC) can be attributed to the predominance of HPI fraction (77% as DOC) in the cellular matter, since hydrophilic compounds are generally less prone to coagulation compared to hydrophobic matter (Ghernaout et al., 2010; Ma et al., 2012). The COM treatability corresponds to removal efficiencies observed for largely hydrophilic (57% or more) AOM derived from other species in the study by Henderson et al. (2010). AOM of *C. vulgaris*, *M. aeruginosa* and *Asterionella formosa* (diatom) at concentrations of 1.5 ± 0.15, 0.6 ± 0.01 and 1.0 ± 0.2 mg L^{-1} as C showed 71%, 55% and 46% removals, respectively. Furthermore, COM of *M. tenuissima* comprises a high portion of non-proteinaceous matter (57% DOC) of mainly low-MW organics (80% DOC <10 kDa). The compounds with low MWs proved to be less amenable to coagulation if they are either of saccharide (Bernhardt et al., 1985) or proteinaceous nature (Pivokonsky et al., 2012). This tendency was further confirmed for the proteinaceous part of COM by HPSEC analyses before and after coagulation, under suitable reaction conditions (Fig. S1). COM of MWs >10 kDa was entirely removed, while lower MWs remained uncoagulated. Interestingly, recorded HPSEC spectra of residual proteinaceous COM were almost identical for all types of jar tests, i.e. COM and COM/cell mixtures in presence and absence of a coagulant. This implies that dominantly high-MW (>10 kDa) COM

