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Coagulation of organic matter produced by phytoplankton

Ph.D. Thesis

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Prohlášení

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In Prague 2014

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Jana Načeradská

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ABSTRACT

This dissertation thesis focuses on the removability of algal organic matter (AOM) by coagulation during water treatment and also on the influence of AOM on the coagulation of other substances present in source water. Special emphasis is put on the description of coagulation mechanisms.

The effectiveness of AOM removal by coagulation was investigated by coagulation tests performed with optimized doses of coagulants (aluminium or ferric sulphate) under different pH values. Peptides and proteins contained in cellular organic matter of cyanobacterium *Microcystis aeruginosa* were used in the experiments since they have been previously reported to disturb the coagulation process. Moreover, peptides and proteins underwent coagulation experiments together with kaolin particles, representing clay particles in turbid waters, in both the presence and absence of coagulants to investigate the effect of AOM on the coagulation of turbid waters. To enable the description of coagulation mechanisms, AOM were characterised in terms of charge, functional groups, molecular weight and ability to form dissolved complexes with coagulant metals.

The experimental results demonstrated that the removability of peptides and proteins is greatly dependent on pH value and on the properties of the involved particles or molecules. The highest removal rates were achieved at such pH values, at which coagulants form positively charged hydroxopolymers that interact with negatively charged functional groups of peptides and proteins (pH 5 - 6.5 for aluminium and 4 - 6 for ferric coagulant). Similar results were achieved in the case of experiments with peptides and proteins and kaolin particles, during which amphoteric peptides and proteins interacted with both kaolin and coagulant hydroxopolymers, and peptides and proteins were shown to contribute to the coagulation of kaolin particles. The negative impact of peptides and proteins on the coagulation by formation of complexes with coagulants was observed, though at a narrow pH range (about 6.8 for Al and 6 for Fe). Furthermore, it was shown that high-molecular weight (MW) proteins (MW > 10 kDa) are easily removable by coagulation, whereas adsorption onto activated carbon is appropriate for the removal of low-MW peptides.

To conclude, coagulation was shown to be effective in removing high-MW AOM under optimised reaction conditions, and to be a useful pre-treatment step before technologies that are able to remove low-MW organic compounds.

ABSTRAKT

Překládaná disertační práce se zabývá odstranitelností organických látek produkovaných řasami a sinicemi (AOM - algal organic matter) koagulací při úpravě vody a také vlivem AOM na koagulaci dalších látek přítomných v surové vodě. Zvláštní důraz je kladen na popis mechanismů koagulace.

Účinnost odstranění AOM koagulací byla posuzována za pomoci koagulačních testů prováděných s optimalizovanými dávkami koagulačních činidel (síranu hlinitého nebo železitého) při různých hodnotách pH. V experimentech byly použity peptidy a proteiny obsažené v buněčném materiálu sinice *Microcystis aeruginosa*, o kterých bylo již dříve zjištěno, že narušují koagulační proces. Za účelem posouzení vlivu AOM na koagulaci zakalených vod byly dále provedeny koagulační testy s peptidy a proteiny společně s kaolinovými částicemi, reprezentujícími jílové částice v zakalených vodách, jak s koagulačními činidly, tak bez nich. Aby bylo možné popsat koagulační mechanismy, AOM byly charakterizovány z hlediska náboje, funkčních skupin, molekulových vah a schopnosti tvořit rozpuštěné komplexy s koagulačními činidly.

Výsledky experimentů ukázaly, že odstranitelnost peptidů a proteinů je značně závislá na hodnotě pH a vlastnostech koagulujících částic či molekul. Nejvyšší míry odstranění bylo dosaženo při takových hodnotách pH, při kterých koagulační činidla tvoří kladně nabité hydroxokomplexy interagující se záporně nabitými funkčními skupinami peptidů a proteinů (pH 5 - 6,5 pro hlinitý a 4 - 6 pro železitý koagulant). Obdobných výsledků bylo dosaženo i v případě experimentů s peptidy a proteiny a kaolinovými částicemi, v průběhu kterých amfoterní peptidy a proteiny interagovaly jak s kaolinem tak s hydroxopolymery koagulačních činidel a přispívaly tak ke koagulaci kaolinových částic. Bylo zjištěno, že peptidy a proteiny negativně ovlivňují koagulaci tvorbou komplexů s koagulačními činidly, nicméně pouze v úzkém rozsahu pH (okolo 6,8 v případě Al a 6 v případě Fe). Dále bylo prokázáno, že vysokomolekulární proteiny (s molekulovou hmotností nad 10 kDa) jsou snadno odstranitelné koagulací, zatímco nízkomolekulární peptidy je možné odstranit adsorpcí na aktivním uhlí.

Lze tedy říci, že koagulace je za vhodně nastavených reakčních podmínek účinným nástrojem pro odstraňování vysokomolekulárních AOM a může být vhodným krokem před použitím technologií odstraňujících nízkomolekulární organické látky.

CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

a_m	Langmuir parameter of adsorption capacity at maximum
AC	Activated carbon
AOM	Algal organic matter
b	Langmuir constant
BET	Brunauer-Emmett-Teller method
BJH	Barrett-Joyner-Halenda method
BC	Binding capacity
C_0	Initial concentration of adsorbate
C_e	Equilibrium concentration of adsorbate
C_t	Concentration of adsorbate at specific time
CC	Cell counting
CHPI	Charged hydrophilic fraction
COM	Cellular organic matter
CV	Coefficient of variation
d	Diameter
DAD	Diode array detector
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DOC_{NT}	Non-protein dissolved organic carbon
DOC_P	Protein dissolved organic carbon
DOC_T	Total dissolved organic carbon
DOM	Dissolved organic matter
EC	Electrical conductivity
EOM	Extracellular organic matter
ESI+	Positive electrospray ionization
FTL	Filtrasorb TL830
GAC	Granular activated carbon
GSM	Geosmin
HAA	Haloacetic acid
HPI	Hydrophilic fraction
HPLC	High performance liquid chromatography
HPO	Hydrophobic fraction
HPSEC	High performance size exclusion chromatography
IC	Inorganic carbon
IEF	Isoelectric focusing
IOM	Intracellular organic matter
IS	Ionic strength
K_f	Freundlich parameter of adsorption capacity
k_1, k_2	Kinetic rate constants
LC/MS/MS	Liquid chromatography with mass spectrometry
m	Adsorbent dose/mass
MC	Microcystin
MC-LR	Microcystin-LR
MC-RR	Microcystin-RR
MC-YR	Microcystin-YR

MF	Microfiltration
MIB	2-methylisoborneol
MH	Molekulová hmotnost
MW	Molecular weight
n	Freundlich constant
N_r	Number of a specific functional group
N_t	Normality of titration agent
NHPI	Neutral hydrophilic fraction
NOM	Natural organic matter
NTU	Nephelometric turbidity unit
N1240	Norit 1240
OD	Optical density
p	Adsorbate pressure
p_0	Saturated vapour pressure of adsorbate
pE	Equivalence point
pI	Isoelectric point
pI_T	Total isoelectric point
pK_a	Acid dissociation constant
PAC	Powdered activated carbon
PACl	Polyaluminium chloride
pH	Potential of hydrogen
pH_{pzc}	pH of the point of zero charge
PIC	Picabiol 12x40
PSD	Pore size distribution
Q	Surface charge
q_e	Adsorbed amount at equilibrium
q_t	Adsorbed amount at specific time
r	Correlation constant
R^2	Coefficient of determination
RPM	Revolutions per minute
S_{BET}	Specific surface area measured by the Brunauer-Emmett-Teller method
S_{meso}	Surface area of mesopores
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SOM	Surface-retained organic matter
SUVA	Specific ultraviolet absorbance
T	Time
Tu	Turbidity
TBA	Terbuthylazine
TC	Total carbon
THM	Trihalomethane
TOC	Total organic carbon
TON	Total organic nitrogen
T&O	Taste and odour
TPI	Transphilic fraction

UF	Ultrafiltration
UV_{254}	Ultraviolet absorbance at 254 nm
UV/VIS	Ultraviolet-Visible Spectroscopy
V	Solution volume
V_0	Initial volume of titration agent
V_{micro}	Volume of micropores
V_t	Volume of titration agent at specific time
V_{total}	Total pore volume

1. INTRODUCTION

Natural organic matter (NOM) is ubiquitous in rivers and reservoirs supplying drinking water treatment facilities and represents one of the major impurities which have to be removed by water treatment process. NOM is a complex mixture of organic compounds that originate from allochthonous input dominated by humic substances (mostly humic and fulvic acids) derived from vegetative debris from terrestrial sources within a catchment and autochthonous input including mainly compounds derived from algae and cyanobacteria, i.e. algal organic matter (AOM). During the summer season, when algae can dramatically increase populations on relatively rapid timescales, AOM may comprise an essential part of NOM in surface waters and consequently affect the treatment processes (Ozawa et al., 2005; Dyble et al., 2008; Henderson et al., 2008a; Zhang et al., 2010a; Li et al., 2011). As well as humic substances, 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) (Leenheer and Croué, 2003). Moreover, AOM may contain harmful cyanobacterial toxins (Harada, 2004).

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 dissolved AOM. It arises extracellularly via metabolic excretion, forming extracellular organic matter (EOM) or when algal cells die and subsequently lyse, forming cellular organic matter (COM). COM is released during the population growth and decline (Nguyen et al., 2005; Pivokonsky et al., 2006) and also during the water treatment process, especially when pre-oxidation methods that induce the cell rupture are used (Ma and Liu, 2002; Ma et al., 2012; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013). The highest concentrations of dissolved AOM are present in raw water after the collapse of an algal bloom in the form of COM released from damaged cells.

The increased concentrations of AOM in raw water can seriously impair water treatment process efficiency (Ozawa et al., 2005; Dyble et al., 2008; Henderson et al., 2008a; Zhang et al., 2010a; Li et al., 2011) and may even lead to the collapse of water treatment plant (Zhang et al., 2010a). 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., 2012), membrane fouling (Amy, 2008; Qu et al., 2012; Zhang et al., 2013a, b; Zhou et al., 2014b), decreased adsorption efficiency for micropollutants and low-molecular weight

compounds onto activated carbon (Hnatukova et al., 2011), and disinfection by-product formation (Nguyen et al., 2005; Fang et al., 2010; Lui et al., 2011; Yang et al., 2011; Li et al., 2012; Wert et al., 2013).

2. LITERATURE REVIEW

2. 1. Algal organic matter

The AOM comprises a wide range of organic compounds, such as saccharides and polysaccharides, nitrogen-containing compounds (amines, amino sugars, amino acids, peptides, proteins, nucleic acids), various organic acids (e.g. glycolic acid, uronic acids), alcohols, aldehydes, ketones, lipids and fatty acids (Hellebust, 1974; Fogg, 1983; Leenheer and Croué, 2003; Huang et al., 2007b). Studies that performed algae cultivation under laboratory conditions showed that the AOM composition 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; Henderson et al., 2008a; Leloup et al., 2013). It should be noted that the amount and character of organics released by algal cells into the surrounding environment is influenced by a range of factors, such as nutrient status or the presence of toxic substances (Hino, 1988; Myklestad, 1995; Wilhelm, 1995; Benderliev, 1999; Huang et al., 2007b; Mohamed, 2008; Huang et al., 2012; Kong et al., 2014). For instance, the study of Mohamed (2008) showed that green algae *Chlorella vulgaris* and *Scenedesmus quadricauda* released an increased amount of polysaccharides as a protective response against oxidative stress induced by cyanobacterial microcystins.

Several AOM properties were reported to be important from the perspective of water treatment, specifically hydrophobicity, SUVA, surface charge, molecular weight (MW), and content of saccharide-like and protein-like substances (Pivokonsky et al., 2006; Henderson et al., 2008a). These properties have a substantial effect on the tendency of organic substances derived from algae to interact between themselves, with coagulants, with other particles and molecules present in raw water, with activated carbon and membrane surfaces, with ion exchange resins and also with disinfectants. The AOM properties then influence not only removability of AOM by water treatment processes, but also the working life of adsorbers and membranes as well as the procedures used to restore the system productivity, such as membrane backwashing and chemical cleaning.

2.1.1. Hydrophobicity

Most studies that investigated AOM hydrophobicity divided this matter into three fractions: hydrophobic (HPO), hydrophilic (HPI) and transphilic (TPI) according to the methodology developed for NOM of humic character (Aiken et al., 1992; Malcolm and MacCarthy, 1992; Croué et al., 1993; Bolto et al., 1998; Carroll et al., 2000). Some of them also divided HPI fraction into neutral hydrophilic (NHPI) and charged hydrophilic (CHPI) fractions (Li et al., 2011; Li et al., 2012). The theory of the fractionation is based on an assumption that some group of organic compounds (e.g. humic acids or proteins) attaches itself only or mostly to a one type of ion exchange resin (anion or cation) under given pH value. It is believed that HPI fraction contains carbohydrates, hydroxy acids, low-MW carboxylic acids, amino acids, amino sugars, peptides, low-MW alkyl alcohols, aldehydes and ketones, while the HPO fraction includes hydrocarbons, high-MW alkyl amines, high-MW alkyl carboxylic (fatty) acids and aromatic acids, phenols and humic substances (Edzwald, 1993; Penru et al., 2013). The TPI fraction, which usually comprises only a small portion of AOM, is assumed to contain weakly hydrophobic compounds, such as fulvic acids (Carroll et al., 2000).

It was shown that AOM is predominantly hydrophilic (Henderson et al., 2008a; Huang et al., 2012; Li et al., 2011; Li et al., 2012; Qu et al., 2012; Chon et al., 2013; Leloup et al., 2013; Zhou et al., 2014a) and COM contains larger portion of HPI fraction and smaller portion of HPO fraction than EOM does (Li et al., 2012; Zhou et al., 2014a). For example, Li et al. (2012) found that COM of *Microcystis aeruginosa* contains 86% of the HPI fraction (76% of NHPI and 10% of CHPI), while the HPI compounds of its EOM accounted for 63% (53% of NHPI and 10% of CHPI). These results compare well with the findings of Li et al. (2011) that cyanobacterial EOM (species not reported) contained 79% of the HPI fraction (57% of NHPI and 22% of CHPI).

Some authors determined polysaccharide-like and protein-like substances, which form the majority of AOM, in all three fractions (HPI, HPO and TPI) (Qu et al., 2012; Zhou et al., 2014a). It is probably given by the fact that both polysaccharides and proteins differ in properties like charge, isoelectric point or content of acid/basic/polar functional groups and are therefore captured by different ion-exchange resins. Moreover, the study by Lui et al. (2011) assumed that AOM contains both hydrophilic and hydrophobic proteins. The hydrophilic ones are represented by cytosolic proteins soluble in water, while hydrophobic ones include membrane proteins which are characterised by hydrophobic domains that

transverse membranes and are difficult to solubilize (practically insoluble) in aqueous solutions (Molloy et al., 1998). It is therefore unclear to what extent these hydrophobic proteins could be present in dissolved AOM.

2.1.2. SUVA

AOM was found to exhibit low values of specific UV absorbance (SUVA) which is in accordance with AOM low hydrophobicity (Nguyen et al., 2005; Henderson et al., 2008a; Fang et al., 2010; Huang et al., 2012; Li et al., 2012). SUVA is defined as the UV absorbance of a water sample at a given wavelength normalised for dissolved organic carbon (DOC) concentration. It reflects the content of aromatic structures and conjugated double bonds in organic matter and correlates with the hydrophobicity of organic molecules (Leenheer and Croué, 2003). SUVA can be a useful parameter for estimating the dissolved aromatic carbon content in aquatic systems and thus the chemical nature of the DOC at a given location. While aromatic structures are quite numerous in humic substances, they are very rare in AOM. It is generally accepted that: (1) SUVA of about 4 or greater indicates the NOM is dominated by aquatic humic matter, (2) SUVA of 2 – 4 indicates the NOM is composed of a mixture of aquatic humic matter, EOM and COM from algae, and (3) SUVA < 2 indicates the NOM is composed largely of algal derived EOM and COM (Edzwald and Tobiason, 1999).