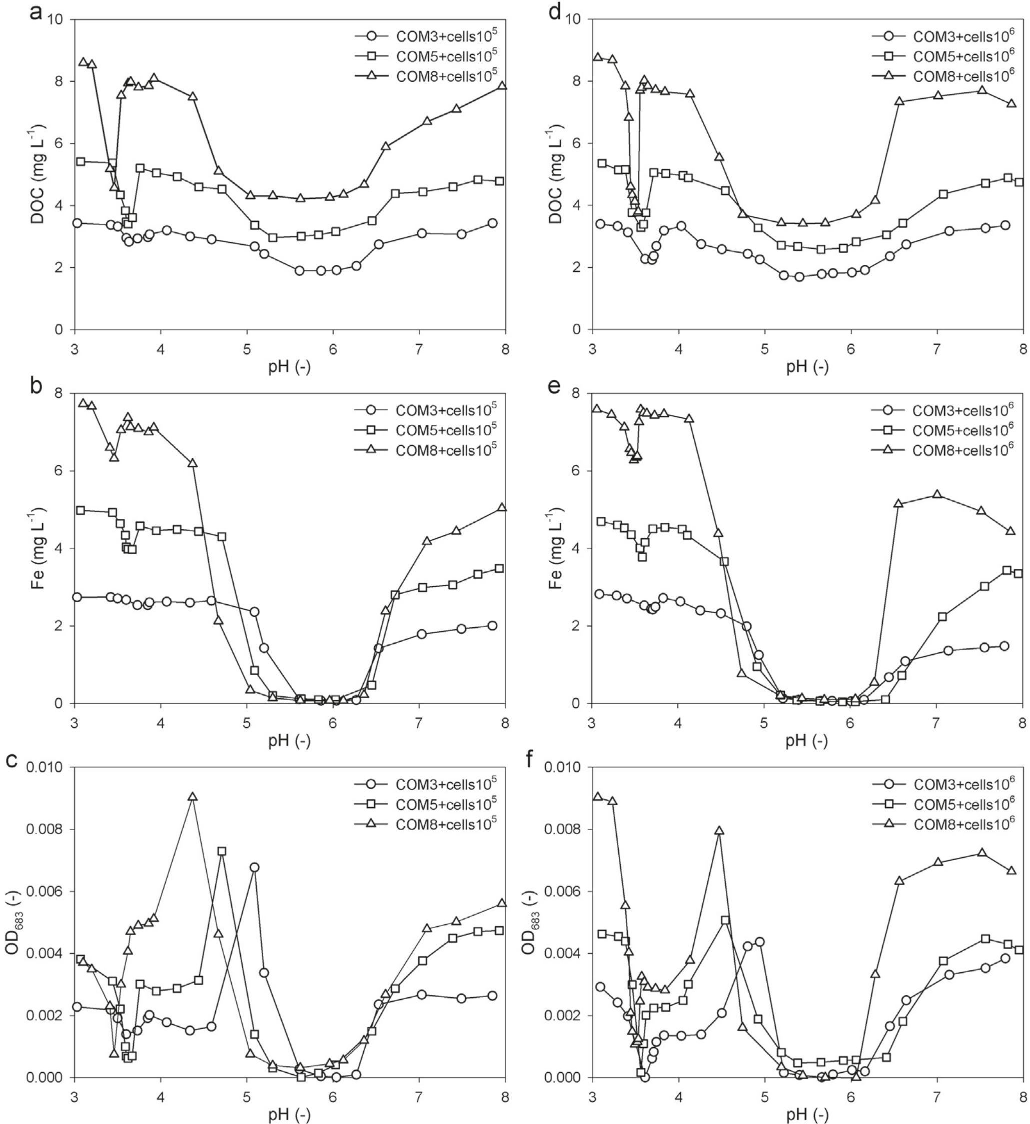

Fig. 4. Dependence of residual DOC, Fe and OD_{683} on pH value for coagulation of COM/cell mixtures of 3, 5 and 8 mg L^{-1} DOC and 10^5 (a, b, c) and 10^6 cells mL^{-1} (d, e, f).

participates in the interactions with cells.

3.5. Interaction mechanisms

What seems to be decisive for an effective coagulation is the charge distribution in the system (Bernhardt et al., 1985; Henderson et al., 2010; Pivokonsky et al., 2012).

At the optimal pH for COM removal (approx. 5–6), COM succumbs to gradual charge neutralisation (CN) by polynuclear cationic Fe-hydroxopolymers (Pivokonsky et al., 2012), since CN becomes significant at a slightly acidic pH and relatively low coagulant doses (e.g. pH 4–6, 0.01–1.0 mmol L^{-1} Fe for inorganic colloids) (Johnson and Amirtharajah, 1983; Duan and Gregory, 2003). At this pH, COM bears a significant amount of negatively charged functional groups, assigned to the dissociation of prevailingly carboxyl groups as derived from a titration curve depicted in SD (Fig. S2). Adding a coagulant results in its attraction with deionised functional COM groups and facilitation of COM destabilisation. Moreover, limited removal was obtained also at around neutral pH 6–7. At this pH, COM can adsorb onto the cationic surface of colloidal Fe-oxide-hydroxides, forming not only hydrogen or covalent bonds, but also facilitating ligand exchange (Bernhardt et al., 1985). Nevertheless, several studies (Bernhardt et al., 1985; Pivokonsky et al., 2009, 2012) showed that the extent of AOM removal by adsorption mechanism is usually less significant, and adsorption is effective only if the ratio between DOC and a coagulant is relatively low (e.g. <0.33 for Fe). As the presented coagulation tests were performed under DOC:Fe ratio ~1, we considered adsorption as a marginal coagulation mechanism. Within the given pH range (6–7), gradual restabilisation takes place instead; COM extensively occupies Fe-oxide-hydroxide surface, raises its negative charge density, and causes consequential charge reversal and stabilisation (Bernhardt et al., 1985; Pivokonsky et al., 2012).