The study by Henderson et al. (2008a) showed that SUVA values determined for EOM are dependent on the growth phase of algae and cyanobacteria. EOM obtained during the exponential growth phase had a SUVA of 1.7 m^{-1} mg^{-1} L for cyanobacterium *Microcystis aeruginosa* and diatom *Asterionella formosa* and 1.5 m^{-1} mg^{-1} L for green alga *Chlorella vulgaris*, while stationary phase EOM had a SUVA of 0.5 m^{-1} mg^{-1} L for all three species. Similarly low SUVA values were determined also for COM (Fang et al., 2010; Li et al., 2012).

2.1.3. Charge

Charge is another property which predetermines the interactions, especially electrostatic ones, of organic molecules with other substances. EOM as a whole was found to bear a negative charge throughout a wide range of pH values (Bernhardt et al., 1985; Bernhardt et al., 1986; Bernhardt and Clasen, 1991; Henderson et al., 2008a). It can be concluded that this also applies to COM since it comprises the same or similar compounds as EOM, though in different amounts. The negative charge of the molecules can be attributed to the acidic

functional groups, such as –OH, –COOH, –SH, that can occur in deprotonated form. Besides negatively charged groups, some organic molecules, e.g. proteins, carry also positively charged ones, such as $–NH_3^+$ and $=NH_2^+$ (with pK_a values of 10.28 and 12.48, respectively) (Creighton, 1993). The charge of AOM is dependent on pH value and can even reach positive values under very low pH conditions. For example, Henderson et al. (2008a) observed a rapid shift from negative to positive electrophoretic mobility of EOM of *Microcystis aeruginosa* and *Chlorella vulgaris* between pH 1 and 4 that can be attributed to the ionisation of carboxylic groups (–COOH) of EOM. On the other hand, electrophoretic mobility decreased at alkaline pH values due to dissociation of peptide/protein amino groups ($–NH_2$ and =NH). Moreover, it was shown that the charge density of EOM changes with the species and the growth phase (Bernhardt et al., 1985; Paralkar and Edzwald, 1996; Henderson et al., 2008a). For instance, charge density of *M. aeruginosa* EOM at pH 7 decreased from 0.2 meq g^{-1} of C at exponential growth phase to 0.1 meq g^{-1} of C at stationary growth phase, while that of *C. vulgaris* EOM at pH 7 increased from 0.9 meq g^{-1} of C at exponential growth phase to 3.2 meq g^{-1} of C at stationary growth phase (Henderson et al., 2008a).

2.1.4. Molecular weight

Another important AOM property from the perspective of water treatment is molecular weight (MW) as it was ascertained that high-MW organic compounds of both allochthonous (humic substances) and autochthonous origin (algae-derived organics) are removed by coagulation more efficiently than low-MW ones (Bernhardt et al., 1985; Pivokonsky et al., 2009b; Matilainen et al., 2010). On the other hand, 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., 2013a, b; Zhou et al., 2014b). Molecular weight is also crucial for the removability of organic matter by adsorption onto activated carbon (Matilainen et al., 2006; Hnatukova et al., 2011; Velten et al., 2011). AOM was shown to comprise compounds with MWs ranging from several hundreds of daltons to hundreds of kilodaltons (Chrost and Faust, 1983; Pivokonsky et al., 2006; Henderson et al., 2008a; Fang et al., 2010; Huang et al., 2012; Li et al., 2012; Wang et al., 2013; Zhang et al., 2013a). Furthermore, COM was found to have larger proportions of high-MW compounds than EOM does and EOM produced in exponential growth phase contains more high-MW material than EOM from stationary growth phase (Pivokonsky et al.,

2006; Fang et al., 2010). It is believed that an important part of AOM is formed by polymers, such as polysaccharides and proteins (Lewin, 1956; Chrost and Faust, 1983; Hoyer et al., 1985; Myklestad, 1995; Maksimova et al., 2004; Henderson et al., 2008a; Qu et al., 2012b; Kong et al., 2014).

2.1.5. Polysaccharide and protein content

The content of saccharide-like and protein-like substances in AOM has been investigated. Lewin (1956), for example, stated that 25% of extracellular dissolved organic matter (DOM) produced by *Chlamydomonas mexicana* was formed by polysaccharides. Similarly, Maksimova et al. (2004) reported that extracellular carbohydrates (mostly polysaccharides) of green alga *Chlorella pyrenoidosa* S-39 comprised 20 – 40% of its EOM. Myklestad (1995) found that carbohydrates, especially polysaccharides, sometimes comprise 80-90% of the total extracellular release of marine algae *Chaetoceros affinis* and *Chaetoceros curvisetus*. The chemical structure of algal polysaccharides is species-specific. They 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).

Protein-like substances comprise up to 42% of EOM depending on the species and age of culture. COM contains even a higher portion of peptides and proteins than EOM does (Pivokonsky et al., 2006; Henderson et al., 2008a; Fang et al., 2010; Huang et al., 2012). For example, Fang et al. (2010) reported that COM of *M. aeruginosa* contained a higher concentration of total organic nitrogen (TON) (230 mg N/mg DOC) in comparison with EOM (180 mg N/mg DOC). In the case of some species (e.g. *M. aeruginosa*), proteinaceous DOC can even exceed 50% of the total DOC in COM (Pivokonsky et al., 2006).

2. 2. Removal of AOM in drinking water treatment

A wide range of technologies are available for treatment of drinking water sources containing algal organic matter. Coagulation followed by separation steps like sedimentation or dissolved air flotation and/or filtration and final disinfection represents conventional water treatment. Several newer processes for improving the removal of AOM have been investigated. The most promising ones include adsorption onto activated carbon (AC) and

membrane filtration. Since different strategies are effective for different types of organic compounds, these methods are often used in combination.

Coagulation is regarded as an effective technology when removing DOM and NOM. Coagulation with Al or Fe salts is specifically good at removing algae cells and hydrophobic and high-MW organic matter such as humic acids (Henderson et al., 2008b, 2010; Ghernaout et al., 2010; Edzwald and Haarhoff, 2011). However, it is widely accepted that if the water is hydrophilic in nature, such as water containing AOM, much lower removal efficiencies would be achieved (Ghernaout et al., 2010). To illustrate, Henderson et al. (2010) observed good cell removal (94 – 99%) for *M. aeruginosa*, *C. vulgaris*, *Asterionella formosa* and *Melosira* sp., while AOM removal efficiency was 46-71%. Furthermore, the study by Cheng and Chi (2003) demonstrated that the removal efficiency of DOC from three water reservoirs, suffering from eutrophication and blooming of algae, decreased as the percentage of DOC with molecular weight less than 5000 Da increased. In addition, conventional treatment using coagulation is able to remove algal cells and therefore the cell-bound cyanotoxins, but it is believed to be largely ineffective for removing extracellular soluble toxins (Himberg et al., 1989; Lahti and Hiisvirta, 1989; Hitzfeld et al., 2000; Schmidt et al., 2002). These require more advanced treatment, for example, activated carbon and/or membrane filtration, or oxidants such as ozone (Hitzfeld et al., 2000; Dixon et al., 2011). The main merits of coagulation are ease of operation and low cost. Pre-oxidation, especially with ozone, chlorine or potassium permanganate, may be applied prior to coagulation to enhance the removal of AOM and algal cells (Hoyer et al., 1987; Paralkar and Edzwald, 1996; Ma and Liu, 2002; Henderson et al., 2008b; Ma et al., 2012; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013). However, the use of oxidants can also cause damage to algal cells and promote the release of COM, including intracellular cyanotoxins and taste and odour compounds (Ma et al., 2012; Coral et al., 2013). Moreover, the usage of chlorine is limited by the generation of DBPs. Coagulation of AOM, which is the focus of this thesis, and pre-treatment by means of pre-oxidation are further elaborated in section 2.3.

Adsorption onto activated carbon (AC) is frequently integrated in the water treatment chain as an effective treatment for organic impurities of both natural and anthropogenic origin (Moreno-Castilla, 2004; Delgado et al., 2012), such as taste and odour compounds, NOM, DBPs, cyanobacterial toxins, pharmaceuticals and pesticides (Bjelopavlic et al., 1999; Newcombe, 2006; Humbert et al., 2008; Bond et al., 2011; Ho et al., 2011; Zhang et al.,

2011b; Delgado et al., 2012). Granular activated carbon (GAC) and powdered activated carbon (PAC) are the most frequently used types of AC. GAC is employed continuously in different types of filters/adsorbers as a final polishing step after coagulation and before disinfection. PAC is typically applied only for short periods of unexpected deterioration in raw water quality, when it is dosed directly to the treated water, usually before or simultaneously with coagulants. It may be also used in combination with membrane filtration (ultrafiltration and microfiltration). Most of adsorption studies that dealt with the removal of NOM focused on humic and fulvic material (Matilainen et al., 2006; Kristiana et al., 2011; Velten et al., 2011; Gibert et al., 2013) or generally on DOM passed 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 (Matsushita et al., 2008; Campinas and Rosa, 2010; Hnatukova et al., 2011; Zhang et al., 2011a; Zhang et al., 2011b). Special attention has been paid to the adsorption of cyanobacterial taste and odour compounds (Matsushita et al., 2008; Zhang et al., 2011b) and toxins, especially commonly occurring microcystins or saxitoxins (Pendleton et al., 2001; Campinas and Rosa, 2006; Huang et al., 2007a; Dixon et al., 2011; Ho et al., 2011; Zhang et al., 2011a). Several studies investigated the use of PAC in combination with membrane filtration (ultrafiltration and microfiltration) which was shown to be a suitable treatment method for the control of cyanobacterial cells and their products (Matsushita et al., 2008; Campinas and Rosa, 2010; Zhang et al., 2011a). Zhang et al. (2011a) found that the addition of PAC to the immersed ultrafiltration (UF) reactor enhanced the removal of DOC, UV_{254}, and microcystins, but had little effect on the rejection of hydrophilic high-MW AOM, such as carbohydrates and proteins. Similarly, study by Campinas and Rosa (2010) showed that PAC in combination with UF can increase hydrophobic AOM rejection, but it is apparently ineffective in adsorbing the highly hydrophilic EOM compounds. In general, studies on AOM adsorption observed preferential adsorption of lower-MW compounds compared to higher-MW ones (Campinas and Rosa, 2010; Hnatukova et al., 2011; Zhang et al., 2011a).

Membrane filtration (microfiltration, ultrafiltration and sometimes nanofiltration) has been increasingly used for drinking water treatment in recent years. The membrane filtration process offers several advantages such as a small footprint, compact module, easy maintenance and superior water quality. A major drawback of membrane filtration is membrane fouling (reversible and irreversible) which results in increase in transmembrane

pressure and flux decline. Several studies have claimed that the non-humic fraction of NOM prevailed over the other fractions in membrane fouling. Fouling is strongly dependent on organic matter properties such as hydrophobicity, molecular weight, and charge density (Fan et al., 2001; Zularisam et al., 2011), as well as solution properties such as pH, ionic strength, and divalent cations concentration (Lim and Mohammad, 2010; Myat et al., 2012). Fouling studies on AOM have reported that neutral hydrophilic components of AOM, being considered to contain biopolymers, are more significant foulants than hydrophobic ones (Carroll et al., 2000; Lin et al., 2000; Amy, 2008). Specifically, protein- and polysaccharide-like substances were found to be the major foulants in studies investigated EOM extracted from a number of algal species (Chiou et al., 2010; Zhang et al., 2010b; Qu et al., 2012a; Villacorte et al., 2013). Moreover, membrane properties (Elimelech et al., 1997) also have a significant impact on membrane fouling. It was shown that strongly hydrophobic membranes tend to foul, causing a much greater flux reduction than hydrophilic membranes while hydrophilic membranes have better permeability (Nakatsuka et al., 1996). Fouling by AOM can be controlled via pre-treatment that reduces the amount of biopolymers, e.g. coagulation and/or ion exchange and selection of hydrophilic membranes (Myat et al., 2012).

2. 3. Coagulation of AOM

Most of the investigations about the coagulation of algae-laden waters have focused on algal and cyanobacterial cells, which is summarised in reviews of Henderson et al. (2008b) and Ghernaout et al. (2010). Some studies have also considered the influence of AOM on the coagulation of cells (Bernhardt and Clasen, 1991; Dolejs, 1993; Henderson et al., 2010; Ma et al., 2012b; Vandamme et al., 2012; Zhang et al., 2012; Garzon-Sanabria et al., 2013; Wang et al., 2013). They found that the cells are easily coagulated in the pH range where coagulants (most commonly Al and Fe hydrolysing salts or prepolymerized Al/Fe coagulants) bear positive charge and can neutralize the slightly negatively charged surface of cells. The studies of Bernhardt and Clasen (1991), Henderson et al. (2008b; 2010) and Zhang et al. (2012) 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; Zhang et al., 2012) and is a result of both cell surface area and EOM concentration and character

(Henderson et al., 2010; Zhang et al., 2012). Henderson et al. (2010) found that EOM was removed with lower efficiency than cells. They observed good cell removal (94-99%) for *M. aeruginosa*, *C. vulgaris*, *Asterionella formosa* and *Melosira* sp., while EOM removal efficiency was 46-71%. Coagulant demand was reported to be highly dependent on charge density which appears to have a stronger influence than the cell surface area does. 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.

Under certain circumstances, the amount of dissolved EOM and COM can predominate over other impurities in source water. This may happen during the die-off and decomposition of algal bloom (Zhang et al., 2010a). Studies on the coagulation of dissolved AOM in the absence of cells are very rare (Bernhardt et al., 1985, 1986, 1991; Paralkar and Edzwald, 1996; Widrig et al., 1996; Pivokonsky et al., 2009a, b). The first major investigation into coagulation of AOM was undertaken by Bernhardt and coworkers (1985, 1986, 1991). They investigated the coagulation of EOM of MW > 2 kDa excreted by several algal species and its influence on the coagulation on quartz particles, which will be discussed in section 2.3.1. that describes the influence of AOM on coagulation of other particles in source water. Paralkar and Edzwald (1996) investigated the behaviour of EOM as a coagulant without the addition of any other coagulants. Their findings are discussed in section 2.3.2. The study by Widrig et al. (1996) examined the removal of EOM derived from two common green algae species, *Scenedesmus quadricauda* and *Dictyosphaerium pulchellum* and blue-green alga *Microcystis aeruginosa* obtained at exponential growth phase. The initial DOC concentration used in coagulation tests was 6.0 mg L^{-1}. Two coagulants, aluminium sulphate (alum, $Al_2(SO_4)_3$. $14H_2O$) and ferric chloride ($FeCl_3$. $6H_2O$), were used at doses ranging from 0.0074 to 2.96 mM as metal (corresponding to 0.25 – 100 mg L^{-1} as AI(III), and 0.51 - 206 mg L^{-1} as Fe(lll)). Tests were performed at two pH values, 5 and 8. The results showed that EOM removal was primarily a function of algal source, coagulant dose and pH value. Alum and ferric chloride and were found to perform quite similarly. Alum performed slightly better at pH 8, while ferric chloride performed better at pH 5, though improved removal was only on the order of 5%. In all tests, overall removals were improved at pH 5 compared to pH 8. Under acidic conditions (pH = 5) and high coagulant dose (2.96 mM as metal), 20 – 50% DOC reductions were achieved. Removal of DOC improved in the order of *M. aeruginosa* (about 20%), *S. quadricauda* (about 25%), and *D. pulchellum* (up to 50%). On the other hand, DOC

removals at pH 8 were only in the range of 5 – 10%. Henderson et al. (2010) observed greater removal efficiencies for EOM of *M. aeruginosa* (55%), *Chlorella vulgaris* (71%) and *Asterionella formosa* (46%) than Widrig et al. (1996) probably due to the fact that Henderson et al. (2010) used EOM extracted in stationary growth phase while Widrig et al. (1996) performed experiments with EOM from exponential growth phase. Henderson et al. (2010) also reported much lower doses of Al required to achieve maximum removal of EOM than Widrig et al. (1996) did. They were 0.8, 1.2 and 1.5 mg (0.03, 0.044 and 0.056 mM) Al per mg DOC at pH 7 for *C. vulgaris*, *M. aeruginosa* and *A. formosa*, respectively.