To remove the cells, optimum pH conditions are found at about neutral pH (6–8), i.e. below or close to the Fe-oxide-hydroxides point of zero charge (pzc), attaining a slightly lower value than pH 8 (Duan and Gregory, 2003). These conditions indicate that the single cells preferably interacted with Fe-oxide-hydroxides. This phenomenon can be explained by the relatively large size of both cells and Fe-oxide-hydroxide particles. The Fe-oxide-hydroxides with moderately decreasing positive charge can adsorb onto the cell surface in accordance with the charge neutralisation

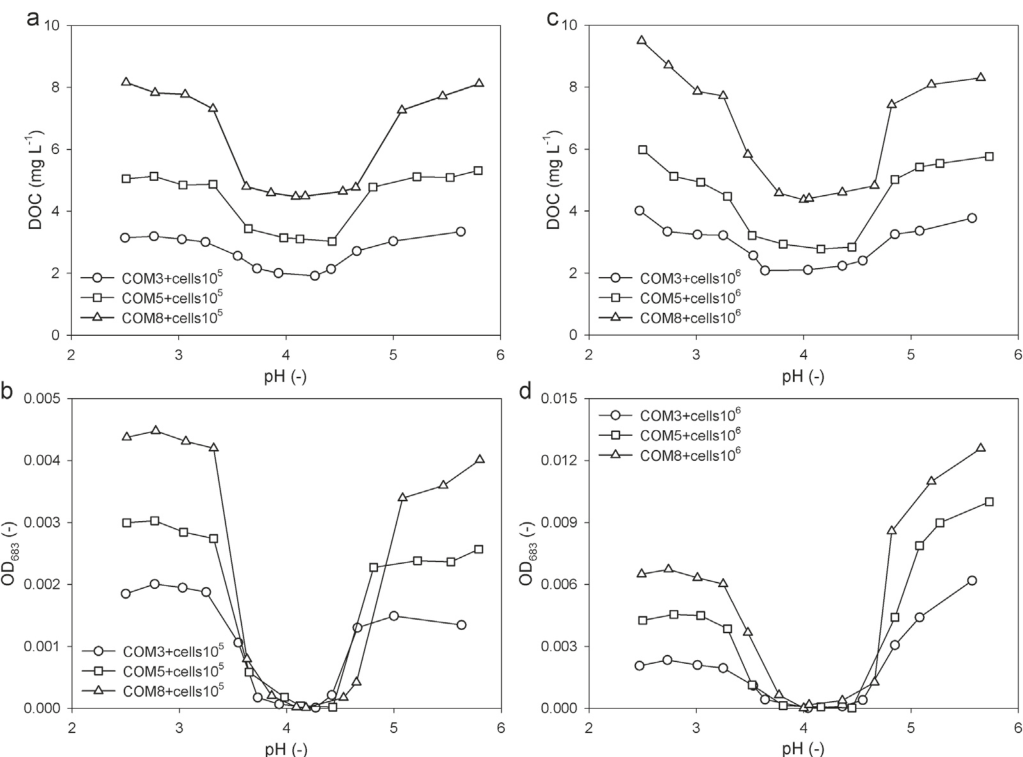

Fig. 5. Dependence of residual DOC and OD_{683} on pH value for coagulation of COM/cell mixtures of 3, 5 and 8 mg L^{-1} DOC and 10^5 (a, b) and 10^6 cells mL^{-1} (c, d) with no coagulant.

mechanism. On the other hand, relatively smaller Fe-hydroxopolymers would occupy the entire cell surface, stabilise it, and then prevent the particles from interacting. Although Garzon-Sanabria et al. (2013) reported the CN and sweep flocculation (SF) mechanisms at pH 5.3 using 50 mg L^{-1} $AlCl_3$ and Gonzalez-Torres et al. (2014) found the CN and CN-SF at pH 6 and 7 using 1 mg L^{-1} Fe or SF mechanisms at pH 6 and 7 using 7.3 mg L^{-1} Fe, it seems that SF tends to be more influential at higher coagulant doses and under neutral to alkaline pH (Johnson and Amirtharajah, 1983; Duan and Gregory, 2003).

Optimum coagulation of the COM/cell mixture is found around pH 5–6, for which both COM and cells are negatively charged. Therefore, they electrostatically interact with cationic Fe-hydroxopolymers. In addition, COM polyanions themselves probably attached the negative cell surface, thereby favouring agglomeration of the cells. The adsorption may involve electrostatic attractions, hydrogen or covalent bonding (Bernhardt et al., 1985). Ligand exchange between COM and cell surface functional groups as described for adsorption of EOM at quartz particle surfaces (Bernhardt et al., 1985) would be implausible in the case of biological surfaces. Due to covalent bound to carbon, the surface hydroxyl groups do not participate in the exchange (Campbell et al., 1997). Fe-hydroxopolymers may then bind to either vacant areas remaining on the cell surface or to COM bound to the cell surface. Particles charged this way can then aggregate through inter-particle bridging, when a positively charged area interacts with a negatively charged domain of another particle. Inter-particle bridging is particularly ascribed to high-MW COM (Henderson et al., 2010; Ma et al., 2012). These polymer chains (both adsorbed COM and SOM at the cell surface) may protrude from the cell surface, overcome the repelling electrostatic forces of particles, and thus trigger their aggregation (Bernhardt et al., 1985; Henderson et al., 2010; Ghernaout and Ghernaout, 2012). The enhancing effect of AOM on the particle removal was already reported by Bernhardt et al. (1985). Low EOM concentrations (0.1–2 mg L^{-1} C) of the green alga *Dictyosphaerium pulchellum* served as non-ionic and anionic polyelectrolytes and consequently improved flocculation of quartz particles and even decreased the coagulant demand. By contrast, increased EOM (>2 mg L^{-1} C) required a triplicate dose of iron ($FeCl_3 \cdot 6H_2O$). Similarly to harvesting *N. salina* at EOM carbohydrate concentration of 8–19 mg L^{-1} C (requiring 3-fold more $AlCl_3$ than for single cells, pH 5.3, 90% flocculation efficacy) (Garzon-Sanabria et al., 2013), Vandamme et al. (2012) needed almost 6-fold more alum (115 mg L^{-1} Al) to flocculate *C. vulgaris* in presence of EOM carbohydrates (5 mg L^{-1} C) (pH 5.5, 85% flocculation efficacy). Contrarily, our study proved that COM concentrations of 3–8 mg L^{-1} DOC also contribute to cell removal and favour the aggregation at relatively low Fe doses, under optimized reaction pH. This discrepancy of the above results can be assigned to the dissimilarities between EOM and COM, such as protein and non-protein content (Henderson et al., 2008; Pivokonsky et al., 2014). Moreover, the previous studies did not take into consideration AOM as the only coagulant and the effect of pH adjustment. Hence, they performed the tests at only one or two selected pH values and mostly considered the dependence of coagulant doses.

The high removal rate of both COM and cells for pH < 4.5 is supported by the studies on adsorption of humic/fulvic acids on algal cell surfaces. Campbell et al. (1997) and Knauer and Buffle (2001) observed an increased accumulation of the organic matter at the cell surface when the pH decreased to 4. This effect probably resulted from the amphoteric character of COM as indicated by the titration curve (Fig. S1). If the pH drops close to or below the dissociation constants of carboxyl groups, COM is progressively protonated and as a consequence, the electrostatic repulsions with cells decrease. COM is then attached to the cells and reduces their still negative net surface charge. Campbell et al. (1997) proposed adsorption mechanisms of humic/fulvic acids onto algal cell surface through electrostatic interactions and hydrogen bonding, which

can also be applied in the case of hydrophilic functional groups, present in COM and on the cell surface. Such charged particles may further interact by inter-particle bridging and enable an aggregation as discussed above. Campbell et al. (1997) further discussed the potential of hydrophobic bonds. However, considering the highly hydrophilic COM nature, in contrast to the hydrophobic humic/fulvic substances, this interaction would require substantial alteration of COM conformation. This is why hydrogen bonding seems to be a more plausible mechanism.