Pivokonsky et al. (2009b) investigated the coagulation of COM produced by *M. aeruginosa* by ferric sulphate. Two DOC initial concentrations of 3 and 7 mg L^{-1} were used in coagulation tests. The lowest residual COM concentrations were achieved in the pH range of 4.5 – 6.5. In case of low initial DOC concentrations (3 mg L^{-1}), the highest COM removal rates of about 60% were achieved in the pH range of 4.5 – 6.5 at iron dose of 6 mg L^{-1}. For high initial DOC concentrations (7 mg L^{-1}), the lowest residual COM concentrations were achieved in the same pH range (4.5 – 6.5). Specifically, the highest DOC removal (52%; residual DOC = 3.35 mg L^{-1}) was observed at pH 6 and corresponded to an iron dose of 26 mg L^{-1}. These optimum dose values are significantly lower than those obtained by Widrig et al. (1996) for initial DOC concentration of 6 mg L^{-1}. This can be given by the fact that Widrig et al. (1996) used EOM from exponential growth phase and Pivokonsky et al. (2009b) used COM, the composition of which are likely to differ. Pivokonsky et al. (2009a, b) also ascertained that protein-like substances were removed with higher efficiency than other organic substances contained in COM. Moreover, proteins of higher molecular weight were removed more efficiently. This is consistent with the findings of Widrig et al. (1996) that coagulation selectively removed nitrogen-containing compounds as shown by pyrolysis-GC-MS analysis of residual organic matrix. Widrig et al. (1996) also stated that the higher DOC removals achieved in *D. pulchellum* waters compared to other two species may be associated with a macromolecular matrix containing proteinaceous and fatty acid material of relatively high molecular weight. Furthermore, these results are in accordance with the study by Cheng and Chi (2003) on the coagulation of eutrophic waters who found that large organic molecules (> 5000 Da) were removed more easily by coagulation than small ones. In addition, Hoyer et al. (1985) also demonstrated that coagulation of the EOM sample which had undergone aeration and subsequent decomposition of the polysaccharides by bacteria provided lower

EOM removals.

Another study that focused on the coagulation of algae-laden waters was taken by Hu et al. (2006). They used water samples collected during summer from the eutrophic Guanting reservoir in China, a potential drinking water source for Beijing. The water sample parameters were: turbidity 14.5 NTU (Nephelometric Turbidity Units), DOC 16.6 mg L^{-1} and SUVA 0.75 m^{-1} mg^{-1} L, which indicates that most of DOC was of algal origin. However, organic matter contained in water samples was not further characterised and no algal species occurring in the reservoir were reported. Three coagulants, aluminium chloride ($AlCl_3$. $6H_2O$), commercial polyaluminium chloride ($PACl_1$) and polyaluminium chloride prepared with the method of an electrolysis process ($PACl_2$) were used in the coagulation experiments at concentrations 2 and 8 mg L^{-1} Al within the pH range from 4 to 9. All coagulants were effective in removing both turbidity and DOC with removal efficiencies of 85 – 95% in case of Al dose 8 mg L^{-1} and pH values 4, 5 and 6. Removal efficiencies then decreased with increasing pH value. Aluminium chloride was slightly more effective than polyaluminium chloride and reached efficiencies of about 95% at pH values 5 and 6 for turbidity removal and more than 90% at pH values 5 and 6 for DOC removal. Satisfactory turbidity and DOC removals were also achieved at pH 4, but at this pH value, high portion of Al monomeric species were detected for $AlCl_3$. $6H_2O$ and $PACl_1$ indicating that high concentrations of residual Al would be present in treated water. On the other hand, $PACl_2$ exhibited high portions of medium and large Al polymers and low portion of monomeric Al at all pH values. At pH 5, monomeric Al species comprised about 15% of $AlCl_3$. $6H_2O$ and $PACl_1$ and about 5% of $PACl_2$. Low portion of monomeric Al for all three coagulants was detected at pH values 6 and 7. The pH value 6 seemed, therefore, to be the most suitable for coagulation using $AlCl_3$. $6H_2O$ and $PACl_1$, while $PACl_2$ can be used in a wider pH range, but with lower efficiencies. In summary, the study suggests that traditional coagulants may be a better selection for eutrophic water treatment than inorganic polymer flocculants and that pH control is necessary to maximize turbidity and DOC removals.

The studies of Widrig et al. (1996), Pivokonsky et al. (2009b) and Hu et al. (2006) demonstrated that optimum pH values for the treatment of waters containing AOM lie in the acidic range (4.5 – 6) depending on coagulant used and AOM composition. Acidic pH values (5 – 6.5 for aluminium-based and 4.5 – 6 for ferric-based coagulants) were also reported to be effective for the removal of NOM, the majority of which was formed by humic substances

(Ebie and Amano, 1993; Matilainen et al., 2010). These results are consistent with coagulation theory which states that at acidic pH, favourable charge interactions between NOM and Al- or Fe-hydroxopolymers produce more efficient DOC removals than the charge interactions and adsorption to Al or Fe hydroxide precipitate occurring at higher pH values (Dempsey et al., 1984; Amirtharajah and O'Melia, 1990). The results of the study by Widrig et al. (1996) indicate that EOM produced during the exponential growth phase is more difficult to coagulate than EOM produced during stationary growth phase. This is probably because stationary-phase EOM contains more high-MW compounds, which are easier to coagulate, than exponential-phase EOM (Pivokonsky et al., 2006), as stated in section 2.1.4.

2. 3. 1. The impact of AOM on coagulation of other particles

The studies that dealt with the influence of AOM on the coagulation of other particles present in source water can be divided into two groups. The first group are the ones that focused on the coagulation of algal cells in the presence of EOM produced by cells and/or COM released after pre-oxidation of the cells (Ma and Liu, 2002; Henderson et al., 2010; Ma et al., 2012a, b; Zhang et al., 2012; Garzon-Sanabira et al., 2013; Pranowo et al., 2013; Wang et al., 2013) that is elaborated in section 2.3.3. It was found that presence of AOM increased the amount of required coagulant (Henderson et al. 2010; Vandamme et al., 2012; Zhang et al., 2012; Garzon-Sanabria et al., 2013). On the other hand, some studies observed a positive influence of high-MW EOM as well as COM released after pre-oxidation on the coagulation of algae cells since algal biopolymers acted as a polymer coagulant aid (Ma and Liu, 2002; Henderson et al. 2010; Ma et al. 2012a, b; Pranowo et al. 2013; Wang et al. 2013).

The second group of studies was undertaken by Bernhardt and coworkers (Bernhardt et al. 1985, 1986, 1991) who investigated the effect of EOM of MW > 2 kDa excreted by several algal species on the coagulation of inorganic quartz particles. These particles represented the turbidity causing inorganic particles that are often present in raw water and they usually bear a negative charge in a wide range of pH values. Bernhardt et al. (1985) concluded that EOM contained a large concentration of non-ionic and anionic polyelectrolytes, i.e. neutral and acidic polysaccharides (polyuronic acids) and non-saccharide macromolecular compounds, such as proteins. EOM polymers were able to 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. This led to an increase in the negative charge of the quartz

particles determined by electrophoretic mobility measurements. Moreover, proteins contained in EOM also bear positively charged functional groups that can electrostatically interact with negatively charged surface of quartz particles, although Bernhardt et al. (1985) does not take this into consideration. The negatively charged particles formed by EOM and quartz particles were then removable by ferric coagulants in the pH range, where Fe formed hydrolytic products bearing a positive charge, i.e. at pH about 5 – 6.5 (the exact pH values differed in algal species). At higher pH values, Fe hydrolytic products lose their positive charge and then form anion complexes with increasing pH. As a result, electrostatic repulsions between negatively charged particles led to system stabilization. The lack of positive charge in the system can be compensated by the addition of calcium ions, which enables efficient coagulation even at pH values > 6 as demonstrated by Bernhardt et al. (1986). Bernhardt et al. (1985) pointed out that EOM behaves as a coagulation aid only at low concentrations (< 2 mg L^{-1} DOC). However, the more precise statement is that the influence of EOM on the coagulation of quartz particles is dependent on the coagulant/DOC concentration ratio (Bernhardt et al. 1985; Bernhardt et al., 1991). 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. Above this ratio, a partial coating of the quartz particles surface by EOM polymers can lead to particle aggregation through polymer bridging and aid coagulation. Below this ratio, large coverage of quartz particles by EOM polymers enhances the stability of dispersed system by steric and charge stabilization (Bache and Gregory, 2007) and impairs coagulation. Similar results were obtained also in case of alginic acid, an polyuronic acid composed of two uronic acids (poly-D-mannuronic acid and L-guluronic acid), which was used as a model compound for EOM acidic polysaccharides (Bernhardt et al., 1985, 1986, 1991).

2. 3. 2. AOM as a coagulant

Natural polymeric materials are increasingly used in water and wastewater treatment as a coagulant or coagulant aid. The main advantages of natural polyelectrolytes are relatively high effectiveness, low cost, safety for human health and biodegradability. The primary coagulation mechanism is ‘bridging’ which involves adsorption of the polyelectrolyte molecule from solution onto the surface of two or more suspended particles. The most commonly used natural polymers are chitosan, alginate, starch and extract from seeds of

Moringa oleifera (Bratby, 2006). Alginates are heteropolysaccharides which widely occur in seaweeds, algae and bacteria. They have a linear co-polymer structure, comprised of two sugar residues, (1-4) linked β-D-mannuronic and α-L-guluronic acid. Since alginates bear a strong resemblance to algal and cyanobacterial products, and are sometimes used as model compounds for AOM polysaccharides, it can be assumed that some compounds contained in AOM may also serve as coagulants.

The study by Devrimci et al. (2012) showed that calcium alginate proved to be successful coagulant for turbid waters, containing clay (predominantly smectite). The initial turbidities of water samples were 150 NTU, 80 NTU and 10 NTU. The coagulation experiments were conducted at pH 7.3 ± 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 clay surface. The role of calcium in coagulation was explained due to its ability to compress the double layer and to reduce repulsive forces between colloid/colloid, polymer/colloid and polymer/polymer pairs. Adding calcium first eased up the approach of alginate to particles. Additionally, calcium ion can form complexes with certain ionogenic groups on the polymer and on the particle surfaces (Gregor et al., 1996). The efficiency of coagulation using alginate was thus not only dependent on the initial turbidity, but also on calcium concentration.

Many studies that focused on the processes in soils demonstrated that natural organic compounds, such as mono- and polysaccharides, amino acids, proteins, nucleic acids and humic substances 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). Organic compounds that bear a charge (e.g. acidic polysaccharides, proteins) can interact with clays, which usually also bear a surface charge, by electrostatic interactions. These are affected by pH of the solution, especially in case of amphoteric organic compounds (Pinck, 1962). For neutral compounds, several molecular mechanisms for adsorption via weak bonds were proposed: (1) substitution of interlayer water molecules solvating exchangeable cations (Parfit and Greenland, 1970), (2) hydrogen bonds between macromolecule hydroxyl and clay surface oxygens, or (3) hydrogen bonds between hydroxyl groups on sheet edges and macromolecule (Labille et al., 2005). To illustrate, Labille et al. (2005) ascertained that bacterial polysaccharides are able to attach to and subsequently coagulate Na-montmorillonite

particles. They used polysaccharides of bacterial origin, i.e. dextran, succinoglycan, YAS34, xanthan, MWAP71, rhamsan, RMDP17, that are present in soils and form organomineral aggregates with clays. Dextran represents non charged polysaccharide composed of chains of glucose moieties. When dosed at 0.1 and 1 mg L^{-1} to Na-montmorillonite 0.005% w/w suspension, no measurable effect on the size of colloidal particles was observed, even though adsorption of dextran on Na-montmorillonite was demonstrated by several studies (Parfit and Greenland, 1970; Guidi et al., 1977). Aggregates were not formed in this case probably because the applied mechanical shear (velocity gradient of 175 s^{-1}). However, when $CaCl_2$ was added into the suspension in order to obtain a final concentration of 1.25 x 10^{-3} M, coagulation was induced by screening electrostatic repulsions. It decreased interparticle distances and allowed the formation of polysaccharide bridges between clay particles. The coagulation of Na-montmorillonite particles in the presence of $CaCl_2$ (1.25 x 10^{-3} M) by succinoglycan, which is negatively charged due to acidic moieties, was significantly affected by the pH of the medium. Increasing pH tended to decrease the coagulation rate. At pH bellow 6, electrostatic interactions between amphoteric edge surface of clay platelets, which are positively charged at these pH values, and polysaccharide carboxyl groups resulted in the coagulation. At higher pH values, deprotonated sheet edges of Na-montmorillonite prevented such interactions and decreased coagulation rate was observed. The study further showed that higher coagulation rates were observed for acidic polysaccharides which bear acidic moieties located on side chains (succinoglycan and YAS34) than for polysaccharides which bear acidic moieties on backbone (RMDP17). This is probably because acidic side chains are more accessible to clay particles than those located on the backbones. Short side chains in case of polysaccharide MWAP71 prevented favourable exposure of acidic moieties and led to lower coagulation rates compared to those of succinoglycan or YAS34. Moreover, higher charge density and mass per repeat unit hindered coagulation by steric and coulombic effects, as shown in case of xanthan.

The studies on the effect of AOM as a coagulant are very rare. As mentioned in section 2.3.1., Bernhardt et al. (1985, 1986) demonstrated that EOM can serve as a coagulant aid in the coagulation of quartz particles. However, they used ferric salt as the main coagulant in their experiments 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 x 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 concentration of 0.01 mg L^{-1} DOC. Reductions in the optical density and particles numbers were observed after 120 minutes of settling. Higher concentrations of EOM from *Cyclotella* sp. resulted in charge reversal, as determined by electrophoretic mobility measurements, and restabilization. EOM from other algae showed charge neutralization at concentrations about 0.1 mg L^{-1} DOC 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 which are able to remove less heavy particles unsuitable for sedimentation, such as sand filtration.

2. 3. 3. The effect of pre-oxidation on the coagulation of AOM

Pre-oxidation of raw water containing algal cells and dissolved AOM with potassium permanganate ($KMnO_4$), potassium ferrate (K_2FeO_4), ozone or chlorine can be used to enhance the coagulation of algae cells (Ma and Liu, 2002; Henderson et al., 2008b; Ma et al., 2012; Coral et al., 2013; Pranowo et al., 2013; Wang et al., 2013). The improvement in algae removal is attributed to cell inactivation, change in external cell architecture, release of COM and the degradation of both EOM and released COM. For instance, Wang et al. (2013) found that the pre-oxidation of *M. aeruginosa* cells (2.32 × 10^9 cells L^{-1}, corresponding to optical density at 680 nm (OD_{680}) = 0.1) by low concentrations of $KMnO_4$ (2 mg L^{-1}) led to the secretion of high-MW compounds with molecular weight above 200 kDa. These molecules easily combine with polyaluminium chloride used as coagulant, which enhanced coagulation and improved the efficiency of algae removal, possibly through adsorptive bridging. However, the release of COM and degradation of EOM/COM may have not only beneficial but also detrimental effects on particles destabilization and aggregation, depending on oxidizer dose, pH value and the properties of EOM/COM (Hoyer et al., 1987; Paralkar and Edzwald, 1996; Henderson et al., 2008b; Ma et al., 2012; Coral et al., 2013; Wang et al., 2013). Overdosing leads to cell lysis, releasing undesirable toxins or taste and odour compounds, and to EOM degradation to the extent that low-MW compounds which may be

difficult to coagulate are formed (Hoyer et al., 1987; Henderson et al., 2008b; Ma et al., 2012). In addition, DBPs can form when using chlorine as pre-oxidizer and also during final disinfection step, if degraded EOM and COM released from damaged cells are not removed by the subsequent treatment (Henderson et al., 2008b). Therefore, attention should be paid to the amount of oxidizer to keep the relative integrity of algal cells to avoid secretion of COM (including toxins).