For a pH exceeding the optimum, all components turn gradually more negative, which results in electrostatic repulsions in the system. The sharp increase of OD_{683}, observed in the tests with single cells at pH around 5.5 and in the tests with COM/cell mixtures at pH 4.5–5.0, is attributable to the influence of iron on OD_{683}. At this given pH, Fe progressively hydrolyses and forms initially stable colloidal precipitates (Duan and Gregory, 2003). The increase in colloidal Fe raises OD_{683} following the decrease of residual Fe. With the pH approaching the pzc, the Fe particles become less stable which evokes their aggregation. This explanation is supported by the analogous behaviour of alum, whose stable precipitates increased turbidity at about pH 4.5 as seen in the study involving hydrolytic reactions of alum (Van Benschoten and Edzwald, 1990).

4. Conclusion

M. tenuissima COM significantly affects the optimum pH for cell coagulation and interaction mechanisms. While the cells preferably adsorb onto Fe-oxide-hydroxides at about neutral pH (6.0–7.7), the COM/cell mixtures undergo charge neutralisation by Fe-hydroxopolymers within moderately acidic pH (5.0–6.5). Although elevated EOM concentrations have been reported to disturb coagulation and increase coagulant demand, our findings demonstrate that COM serves as a cationic polymer flocculation aid in *M. tenuissima* cell suspension. In addition, the simultaneous coagulation slightly lowers the coagulant doses under optimized pH conditions. This supportive effect arises mainly from the inter-particle bridging of organic matter attributable to the involvement of particularly high-MWs organics (>10 kDa) in the adsorption processes. COM may bridge cells mainly on the basis of hydrogen bonds and electrostatic interactions. These attractive forces induce COM/cells coagulation even in the absence of a coagulant at low pH (<4). Nevertheless, to ensure an efficient treatment of algal-laden waters, we suggest adjusting the reaction pH to the interval 5–6, since the coagulation of cells at more acidic pH may disrupt the cell integrity. However, COM/cell simultaneous coagulation/flocculation could be applicable to the flocculation-based harvesting of algal cultures for e.g. biofuel purposes. Its potential for exploiting this mechanism at WTPs or bio-resource technologies will thus require further investigation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.05.070.

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Supplementary Data for

An application of cellular organic matter to coagulation of cyanobacterial cells (*Merismopedia tenuissima*)

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This supplementary data contains the following sections, figures and a table:
S1. MW distribution
S2. Charge characterisation
S3. Cell counting
Fig. S1 Apparent MWs distribution of *M. tenuissima* COM before and after coagulation.
Fig. S2 Titration curve for *M. tenuissima* COM.
Table S1 Optimum coagulation conditions and removal rates for COM and/or cells of *M. tenuissima*.

S1. MW distribution
Prior to the MW analyses, the samples obtained after coagulation were concentrated with a rotary evaporator (23 °C, 1.9 kPa) (Laborota 4002 control HB/G1, Heidolph Instruments, Germany) to reach unified DOC of 100 mg L^{-1} convenient for HPSEC performance. Peaks of low-MW compounds were found to be higher after coagulation and pre-concentration, in comparison to the raw COM sample (**Fig. S1**). This could be explained by the fact that the process of coagulation changed the content and ratio of proteinaceous compounds of different MWs in the samples.

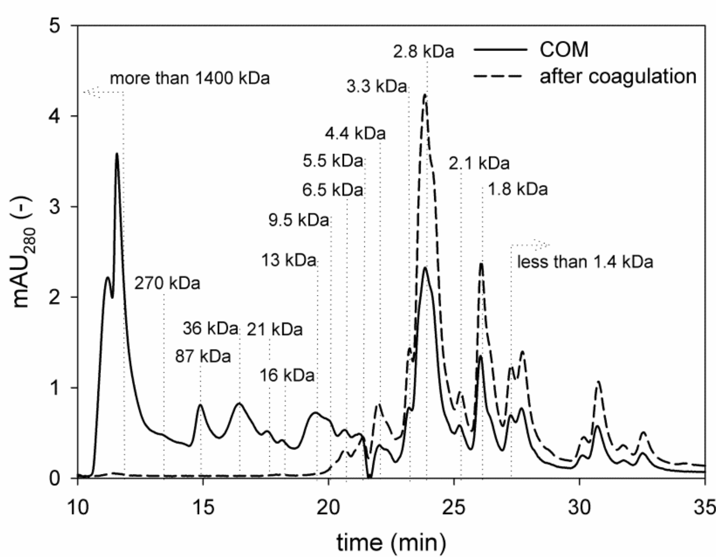

Fig. S1 Apparent MWs distribution of *M. tenuissima* COM before and after coagulation (sample DOC 100 mg L^{-1}).

S2. Charge characterisation

Zeta potential measurement

The cell suspension at the stationary growth phase was subjected to zeta potential measurement by a Zetasizer Nano-ZS (Malvern, UK) in a model environment (1 mM KCl, pH 2-9) at 25 °C. All samples were measured six times with the deviation lower than 10%.

Potentiometric titration

The pH-dependent charge conditions of COM were expressed as the number of the functional groups present, that are able to release or accept a proton depending on pH and therefore bear a charge. These functional groups were quantified by potentiometric titration according to the methodology described in Safarikova et al. (2013). The COM sample (DOC 200 mg L^{-1}) measured at volume of 500 mL was adjusted to an electrolyte concentration of 0.05 M NaCl and pH value of 11 by NaOH. The solution was then titrated to pH 2 using HCl. The amount of titratable acidic groups present in COM was determined as the difference between the COM and the blank titration curve. The pH value was measured using a pH meter SevenCompact^{TM} pH/Ion (electrode InLabR Expert Pro, Mettler Toledo, Switzerland).

The chemical heterogeneity of the complex COM mixture complicates the attribution of detected dissociation constants (pK_a) and equivalence points (pEq) of specific functional groups. Their identification as well as quantification was beyond the scope of this study. As can be observed on the presented titration curve (**Fig. S2**), four buffer regions were identified. The charge equivalent of HCl added at the moderately basic pH range (11.0-8.1 around pK_4=9.8) was assigned to the number of functional groups dissociating in alkaline pH, such as –OH, $–NH_3^+$, $=NH_2^+$, and –SH (Chang, 2005). The change of charge in the neutral-acidic pH range 8.1-2.0 with pK_a=6.4, 3.0 and 2.4 could be entirely attributed to the presence of a carboxyl region (e.g. proteinaceous aspartic acid with terminal α- and side β-COOH of pK_a=2.09 and 3.86 and glutamic acid with α- and γ-COOH of pK_a=2.19 and 4.25 (Chang, 2005) or of saccharide alginic acid, composed of poly-D-mannuronic and L-guluronic acid with –COOH of pK_a=3.38 and 3.65 respectively (Haug, 1961)).

The COM titration curve shows that 1 g DOC contains almost 15 mmol of –COOH dissociating in the pH range 2.0-8.1, and about 4 mmol of basic functional groups dissociating in the pH range 8.1-10.6, depending on their respective pK_a values. Compared to our data for the entire COM, Kopecka et al. (2014) recorded for proteinaceous COM of *M. aeruginosa* nearly 10 mmol of titratable carboxyl groups per 1 g DOC in the pH range 7.1-1.5. Similarly, alginate, a polysaccharide produced by some algae and bacteria, was also estimated to contain about 10 mmol of carboxyl groups per 1 g C (Gregor et al., 1996).