The studies focusing on the oxidation of AOM in the absence of cells are rare (Hoyer et al., 1987; Paralkar and Edzwald, 1996; Widrig et al., 1996). Widrig et al. (1996) investigated the influence of ozonation on the removal of EOM derived from green algae *Scenedesmus quadricauda* and *Dictyosphaerium pulchellum*, as well as blue-green alga, *Microcystis aeruginosa*, extracted at exponential growth phase, on coagulation. They reported that preozonation by 0.8 mg ozone/mg DOC increased EOM removal, which was always higher when preozonation was performed at pH 8 compared to pH 5. Maximum DOC removal with preozonation was increased by approximately 15% for *S. quadricauda*, 12% for *D. pulchellum*, and 5% for *M. aeruginosa* beyond the highest removals for coagulation alone. Maximum removals were always achieved at the highest coagulant dose (2.96 mM of Al of Fe), at a preozonation pH 8 and a coagulation pH 5. The difference in ozonation efficiency at pH 5 and 8 is caused by different pathway of ozone action. At lower pH (pH 5), molecular ozone (O_3) is the primary oxidant and its reactions are highly selective. At alkaline pH value (pH 8), hydroxide ion (OH^-) is an initiator in the ozone decomposition chain reaction, and as pH increases molecular ozone decomposes more rapidly into a series of free radicals. The most important of these is the hydroxyl free radical, a highly nonselective oxidant. It is highly effective in degrading saturated aliphatic molecules, which comprise a significant portion of AOM. The results of pyrolysis-GC-MS showed that macromolecular proteinaceous and fatty acid material was degraded by ozone into smaller units. Similarly, Paralkar and Edzwald (1996) found that ozonation of the EOM of *Chlorella vulgaris* (green algae), *Scenedesmus quadricauda* (green algae) and *Cyclotella* sp. (diatom) showed a consistent trend of decreasing high-MW fractions and increasing medium- and low-MW fractions with increasing dose of ozone. Moreover, EOM from these three species indicated an increase in charge with ozonation. The authors stated that ozone first cleaved the macromolecules and polymers to smaller subunits or monomers. Further ozonation probably led to the formation of functional groups and acidic fractions on the cleaved monomers. Moreover, Paralkar and

Edzwald (1996) investigated the influence of EOM ozonation on the charge neutralization and coagulation of positively charged latex particles. The experiments were performed at pH 7 with both non-oxidized and oxidized EOM from the three species. The concentration of latex particles of mean diameter 3.98 µm was 5 x 10^5 particles m L^{-1}. Applied ozone doses were 1 and 3 mg L^{-1} and EOM was added to latex particles to reach concentrations of 0 – 0.5 mg L^{-1} DOC. The results of the coagulation experiments with non-oxidized EOM are described in section 2.3.2. During the experiments with oxidized EOM, ozone tended to cause an increase in the concentration of EOM needed for charge neutralization of latex particles when compared to experiments with non-oxidized EOM. To illustrate, an ozone dose of 3 mg L^{-1} caused a fivefold increase in *Cyclotella* sp. EOM required for charge neutralization. Contrary to the tests with non-oxidized EOM of *Cyclotella* sp., the particles formed by oxidized EOM adsorbed onto latex particles were not removed by settling efficiently. The authors concluded that EOM after ozonation was probably too small for interparticle bridging since an ozone dose of 3 mg L^{-1} reduced the high-MW fraction of *Cyclotella* sp. EOM from 90 to about 30%. Hoyer et al. (1987) used EOM extracted from *Fragilaria* (diatom), *Pseudanabaena* (cyanobacterium), and *Dictyosphaerium* (green alga) during late stationary growth phase and showed that ozone treatment can either support or inhibit coagulation, depending on algae species and ozone dose. The coagulation tests were carried out at pH 6.5 using EOM concentrations of 1.5 and 3.0 mg L^{-1} DOC with 5.0 mg L^{-1} Fe^{3+} as coagulant. For all species investigated, ozone doses > 1 mg/mg DOC caused coagulation disturbance. On the other hand, lower ozone doses enhanced removal of EOM of *Fragilaria* (diatom), *Pseudanabaena* (cyanobacterium). The *Dictyosphaerium* EOM, however, impaired flocculation at doses as low as 0.3 mg ozone/mg DOC.

It can be concluded that beneficial effects of preozonation to enhance the coagulation of EOM have been shown to depend on algal type and age and ozone dose.

2. 3. 4. Formation of complexes between AOM and coagulants

Several studies found out that some AOM substances are able to form dissolved complexes with coagulant metals, which results in the deterioration of coagulation (Bernhardt et al., 1985; Takaara et al., 2005, 2007, 2010; Pivokonsky et al., 2006). These substances were reported to include polysaccharides, lipopolysaccharides, peptides and proteins. Algal polysaccharides, particularly anionic ones containing uronic acids, are able to bind metals

through their carboxyl groups (–COOH). Metal uptake is typically dependent on pH values because binding of metal cations is determined primarily by the state of dissociation of the carboxyl groups (Bernhardt et al., 1985; Kaplan et al., 1987; Kratochvil and Volesky, 1998; Gyurcsik and Nagy, 2000; Hamdy, 2000; De Philippis et al., 2007). Sulfonate groups ($–OSO_3^-$) can also contribute to heavy metal binding, especially at low pH values (Kratochvil and Volesky, 1998). It was demonstrated that alginate (or alginic acid), a model compound for acidic polysaccharides, is able to bind Al^{3+} (Seely and Hart, 1976; Gregor et al., 1996). Similar behaviour was observed in case of anionic lipopolysaccharides on the cell surface of cyanobacterium *M. aeruginosa* that bound to dissolved polyaluminium chloride (PACl) constituents. This resulted in the inhibition of coagulation process (Takaara et al., 2010). Algal peptides and proteins were also found to bind Al^{3+} and Fe^{3+} through their dissociated carboxyl ($–COO^-$) groups (Takaara et al., 2005, 2007; Pivokonsky et al., 2006; Sano et al., 2011). By affinity chromatography followed by HPSEC, Pivokonsky et al. (2006) isolated protein of MW of 60 kDa which formed complexes with Al^{3+} and Fe^{3+} from COM of two cyanobacteria species, *Anabaena flos-aqua* and *M. aeruginosa*. Takaara et al. (2007) identified proteins of MWs between 30 and 70 kDa to be responsible for Al-complexation by affinity chromatography followed by SDS-PAGE.

The formation of coagulant metal-AOM complexes has two consequences. Coagulant metals that were captured in the complexes cannot undergo hydrolysis and form hydroxopolymers necessary for coagulation. For this reason, the dissolved or colloidal residual Al/Fe concentration in the filtrate increases (Bernhardt et al., 1985; Pivokonsky et al., 2006) and coagulant demand rises. Furthermore, AOM functional groups whose interactions with coagulant hydroxopolymers enable coagulation and efficient removal are blocked by coagulant metals and are no longer effective in coagulation process. Metal-organic complexes then tend to remain in solution after the coagulation (Pivokonsky et al., 2006; Takaara et al., 2007).

3. MOTIVATION AND OBJECTIVES

Most studies that investigated the coagulation of natural organic matter focused on the organic matter of allochthonous origin, i.e. humic substances, and only a handful of them dealt with coagulation of AOM. It is widely accepted that dissolved AOM, released as EOM and/or COM into source water, is difficult to remove by coagulation, deteriorates the coagulation process and negatively affects the removal of other substances present in source water. However, a few studies indicate that quite high removal rates can be achieved for AOM under appropriate coagulation conditions. Coagulation is a cost-effective process widely employed by many water treatment plants that have to deal with an increase in algal populations and consequently with increasing concentration of AOM connected with eutrophication of water reservoirs. Moreover, coagulation seems to be an effective pre-treatment to improve the performance and reduce the cost of downstream processes, such as adsorption onto activated carbon or membrane filtration. It is evident that the removability of AOM is dependent on its properties and composition which indicates that their knowledge is essential for understanding AOM treatability and subsequent optimization of the removal/coagulation process.

For these reasons, the aim of this thesis was to investigate the coagulation of AOM and to elucidate its mechanisms. Since the proteinaceous substances were reported to deteriorate coagulation in particular, special emphasis was put on the coagulation of peptides and proteins produced by phytoplankton.

This was achieved by carrying out the following objectives:

1) Investigate the AOM properties, which are important from the perspective of coagulation, such as hydrophobicity, molecular weight, surface charge etc. (Publication 1).

2) Isolate and characterise proteinaceous substances that were reported to disturb the coagulation process from cellular organic matter (COM) of cyanobacterium *Microcystis aeruginosa* (Publication 1, 2, 4).

3) Investigate the coagulation of peptides and proteins produced by *M. aeruginosa*, and to describe the coagulation mechanisms (Publication 2, 3).

4) Quantify the contribution of the formation of dissolved complexes between peptides/proteins of *M. aeruginosa* and coagulants (aluminium and iron) to the

coagulation disturbance and to identify the peptides/proteins which are able to form dissolved complexes with coagulants (Publication 2, 3, 4).

5) Investigate the influence of peptides/proteins of *M. aeruginosa* on the coagulation of kaolin particles which represent the clay particles (aluminosilicates) causing the turbidity of raw water (Publication 4).

6) Identify the peptides/proteins of *M. aeruginosa* which are difficult to remove by coagulation and to present another process which may be effective in removal of these peptides/proteins (Publication 5).

7) Summarise the current knowledge on the impact of algal organic matter on the water treatment processes (Publication 6).

In order to achieve these objectives, several series of laboratory experiments were carried out. Their results have been presented in 6 following papers.

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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.

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

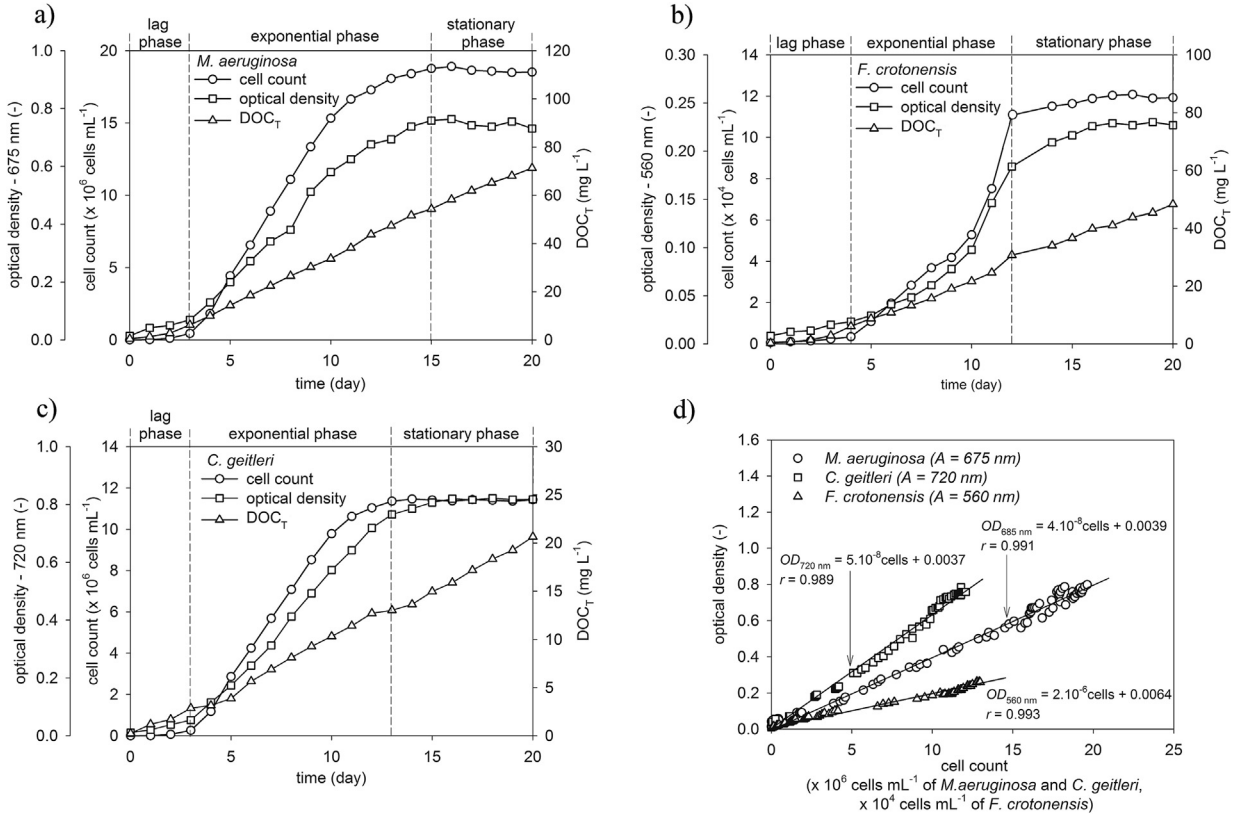

Fig. 1 – Growth curves of *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c) with growth phases marked, and linear correlations between optical densities and cell counts (d).

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

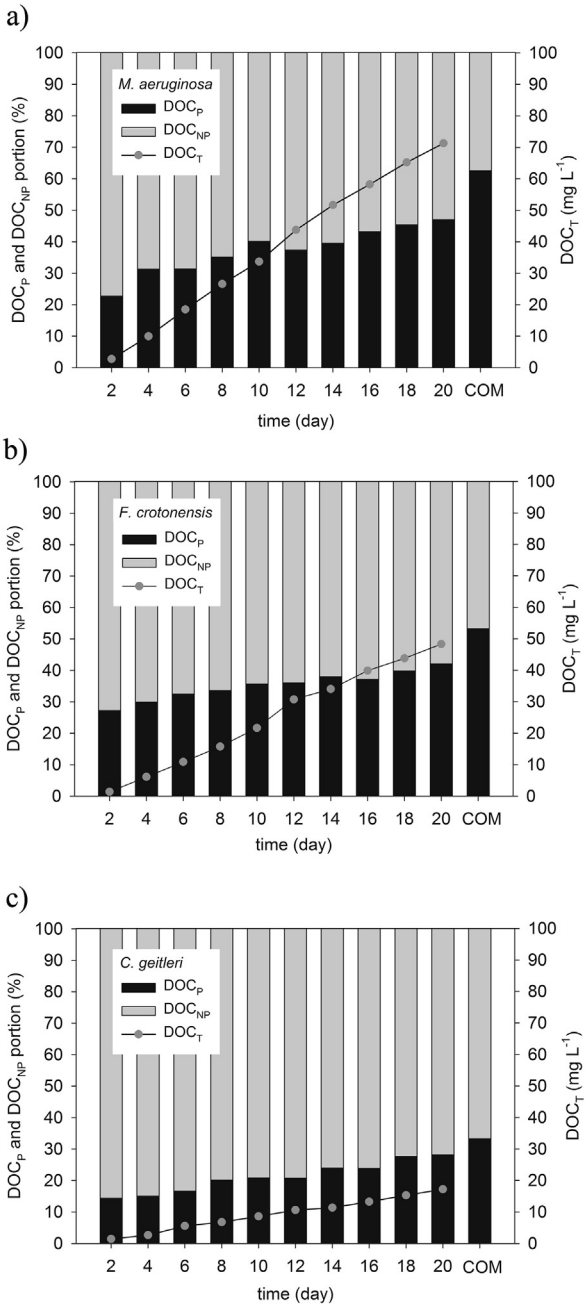

Fig. 2 – Portions of peptide/protein (DOC_P) and non-peptide (DOC_{NP}) organic matter from the total amount of organics released into cultivation media (DOC_T) as a function of cultivation time, and from COM of *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c).