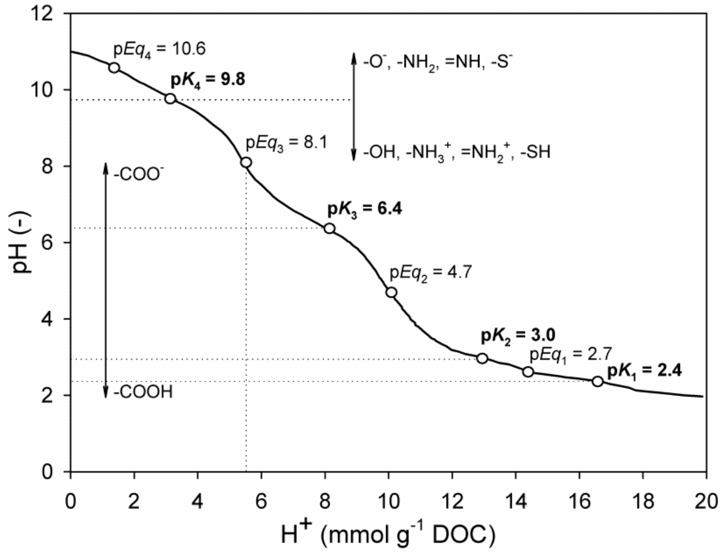

Fig. S2 Titration curve for *M. tenuissima* COM with dissociation constants (pK_1, pK_2, pK_3, pK_4) and equivalence points (pEq_1, pEq_2, pEq_3, pEq_4) assigned to different functional groups.

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S3. Cell counting

The initial cell concentration as well as the residual cell counts measured after the coagulation tests, performed under optimised reaction conditions, were analysed via flow cytometry by means of an Accuri C6 cytometer (BD Accuri Cytometer Inc., USA) equipped with the Solid State Blue Laser (20 mW, 488 nm). To distinguish desirable particles from the background, we used a combination of red fluorescence channel (FL4, 675±12.5 nm), forward scatter (FSC, determining volume parameter) and side scatter (SSC, describing morphological complexity). The sample flow rate was set at 10 µL min^{-1}. To check operational consistency, we used standard CountBright™ Absolute Counting Beads (7 µm) (Thermo Fischer Scientific, USA). The measurement deviation was found to be less than 10% within the triplicate analysis.

Table S1 Optimum coagulation conditions and removal rates for COM and/or cells of *M. tenuissima* with and without a coagulant.

Jar test	COM/cell concentration*	optimum pH (-)	max DOC removal rate (%)**		max cell removal rate (%)†	
single COM	3	5.4-6.2	43		-	
	5	5.3-6.1	46		-	
	8	5.1-6.4	53		-	
single cells	10^5	6-7.7	-		99	
	10^6	6-7.7	-		99	
COM/cell mixtures	$3/10^5$	5.6-6.3	37	5#	98	99#
	$5/10^5$	5.3-6.4	40	32#	99	99#
	$8/10^5$	5.0-6.4	47	43#	99	98#
	$3/10^6$	5.2-6.2	44	25#	99	99#
	$5/10^6$	5.2-6.4	48	34#	99	99#
	$8/10^6$	5.0-6.2	57	53#	99	99#
COM/cell mixtures without Fe	$3/10^5$	3.7-4.4	36		97	
	$5/10^5$	3.7-4.5	40		98	
	$8/10^5$	3.7-4.6	44		97	
	$3/10^6$	3.6-4.4	31		99	
	$5/10^6$	3.7-4.5	44		99	
	$8/10^6$	3.8-4.4	46		99	

*Initial COM (mg L^{-1} DOC) and/or cells concentrations (cells mL^{-1}).

**Removal rates calculated as a DOC percentage ratio before and after the coagulation.

†Cell removal rates gained from flow cytometry calculated as a percentage ratio before and after the coagulation under optimum conditions.

#Removal rates gained at pH <4.

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Cover – Cells of the cyanobacterium *Merismopedia tenuissima* under a light microscope

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