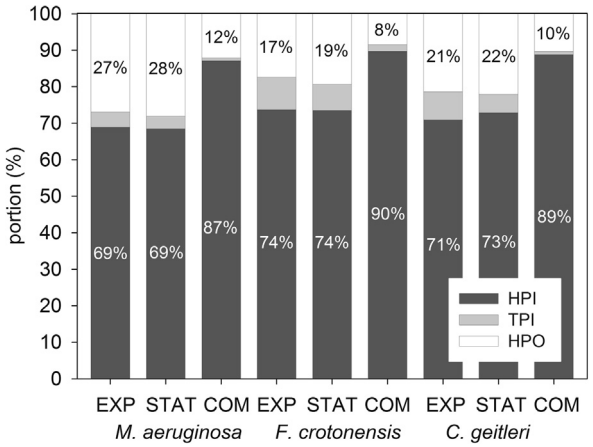

Fig. 3 – Hydrophilic (HPI), transphilic (TPI) and hydrophobic (HPO) fractions of EOM at exponential (EXP) and stationary (STAT) growth phases and of COM.

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

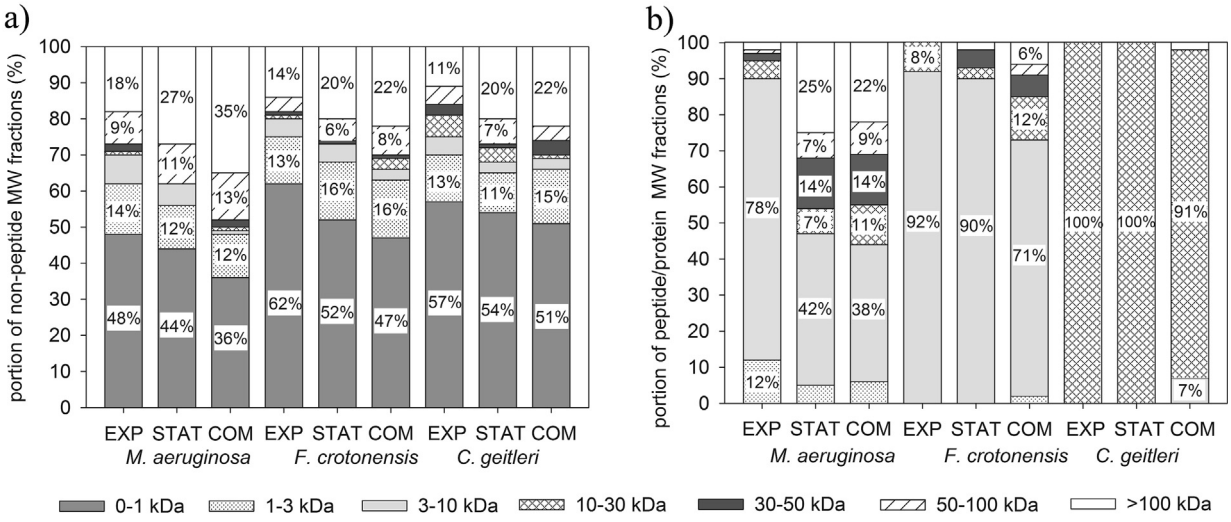

Fig. 4 – Molecular weight (MW) fractionation of non-peptide (a) and peptide/protein (b) portions of EOM at exponential (EXP) and stationary (STAT) growth phases, and of COM determined by centrifugation-driven filtration.

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

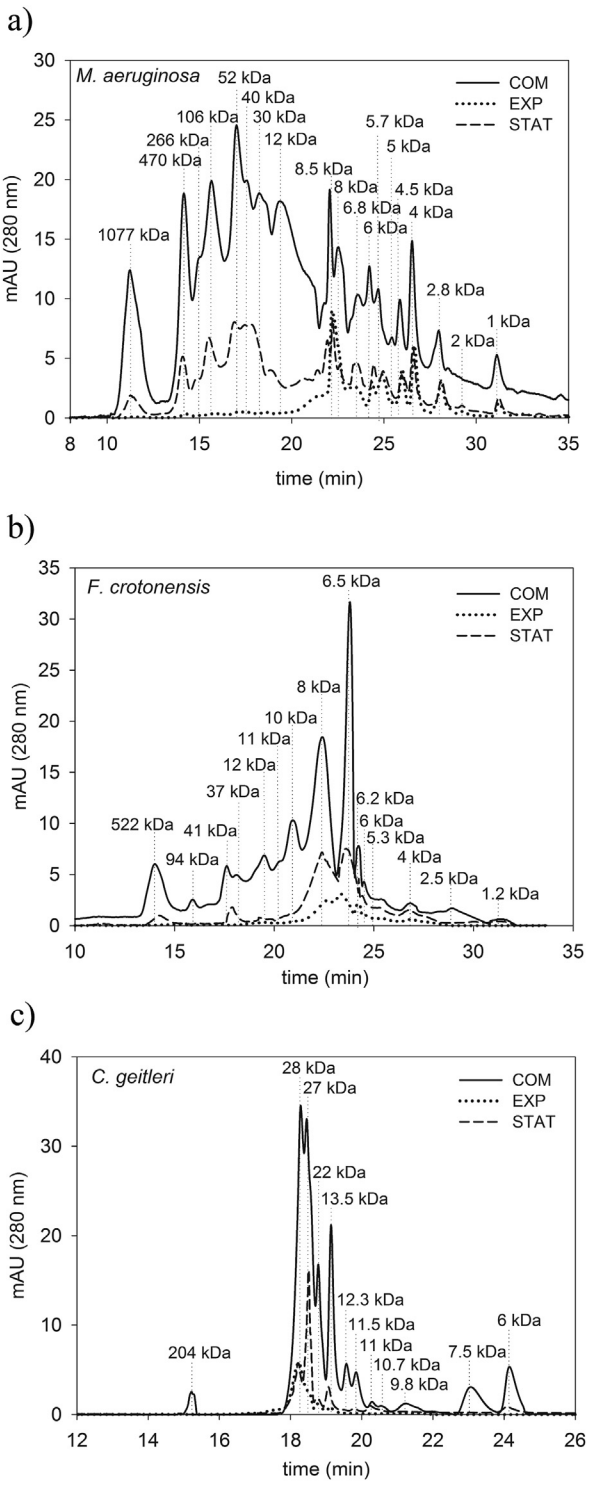

Fig. 5 – Molecular weight (MW) distributions of peptide/protein portions of EOM at exponential (EXP) and stationary (STAT) growth phases, and of COM determined by HPSEC for *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c).

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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Coagulation of peptides and proteins produced by *Microcystis aeruginosa*: Interaction mechanisms and the effect of Fe–peptide/protein complexes formation

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ABSTRACT

This paper focuses on elucidation of the mechanisms involved in the coagulation of peptides and proteins contained in cellular organic matter (COM) of cyanobacterium *Microcystis aeruginosa* by ferric coagulant. Furthermore, coagulation inhibition due to the formation of Fe–peptide/protein surface complexes was evaluated. The results of coagulation testing imply that removability of peptides and proteins is highly dependent on pH value which determines charge characteristics of coagulation system compounds and therefore the mechanisms of interactions between them. The highest peptide/protein removal was obtained in the pH range of 4–6 owing to charge neutralization of peptide/protein negative surface by positively charged hydrolysis products of ferric coagulant. At low COM/Fe ratio (COM/Fe <0.33), adsorption of peptides/proteins onto ferric oxide-hydroxide particles, described as electrostatic patch model, enables the coagulation at pH 6–8. On the contrary, steric stabilization reduces coagulation at pH 6–8 if the ratio COM/Fe is high (COM/Fe >0.33). Coagulation of peptides and proteins is disturbed at pH 6–7 as a consequence of Fe–peptide/protein complexes formation. The maximum ability of peptides/proteins to form soluble complexes with Fe was found just at pH 6, when peptides/proteins bind 1.38 mmol Fe per 1 g of peptide/protein DOC. Complex forming peptides and proteins of relative molecular weights of 1, 2.8, 6, 8, 8.5, 10 and 52 kDa were isolated by affinity chromatography.

1. Introduction

The excess growth of phytoplankton and subsequent production of algal organic matter (AOM) occurring mainly during algal blooms in eutrophicated water areas bring significant problems in drinking water treatment processes (Henderson et al., 2010). The most serious adverse effects of AOM include coagulation inhibition resulting in increased coagulant demand (Bernhardt et al., 1985; Takaara et al., 2010; Ma et al., 2012a), increased membrane fouling (Campinas and Rosa, 2010) and production of hazardous trihalomethanes and haloacetic acids (Fang et al., 2010). AOM is also a source of abnormal odour and taste and, in the case of cyanobacteria, of many toxins (Harada, 2004).

AOM arises extracellularly through metabolic excretion as extracellular organic matter (EOM) or intracellularly during autolysis of cells forming cellular organic matter (COM) (Takaara et al., 2007). This organic cocktail is a mixture of proteins, peptides, amino acids, polysaccharides, oligosaccharides, lipids and various organic acids (Fogg, 1983). The

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AOM composition can be characterised as peptide/protein and non-peptide organic matter (Pivokonsky et al., 2006). It was ascertained that the molecular weight (MW) and concentration of peptide and non-peptide organic matter is highly variable depending on both species and age of the culture (Henderson et al., 2008). For example, the COM of cyanobacterium *Microcystis aeruginosa* comprises about 60% of peptide/protein substances, while EOM is mainly composed of organic matter which is of non-peptide character (polysaccharides, oligosaccharides and monosaccharides). Moreover, the portion of peptide/protein substances forming AOM increases with the ageing of organisms (Pivokonsky et al., 2006).

Water treatment by means of coagulation is very sensitive to the sudden increase of AOM in raw water, which can cause severe problems (Bernhardt et al., 1985; Pivokonsky et al., 2009a; Takaara et al., 2010). Until now, two aspects of coagulation of AOM in drinking water treatment have been investigated: the efficiency of AOM removal from water and the influence of AOM on coagulation of other particulate impurities present in water (Bernhardt et al., 1985; Takaara et al., 2007, 2010). Efficient removal of AOM requires sufficient particle destabilization to take place at a pH value at which the metal hydroxopolymers bear the highest charge and the organic macromolecules provide the most active centres (Bernhardt et al., 1985).

Some authors dealt with the influence of COM on coagulation of other impurities. Takaara et al. (2007, 2010) focused on the inhibitory effect of proteins on coagulation of kaolin suspension. They ascertained that due to their high affinity with metal ions, several proteins inhibit coagulation by the formation of highly soluble coagulant metal–protein complexes. It was found that formation of these complexes gives rise to an increase in dissolved and colloidal aluminium or iron that cannot be effective in the coagulation process, and results in increase of coagulant dose required (Bernhardt et al., 1985; Pivokonsky et al., 2006, 2009a; Sano et al., 2011; Ma et al., 2012a). Therefore, the characterization of peptides and proteins forming dissolved complexes with coagulant and the quantification of the peptide/protein inhibitory effect under different reaction conditions should be carried out. It is a highly desirable task, because the increase in coagulant dose and the decrease in the coagulation efficiency severely reduce the performance of the conventional water treatment process.

The paper deals with the effect of COM peptides and proteins isolated from cyanobacterium *M. aeruginosa* on the coagulation process in drinking water treatment. The aims of the study are:

1) To characterise peptides and proteins contained in *M. aeruginosa* COM and determine which of them are able to form complexes with the metal component of the coagulant (i.e. Fe); furthermore, to quantify their ability to form complexes (amount of Fe that can be incorporated in these complexes); and finally, to explain the mechanisms of complex formation,
2) To evaluate the influence of pH on the peptide/protein removal; and again, to explain the interaction mechanisms between COM peptides and proteins and Fe-hydroxopolymers (coagulant products) which asserts themselves at different pH values.

2. Material and methods

2.1. *M. aeruginosa* cultivation

The cyanobacterium *M. aeruginosa* was used in this study. Inoculum of this strain was obtained through the kind generosity of the Department of Culture Collection of Algal Laboratory, Institute of Botany, AS CR, Czech Republic. The strain of *M. aeruginosa* was cultured according to the methodology described in the literature (Pivokonsky et al., 2006, 2009a). The culture of *M. aeruginosa* was harvested on the 16th day of cultivation during the steady-state growth.

2.2. *COM preparation*

The microorganisms' cells were separated from the growth media by 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 homogenizer (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.

2.3. *COM peptides/proteins characterization*

2.3.1. *Isolation and determination of COM peptide/protein portion*

The COM was characterised in terms of the amount of peptide/protein and non-peptide (especially neutral and charged polysaccharide) organic carbon (Fogg, 1983; Pivokonsky et al., 2006; Henderson et al., 2008). Peptides and proteins were isolated from the COM using $(NH_4)_2SO_4$ as a peptide/protein precipitant. The methodology of peptide/protein precipitation is described in the literature (Dawson et al., 1986).

The peptide/protein precipitate was then separated from the dissolved organic matter by filtration through a 0.22 μm membrane filter (Millipore, USA), and DOC_{NP} was analyzed in the filtrate. The peptide/protein portion DOC_P was calculated as follows:

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

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 (polysaccharide) DOC.

The peptide/protein precipitate was then 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). The stirred cell was operated at 60 RPM with the constant nitrogen pressure of 1 bar. 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}.

2.3.2. *DOC analysis*

Dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC-V_{CPH} analyzer (Shimadzu Corporation, Japan). All

samples were first filtered through a 0.22 μm membrane filter (Millipore, USA). All measurements were conducted in triplicate and errors of measurement were less than 2%.

2.3.3. Molecular weight fractionation

The MW fractionation by a high performance size exclusion chromatography (HPSEC) was performed 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 1100 series, Agilent Technologies, USA) was coupled with a diode array detector (DAD) operated at 280 nm. Prior to HPSEC, the maximum absorption wavelength ($\lambda_{max} = 280$ nm) of COM peptides and proteins was detected in UV–vis absorbance spectra using a UV–vis 8452A spectrophotometer (Agilent Technologies, USA). Therefore, the wavelength 280 nm was used for the COM peptide and protein detection. The HPSEC mobile phase used for the MW fractionation was 0.15 M phosphate buffer (pH 7.0). The flow rate was 1 mL min^{-1} at the temperature of 25 °C and the sample injection volume was 60 μL. The DOC_P concentration of all peptide/protein samples was 100 mg L^{-1} to eliminate potential concentration effect on HPSEC. The system was calibrated using peptide and protein SEC standards (Sigma–Aldrich, USA) of MW range from 224 Da to 900 kDa. A semi-log calibration curve was utilized to calculate the MW ($r = 0.98$). BioRad gel filtration standards of chicken ovalbumin (44 kDa) and bovine gamma globulin (158 kDa) were used as control samples. The standard error was ±0.95 kDa for gamma globulin and ±0.64 kDa for chicken ovalbumin. The reproducibility of the MW fractionation of COM peptide/protein samples was very good with MW deviations of less than 3% from repeated measurements.

2.3.4. Determination of peptide and protein isoelectric point

Isoelectric points (p*I*) of isolated COM peptides and proteins were determined by isoelectric focusing (IEF) which was carried out with a Multiphor II electrophoresis system (Pharmacia, Sweden). The IEF gel (7.5%) was prepared using ampholines of p*I* 2.5–5.0 and 3.5–10.0 (Pharmacia, Sweden). A standard calibration curve with broad-p*I* protein calibration kit, p*I* 3.0–10.0 (Pharmacia, Sweden) was used to determine the isoelectric points. Gels were stained with silver staining kit (BioRad Silver Stain, USA) and activity-stained with 0.005 M guaiacol.

2.4. Evaluation of Fe–peptide/protein complex formation

2.4.1. Screening of COM peptides and proteins having an affinity with iron

Affinity chromatography was used for the detection of peptides and proteins which are able to form soluble complexes with iron used as coagulant. 10 mL of peptide/protein samples were applied to the affinity column (1 mL HiTrap™, Amersham Bioscience Corp., Japan) in which Fe^{3+} ions were immobilized as ligands. The binding buffer for the affinity chromatography was 0.02 M sodium phosphate containing 0.5 M NaCl (pH 7.2; Fe is present in the form of Fe-oxide-hydroxides). The elution buffer was the same as the binding buffer, but its pH was set to 9 when Fe is present in the form of anionic hydroxopolymers. The flow rate of buffer phase was 1 mL min^{-1} and volume of the collected fractions was 15 ml. Then, the affinity chromatographic fractions were desalted and concentrated by an ultrafiltration membrane PLAC 1000 Da (Millipore, USA) using a Solvent Resistant Stirred Cell (Millipore, USA). MW of isolated peptides and proteins was determined by HPSEC. All analyses were performed in triplicate.

2.4.2. Determination of amount of Fe bound in peptide/proteins complexes

In order to quantify the Fe–peptide/protein binding capacity, 250 mL of COM peptide/protein samples with different DOC concentrations ranging from 1 to 8 mg L^{-1} were mixed with defined amounts of pure Fe standard (0.1, 0.5, 1, 1.5 and 2 mg L^{-1} Fe) (Sigma–Aldrich, USA). The experiment was held under pH values ranging from 2 to 10. The pH value was adjusted by 0.1 M HCl and 0.1 M NaOH. Coagulated portions of peptides/proteins and iron were removed by a 0.22 μm membrane filter (Millipore, USA). Subsequently, non-coagulated peptide/protein substances (including soluble Fe–peptide/protein complexes) and iron were separated by an ultrafiltration membrane PLAC 1000 Da (Millipore, USA) using a Solvent Resistant Stirred Cell (Millipore, USA). The amount of Fe bound in peptide/protein soluble complexes was determined as a difference in Fe concentration after filtration through a 0.22 μm and through the ultrafiltration membrane of 1000 Da.

2.5. Coagulation tests

Ultra-pure water with COM peptides/proteins of DOC concentrations of 1, 3, 5 and 8 mg L^{-1} was used in coagulation tests. These concentrations represent the typical COM peptide/protein content in natural surface water (Pivokonska et al., 2008). Jar testing was done with the LMK 8-03 (IH ASCR, Czech Republic) variable speed eight paddle stirrer and 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 on peptide/protein COM coagulation is higher comparing to aluminium coagulants. The tested doses were in the range of 0.018–0.268 mmol L^{-1} Fe (1–15 mg L^{-1} Fe). The pH of raw water samples was adjusted using predetermined amount of 0.1 M HCl and NaOH in order to reach the final suspension pH (after coagulant addition) ranging approximately from 3 to 10. The jar test procedure consisted of 1 min of high intensity mixing ($\overline{G} = 300$ s^{-1}), 15 min of low intensity mixing ($\overline{G} = 80$ s^{-1}) and 60 min of settling. The results of coagulation testing were evaluated by water analysis after the sedimentation of suspension. Primarily, residual Fe, DOC, pH value, MW of residual peptides and proteins were monitored.

3. Results and discussion

3.1. COM peptide and protein characterization

The results of COM characterization show that the COM of *M. aeruginosa* is composed of protein and non-protein organic

matter. The protein portion, determined as DOC_P, accounted for approximately 62.9% of DOC_T in the COM, and the non-protein portion of organic matter made up the balance of 37.1%. This result is in agreement with the literature where the protein portion was measured at about 64% of DOC_T for *M. aeruginosa* during the steady-state growth (Henderson et al., 2008). The COM proteins, which were consequently used in coagulation experiments, were characterized in terms of MW distribution. Fig. 1 shows the HPSEC chromatogram with apparent MW of the isolated COM peptides/proteins. Peptides and proteins of 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 identified as components of COM. The isoelectric points of isolated COM peptides and proteins were determined by isoelectric focusing (IEF). The measured values of peptide/protein pI 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.

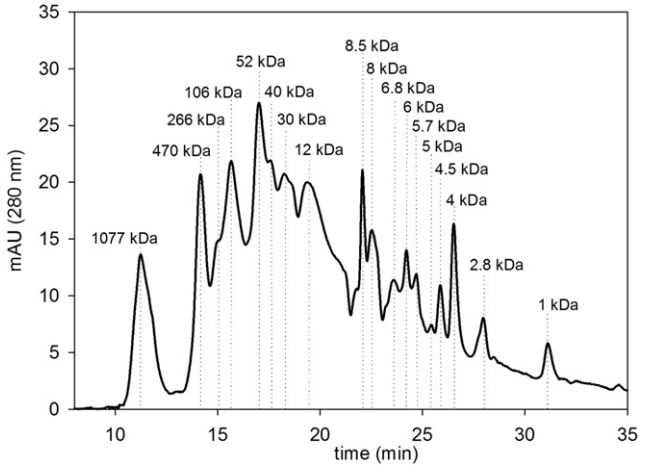

Fig. 1 – Molecular weight distribution of COM peptides and proteins produced by *M. aeruginosa*.

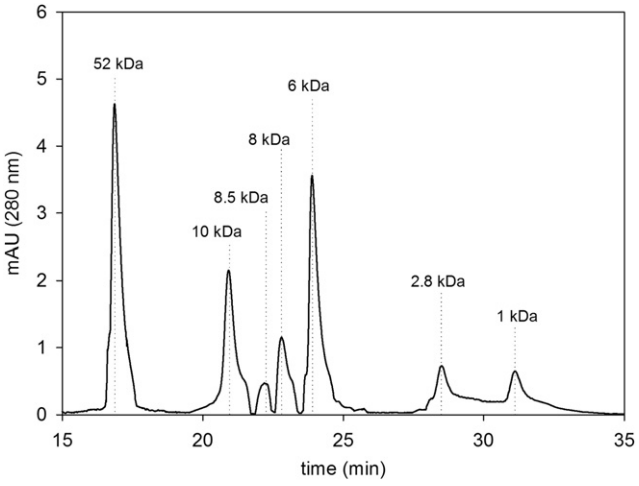

Fig. 2 – Molecular weight distribution of complex forming peptides and proteins isolated by affinity chromatography.

3.2. *Formation of complexes between COM peptides/proteins and iron*

3.2.1. *Complex forming peptides and proteins*

Affinity chromatography (AC) and subsequent HPSEC were used for the detection of peptides and proteins able to form complexes with iron used as coagulant. Fig. 2 shows the peaks of isolated complex forming peptides and proteins of MWs of approximately 1, 2.8, 6, 8, 8.5, 10 and 52 kDa. Birch and Bachofen (1990) observed that the majority of complexing ligands produced by microorganisms are of low molecular weight (MW <10 kDa). Low-MW peptides may include linear and cyclic oligopeptides such as cyanopeptolin (MW approximately 950 Da), aeruginopeptin (MW 1022–1072 Da), micropeptin (MW 885–1110 Da), ferintoic acid (MW approximately 880 Da), microcystin (MW 994 Da) and/or cyanochlorophyll (MW approximately 900 Da) which may be able to bind iron into their molecule (Harada, 2004).

Moreover, it is very likely that low-MW region includes iron-binding siderophores with MW ranging from 500 to 1500 Da. Siderophores are high-affinity Fe-binding compounds secreted by cyanobacteria under iron-limiting culture conditions and they facilitate the transport of ferric ions into cells during periods of iron deficiency (Wilhelm, 1995). They bind themselves to periplasmic binding proteins (MW 8–100 kDa) which assist for the transport of siderophores through the cytoplasmic membrane into cyanobacterial cytoplasm (Krewulak and Vogel, 2008). In our study, Fe–protein complexes with MW 8–52 kDa probably represent the family of these iron transporters.

Furthermore, Fe–protein complexes released from *M. aeruginosa* may well contain cyanobacterial metalloenzymes hydrogenases which have affinity with iron (Tamagnini et al., 2007). Asada et al. (1987) isolated Fe-binding hydrogenase with MW about 50–56 kDa from *M. aeruginosa* cells. The protein of around 60 kDa with high iron affinity was isolated from COM of cyanobacterium *M. aeruginosa* in our previous study (Pivokonsky et al., 2006). These results are in agreement with the study of Takaara et al. (2007) in which proteins of *M. aeruginosa* COM of 43–67 kDa were identified as chelate complex forming substances.

3.2.2. *Mechanisms and evaluation of complex formation*

Fe–peptide/protein surface complexes are predominantly formed by the coordinate bond of dissociated –COOH groups ($-COO^-$) on the surface of peptides and proteins and positively charged Fe-hydroxopolymers and Fe-oxide-hydroxides necessary for coagulation (Bernhardt et al., 1985). The prevailing type of charged Fe-hydroxopolymer depends on a pH value. Fe^{3+} ions occur to about 99% as Fe-hexaaquacomplex $[Fe(H_2O)_6]^{3+}$ in an aqueous medium at pH <2. At pH ~2, due to the release of protons from the ion, the hydrolysis is so advanced that the double nuclear Fe-hydroxocomplex $[Fe_2(OH)_2]^{4+}$ ions are formed. As the pH rises (pH >2), 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 consequently formed (Stumm and Morgan, 1996). Positively charged Fe-hydroxopolymers and Fe-oxide-hydroxides interact by coordinate electrostatic bonds with dissociated –COOH groups ($-COO^-$) of peptide and protein molecules (Sigg and

Stumm, 1981; Stumm and Morgan, 1996). Iron necessary for coagulation is, thus, incorporated in stable soluble or microcolloid Fe–peptide/protein complexes, which results in inhibition of cross-linking and clustering of Fe-hydroxopolymers and increases the coagulant demand accordingly (Bernhardt et al., 1985; Pivokonsky et al., 2006, 2009a; Ma et al., 2012b).

Soluble or microcolloid Fe–peptide/protein complexes can be formed only when carboxyl groups present in peptide side chains are deprotonated, i.e. approximately at pH >4.8 (RCOOH pK_a = 4.756) (Dawson et al., 1986). Other peptide/protein acid functional groups, for instance –SH and –OH groups, have their pKa constants at high pH values (RSH pKa = 8.33 and ROH pKa = 10.07) (Dawson et al., 1986), when Fe-hydroxocomplexes ($[Fe(OH)_4]^-$) are negatively charged. Thus, –SH and –OH groups have no importance to Fe–peptide/protein complexes formation. The connection between complexes formation and charges of Fe-hydroxopolymers and peptide/protein functional groups implies that the Fe-binding capacity (BC) depends on pH value. For the verification of BC, correlation coefficients between bound soluble Fe and COM peptide/protein DOC at pH values 5, 6, 7 and 8 were developed. At all pH values, a good correlation was observed. The correlation coefficients were 0.988 at pH 5, 0.986 at pH 6, 0.983 at pH 7 and 0.991 at pH 8. Fig. 3 shows Fe BC curves for DOC concentration of COM peptides and proteins ranging from 1 to 8 mg L^{-1} at pH 5, 6, 7 and 8. The slopes of these regression lines, i.e. 0.19, 1.38, 1.21 and 0.12, express the Fe-binding capacities (in millimoles of iron per gram of dissolved organic carbon) at corresponding pH values. The maximum amount of Fe–peptide/protein complexes was found at pH 6, when the ability of peptides and proteins to capture the positively charged Fe-hydroxopolymers by means of a coordinate electrostatic interaction reached 1.38 mmol g^{-1} (also Fig. 3). These data are in agreement with Gregor et al. (1996), who have determined the maximum Al binding capacity of 1.6 mmol g^{-1} for EOM at pH 6. Frimmel et al. (1983) have ascertained the iron-binding capacity of 1.2 mmol g^{-1} for fulvic acids. Thus, COM peptides and proteins show practically the same binding capacity for iron cations as fulvic acids, the most complex forming humic natural organic matter.

The influence of pH value on the amount of iron incorporated in organic matter and conditions of Fe–peptide/protein surface complexes formation are shown in Fig. 4. The isoelectric points (p*I*) of complex forming peptides and proteins (see Fig. 2) determined by isoelectric focusing (IEF) were 4.79, 5.12, 5.25, 5.45, 5.62, 5.80 and 6.10. The ascertained values of peptide/protein p*I* indicate that at pH <5.5 (average p*I*), the positive charge of complex forming peptides predominates. Therefore, the formation of soluble Fe–peptide/protein complexes is insignificant (BC ranged from 0.19 mmol g^{-1} at pH 5 to zero value at pH 2), because the positive charge of peptides/proteins interferes with positively charged Fe-hydroxopolymers and repulsion occurs. In the pH range between 5.5 and 7.5, the Fe–peptide/protein complexes formation considerably increases, due to the attractive electrostatic interaction between (negatively charged) deprotonated acid functional groups of peptides/proteins and positively charged Fe-hydroxopolymers. At pH >7.5, iron largely occurs as anionic hydroxocomplexes, e.g. $[Fe(OH)_4]^-$ (Stumm and Morgan, 1996) and peptides and proteins are negatively charged. Thus, the formation of Fe–peptide/protein complexes is significantly reduced owing to the electrostatic repulsion between iron compounds and peptides/proteins (BC ranged from 0.12 mmol g^{-1} at pH 8 to 0.01 mmol g^{-1} at pH 10). A similar mechanism of repulsion at high pH values was described in the case of the interaction between deprotonated carboxyl groups of COM peptides and surface acidic oxygen groups of activated carbon (Hnatukova et al., 2011).

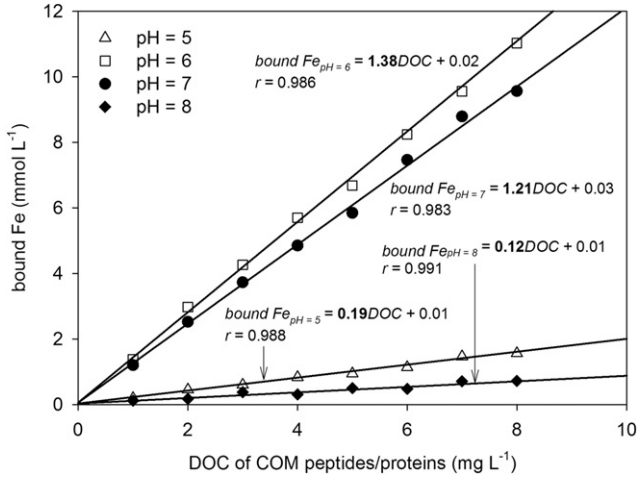

Fig. 3 – Fe-binding capacities of COM peptides/proteins of DOC concentrations 1–8 mg L^{-1} for pH values 5, 6, 7, and 8.

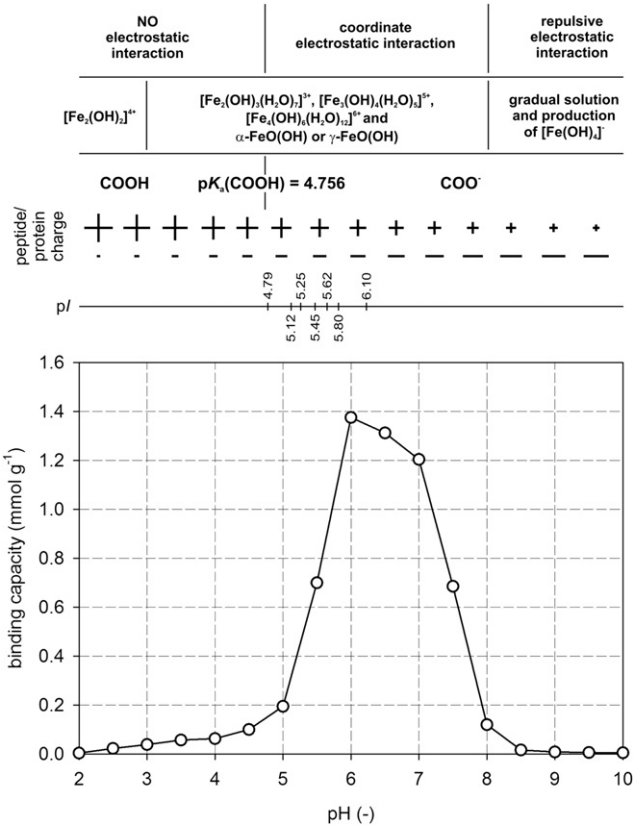

Fig. 4 – Description of Fe-binding capacity as a function of pH and charge characteristics.

3.3. Coagulation of COM peptides/proteins

3.3.1. Influence of pH on COM peptide/protein coagulation

In order to optimise the dose of the ferric coagulant, jar tests with raw water samples containing COM peptides/proteins of DOC concentrations of 1, 3, 5 and 8 mg L^{-1} were performed without pH control. The change of pH was dependent on the iron dose and ranged from 3 to 10. The optimum coagulant dose for DOC removal increased with increasing DOC concentration, while the highest Fe removal was achieved at the same coagulant dose of 0.125 mmol L^{-1} (7 mg L^{-1} Fe) for all initial DOC concentrations. Subsequently, this optimized dose was used in a coagulation testing with pH control which was aimed at describing the influence of the pH value on COM peptide/protein coagulation.

The results of coagulation tests (Fig. 5a) demonstrated that the lowest residual protein/peptide (DOC) concentrations were achieved across the pH range from 4 to 6 for all initial DOC concentrations. Interestingly, the pH range of the minimum residual Fe concentration slightly moved with decreasing initial DOC concentration from pH 4–6 for DOC = 8 mg L^{-1} to pH 5.5–7.5 for DOC = 1 mg L^{-1} (Fig. 5b). The shift of the lower boundary of the pH range optimal for Fe removal from 4 (DOC = 8 mg L^{-1}) to 5.5 (DOC = 1 mg L^{-1}) was simply caused by the low peptide/protein concentration, when there were too few negatively charged sites on the protein side chains to react with positive Fe-hydroxopolymers, and therefore, Fe remained in the solution. The shift of the upper boundary of the pH range optimal for Fe removal from 6 (DOC = 8 mg L^{-1}) to 7.5 (DOC = 1 mg L^{-1}) is discussed in Section 3.3.2. At pH between 6 and 7 (with the maximum at around 6.2), a noticeable increase in residual DOC and iron concentrations was observed. It is attributable to the formation of surface complexes between soluble or microcolloid Fe and peptide/protein deprotonated carboxyl groups, as described in Chap. 3.2. In detail, peptides and proteins used in coagulation tests exhibited the highest productivity in complexes formation at pH between 6 and 7 (binding capacity >1.2 mmol g^{-1}). As a result, Fe ions are bound into the organic molecules and therefore are no longer active in the coagulation process (Bernhardt et al., 1985; Pivokonsky et al., 2006; Takaara et al., 2007). Interestingly, many water treatment plants treat raw water containing COM within the pH range from 6 to 7, which leads to inefficient coagulation.

In addition, iron bound to proteins blocks negative charged sites on the protein surface, and in this way prevents proteins from being coagulated by adsorption and charge neutralization mechanisms. It should be noted that under certain conditions (sufficiently high dose of coagulant, pH >6 etc.), Fe–peptide/protein surface complexes can be efficiently removed during enmeshment – accelerated iron oxide-hydroxides formation (Ma et al., 2012a,b). As seen in Fig. 5, at pH >7 both residual DOC and iron concentrations sharply rise, which implies that coagulation does not take place.

HPSEC analysis provides insight into the variation of protein composition after coagulation tests. Chromatograms in Fig. 6 compare MWs of peptides and proteins after coagulation at pH levels of 5, 6.2 and 8 for initial DOC concentration of 5 mg L^{-1}. These results indicate that high-MW proteins are removed more efficiently at pH value of 5 compared to pH 8. At pH ~5, high-MW proteins of MW >10 kDa were completely removed and only peptides of MW of approximately 1, 2.8, 4.5, 5.5, 6, 8, 8.5 and 10 kDa were identified in the solution. On the contrary, a negligible amount of high-MW proteins was removed during coagulation at pH 8. This observation is consistent with conclusions of other studies (Ma et al., 2012a; Pivokonsky et al., 2009a), in which high-MW COM organics are removed with higher efficiency than low-MW ones. At the pH value of maximum binding capacity (pH ~6.2), peptides and proteins of MW of 1, 2.8, 4.5, 5.5, 6, 8, 8.5, 10 and 52 kDa were identified in the solution after coagulation. The similarity between this spectrum and the chromatogram of complex forming peptides and proteins isolated by affinity chromatography (Fig. 2) indicate that considerable portion of peptides and proteins determined after coagulation at pH 6.2 represent Fe–peptide/protein complexes.

3.3.2. Mechanisms involved in coagulation of COM peptides/proteins

As mentioned above, peptides and proteins contain many functional groups, such as –OH, –COOH, –SH, $-NH_2$, $-CONH_2$ etc., which are able to possess charge under certain pH conditions. The surface charge of peptides and proteins is the source of their reactivity towards their surroundings, and leads to

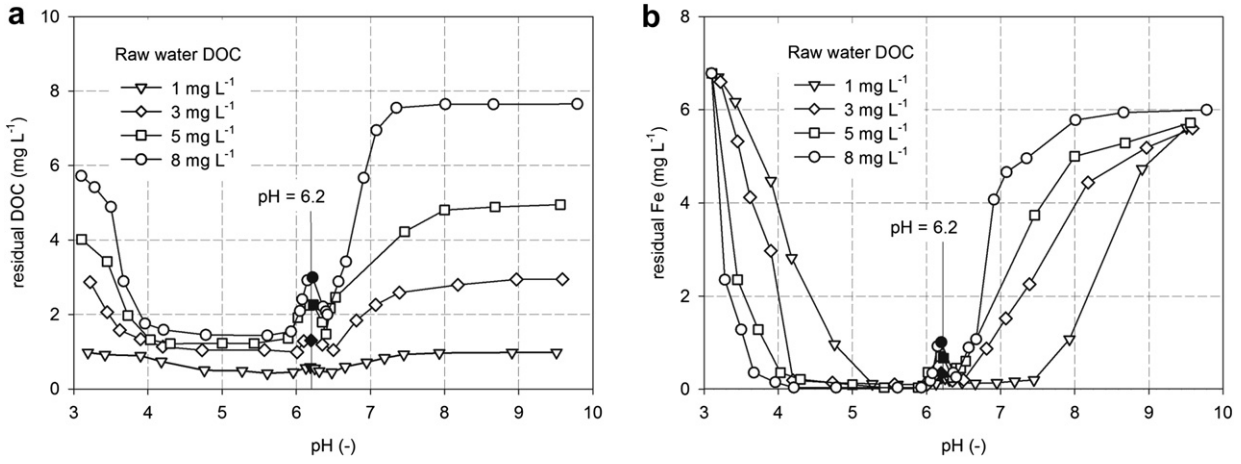

Fig. 5 – Dependence of residual DOC (a) and residual Fe (b) on pH value for initial DOC concentrations of 1, 3, 5 and 8 mg L^{-1}.

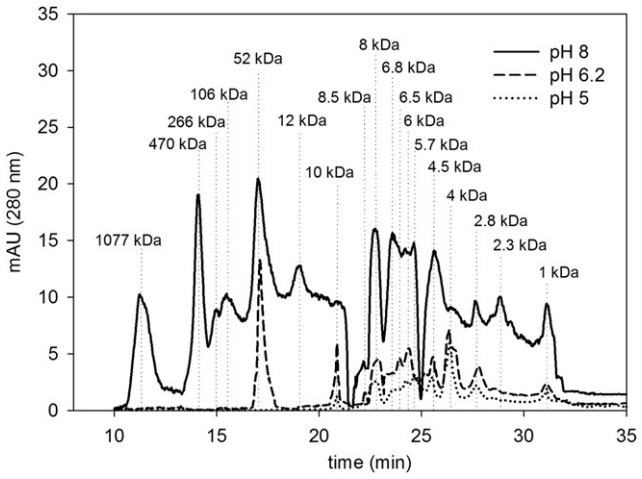

Fig. 6 – Molecular weight distributions of residual peptides and proteins after coagulation tests at pH values 8, 6.2 and 5 for initial DOC concentration of 5 mg L^{-1}.

electrostatic interactions with Fe-hydroxopolymers and Fe-oxide-hydroxides (Burns et al., 1996), and, thus, allows the coagulation by charge neutralization and/or adsorption (Bernhardt et al., 1985). The ascertained values of peptide/protein p*I* (see Chap. 3.1.) indicate that, approximately at pH <4, the positive charge of peptides and proteins prevails, i.e. deprotonated acid functional groups on peptide side chains are rare. Naturally, Fe-hydroxopolymers also bear a positive charge at this pH. As a consequence of prevailing positive charge, the efficiency of coagulation is low.

The highest coagulation efficiency was reached over pH range from 4 to 6, where the electrostatic interactions between negatively charged acid functional groups (especially $-COO^-$ groups) of peptides/proteins and positively charged Fe-hydroxopolymers are enabled. These attractive interactions result in gradual neutralization of peptide/protein surface charge and consequently allow the formation of uncharged micro-aggregates. Neutralization of surface charge is likely to be a major mechanism of coagulation of peptides and proteins.

However, at pH 6–8, the adsorption of COM peptides and proteins on the surface of colloidal Fe-hydroxopolymers and/or Fe-oxide-hydroxides may also be very important. The interaction proceeds mainly in accordance with the *electrostatic patch model* postulated by Gregory (1973). According to this theory, negatively charged peptides and proteins attach themselves to the positively charged colloidal particles of Fe-oxide-hydroxides (pH_{pzc} ~7.5). This results in the development of negatively charged areas on the surface of iron colloidal particles. When so charged particles collide with each other, a simultaneous attachment of oppositely charged areas to the surface of particles occurs. Besides the electrostatic patch model, adsorption may also be explained by the formation of hydrogen bonds between functional groups of peptides/proteins and Fe-oxide-hydroxides (Burns et al., 1996). However, the extent of adsorption during coagulation of organic matter is usually lesser in comparison with the charge neutralisation by electrostatic interactions (Pivokonsky et al., 2009a).

It has been noted that adsorption presumably plays an important role only when the concentration ratio between COM peptides/proteins and iron is low (Bernhardt et al., 1985). In this case, described in Fig. 5b (curve of residual Fe for initial DOC concentration of 1 mg L^{-1}; COM/Fe = 0.14), peptides and proteins can be adsorbed on the surface of colloidal Fe-oxide-hydroxides and may enhance coagulation by the formation of interparticle bridges. In contrast, if the concentration ratio of peptides and proteins is high (see Fig. 5b – curve of residual Fe for initial DOC concentrations of 3, 5 and 8 mg L^{-1}; COM/Fe = 0.43, 0.71 and 1.14, respectively), the whole surface of iron oxide-hydroxide particles can be occupied with COM proteins. It results in an increase in the negative charge on the surface of iron oxide-hydroxide particles and correspondingly, in their steric stabilization and inhibition of the coagulation process (Bernhardt et al., 1985; Liang et al., 2007). Bernhardt et al. (1985) pointed out that steric stabilisation appears to be effective in the inhibition of coagulation if the concentration ratio between AOM DOC and iron is greater than 0.33.

In addition, as already mentioned in Section 3.3.1, coagulation is disturbed by the formation of Fe–peptide/protein surface complexes at pH between 6 and 7. Complexes formation is caused by the coordinate electrostatic interaction between peptide/protein dissociated carboxyl groups and positively charged Fe-hydroxopolymers.

Finally, at pH >8, the charge of Fe-hydroxopolymers turns negative. The repulsive electrostatic interactions between negatively charged peptides and proteins, which bear a considerable amount of ionized functional groups, and anionic Fe-hydroxopolymers, i.e. $[Fe(OH)_4]^-$, lead to a significant reduction in coagulation.

4. Conclusions

The coagulation of *M. aeruginosa* peptides and proteins and the coagulation mechanisms were investigated. The presented results indicate that the removability of peptides and proteins by coagulation was shown to be strongly dependent on pH value. The highest coagulation efficiency was achieved due to the mechanism of charge neutralization in the pH range from 4 to 6. Interestingly, at pH value usually used in common practice for the coagulation of organic matter (pH 6–7), coagulation of peptides and proteins is disturbed by the Fe–peptide/protein complexes formation. The capacity of peptides and proteins to bound iron was found to be the highest at pH 6–7 and was observed to be >1.2 mmol g^{-1}. It was shown that high-MW proteins (MW >10 kDa) are easily removable, whereas coagulation of low-MW peptides proved to be very difficult, even in the pH range of the highest coagulation efficiency. Nearly the same low-MW peptides/proteins as those identified as complex forming ones remain in the water after coagulation. This confirms the idea of complexes formation to be the coagulation inhibition mechanism at this pH.

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Mechanismy koagulace při odstraňování peptidů a proteinů produkovaných fytoplanktonem

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Klíčová slova: Al/Fe-peptidové/proteinové komplexy; koagulace; organické látky produkované fytoplanktonem; peptidy/proteiny; vazebná kapacita.

Obsah

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-metylisoborneol (MIB) a trans-1,10-dimetyl-trans-9-decalol (geosmin, GSM), 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 trihalogenmetanů – THM (např. chloroform a dibromchlormetan) a halogenderivátů kyseliny octové – HAA (např. kyselina dichloroctová a trichloroctová). Do současné doby bylo detekováno přes 700 takových sloučenin vyskytujících se v pitné vodě zpravidla v koncentracích v řádech $\mu g.l^{-1}$, 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 Al/Fe 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], fyziologických 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 polysacharidy 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 *A. flos-aquae* 51% podíl a pouze 29% podíl peptidů/proteinů byl stanoven v případě zelené řasy *S. 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[16,18]. AOM ovlivňují koagulační procesy pomocí řady mechanismů. V závislosti

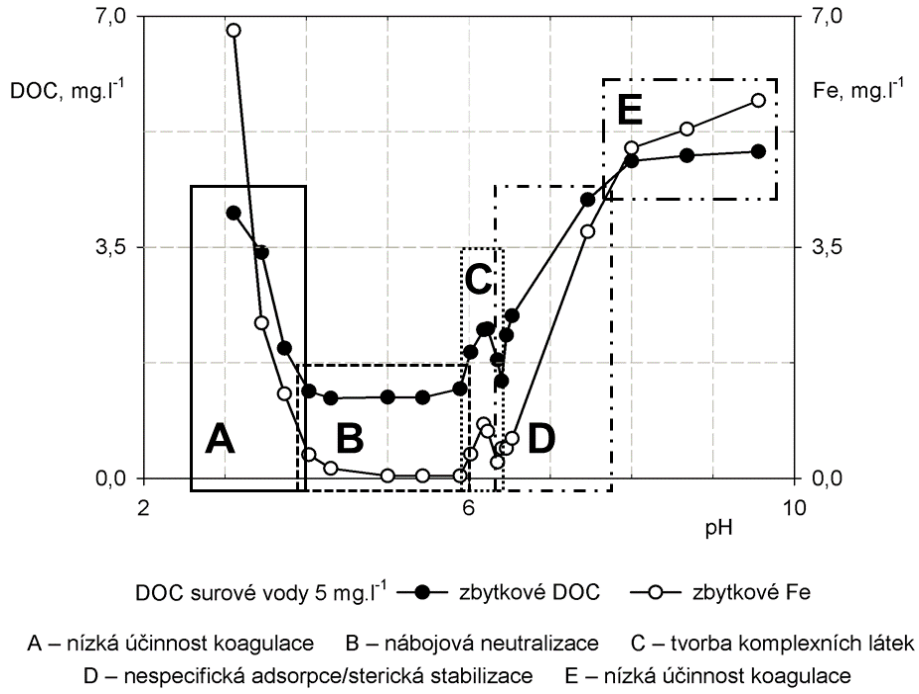
na koncentraci, složení a vlastnostech, jako je například distribuce molekulových hmotností, velikost povrchového náboje či hodnota izoelektrického bodu, vykazují AOM pozitivní i negativní účinky na koagulaci[10]. Při nízkých koncentracích mohou AOM účinnost koagulace zvyšovat, naopak jejich vysoké koncentrace 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[3,10,16,17,18,22] 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[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[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.



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

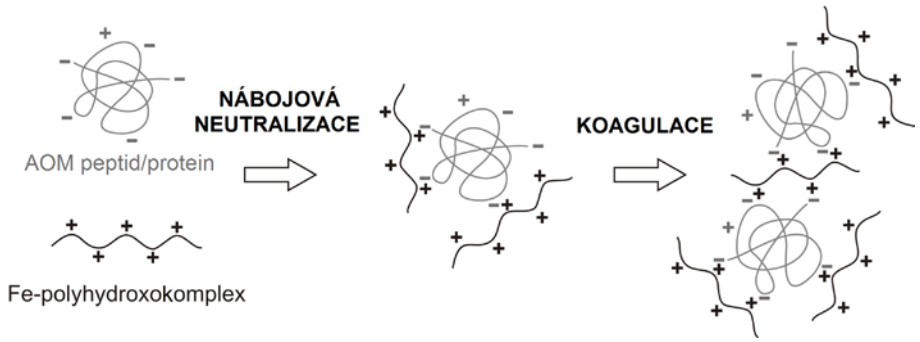
A – Oblast neúčinné koagulace

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 $–NH_3^+$ skupin (počet deprotonizovaných kyselých funkčních skupin je naopak velmi malý) a železo se vyskytuje v rozpuštěném 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 DOC 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 koncentracemi 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]).



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

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

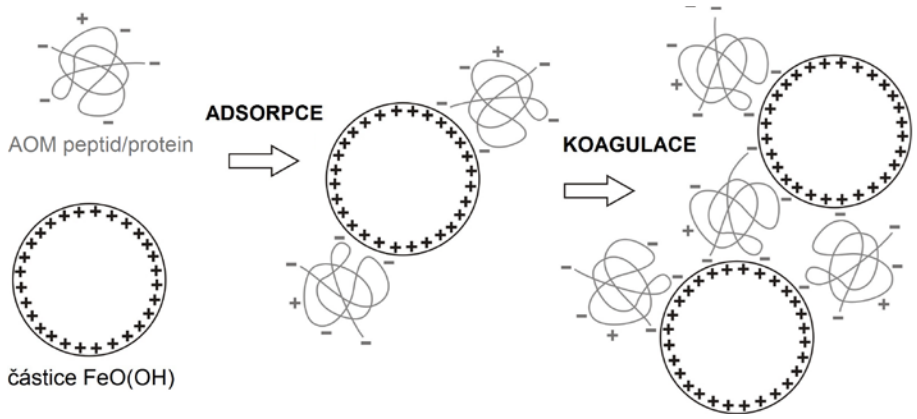
účinnosti koagulace, neúměrnému nárůstu účinné dávky koagulačního činidla a zvýšení zbytkových koncentrací organických látek a koagulantu (Al či Fe) v upravované vodě[3,11,14,16,26]. Nicméně, je třeba zdůraznit, že při dostatečně vysoké dávce koagulantu a pH > 7 lze Al -, resp. Fe-peptidové/proteinové 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

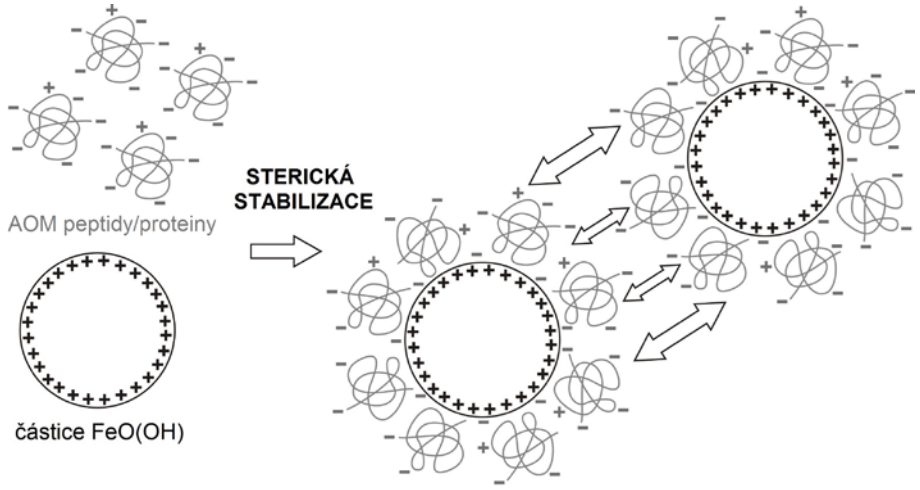
Z obr. 1 je dále patrné, že v oblasti D (pH 6,5-8) dochází k poměrně prudkému nárůstu zbytkových koncentrací DOC i Fe[16,22]. V této oblasti pH se již obvykle tak výrazně neuplatňují klasické elektrostatické interakce vedoucí k postupné nábojové neutralizaci systému, naopak začíná převládat mechanismus 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 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].



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

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:

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

Míra tvorby Al/Fe-peptidových/proteinových komplexů úzce souvisí s hodnotou disociační konstanty karboxylové skupiny vázané na povrchu AOM peptidů a proteinů, respektive 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[16,22] a proteiny o MH 43; 52 a 67 kDa[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), micropeptin (MH 885 až 1110 Da), ale i microcystiny (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 (hydrogenázy) 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[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 DBPs 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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Souhrn v českém jazyce

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(Ústav pro hydrodynamiku AV ČR, v. v. i., Praha; Ústav pro životní prostředí PřF UK, Praha): **Mechanismy koagulace při odstraňování peptidů a proteinů produkovaných fytoplanktonem**

Sezónní zvýšené koncentrace organických látek produkovaných fytoplanktonem (AOM) v surové vodě mohou narušit proces úpravy pitné vody. Cílem této práce je objasnění mechanismů podílejících se na koagulaci peptidových a proteinových látek produkovaných fytoplanktonem. Dle dostupných informací jsou dominantními mechanismy koagulace peptidů/proteinů nábojová neutralizace a nespecifická adsorpce. Maximálního odstranění peptidů a proteinů je dosahováno v rozmezí pH 4-6 v důsledku nábojové neutralizace záporně nabitého povrchu peptidů/proteinů kladně nabitými produkty hydrolýzy železitého koagulačního činidla. Při nízkém koncentračním poměru peptidů/proteinů a koagulačního činidla dochází navíc ke koagulaci peptidů/proteinů v rozsahu pH 6-8 mechanismem nespecifické adsorpce peptidů/proteinů na částicích hydratovaných oxidů železa, který popisuje elektrostatický patch model. Naopak při vysokém poměru koncentrací DOC a Fe zde dochází ke snížení účinnosti koagulace mechanismem sterické stabilizace. Ve slabě kyselé oblasti pH 6 dochází k narušení koagulačního procesu v důsledku tvorby rozpustných Fe-

peptidových/proteinových komplexních látek. Práce ukazuje, že účinnost koagulace a odstranitelnost peptidů/proteinů je silně závislá na hodnotě pH, která určuje nábojové charakteristiky peptidů/proteinů a produktů hydrolýzy koagulačního činidla a tedy i mezi nimi převládající interakce.

Souhrn v anglickém jazyce

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(Institute of Hydrodynamics, Academy of Sciences of the Czech Republic, Prague; Institute for Environmental Studies, Faculty of Science, Charles University in Prague): **Coagulation Mechanisms for Removal of Peptides and Proteins Produced by Phytoplankton**

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

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

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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).

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

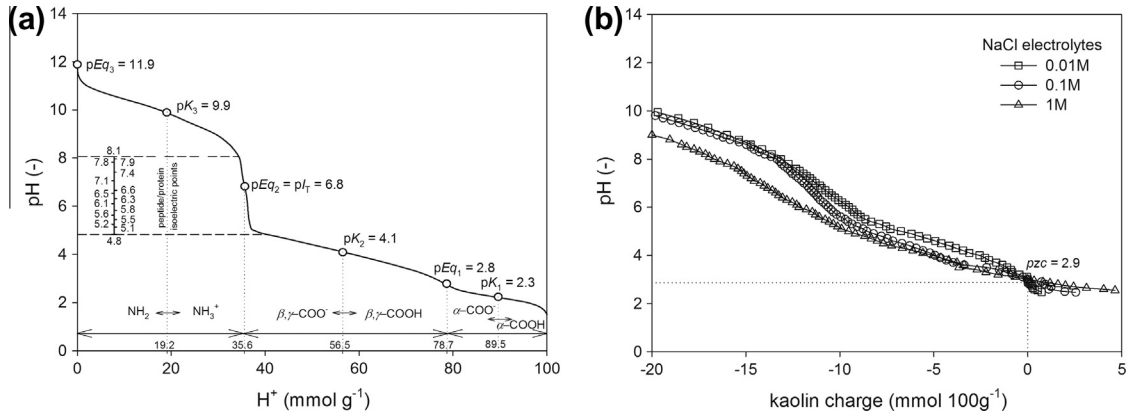

Fig. 1. (a) pH – titration curve for the COM peptide/protein mixture with equivalence points (pEq_1, pEq_2 and pEq_3), dissociation constants (pK_1, pK_2 and pK_3) and total isoelectric point (pI_T). Isoelectric points of single peptides/proteins contained in the mixture are depicted on the left. (b) 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 ≈ 2.9. This is consistent with the values reported in the literature, Coles and Yong [20] found the pzc at pH ≈ 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 ≈ 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.

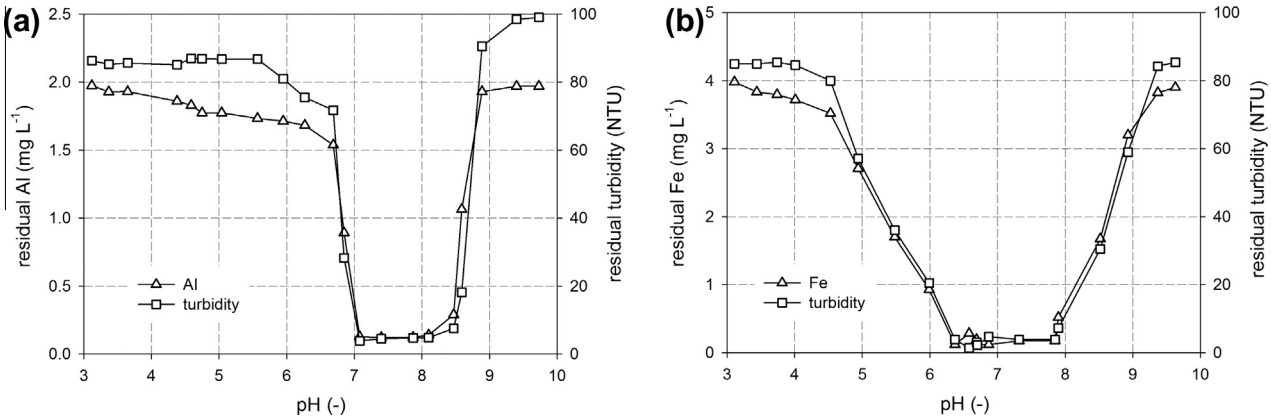

Fig. 2. Coagulation tests with kaolin and either Al (a) or Fe (b) – dependence of residual Al/Fe and turbidity on a pH value.

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

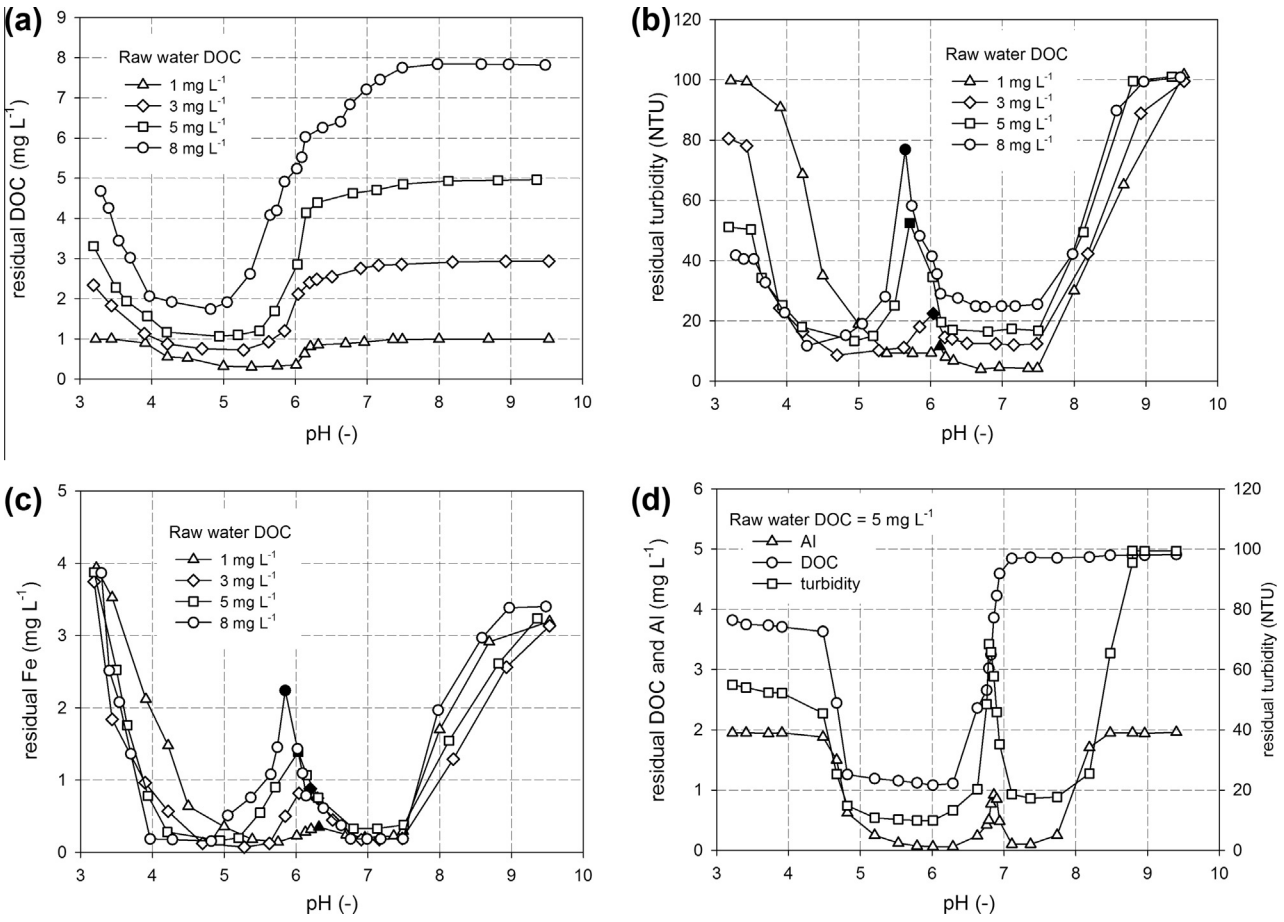

Fig. 3. Coagulation tests with Fe, COM peptides/proteins (initial DOC of 1, 3, 5 and 8 mg L^{-1}) and kaolin – dependence of residual DOC (a), turbidity (b) and Fe (c) on a pH value. (d) Coagulation tests with Al, COM peptides/proteins (initial DOC of 5 mg L^{-1}) and kaolin – dependence of residual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

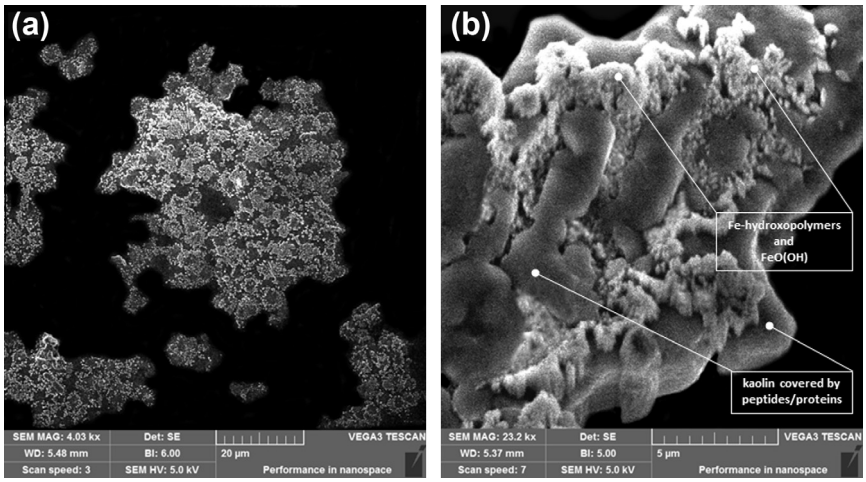

Fig. 4. SEM micrographs of aggregates formed through the interaction between Al/Fe, COM peptides/proteins and kaolin particles – complete (a) and detail (b) picture.

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

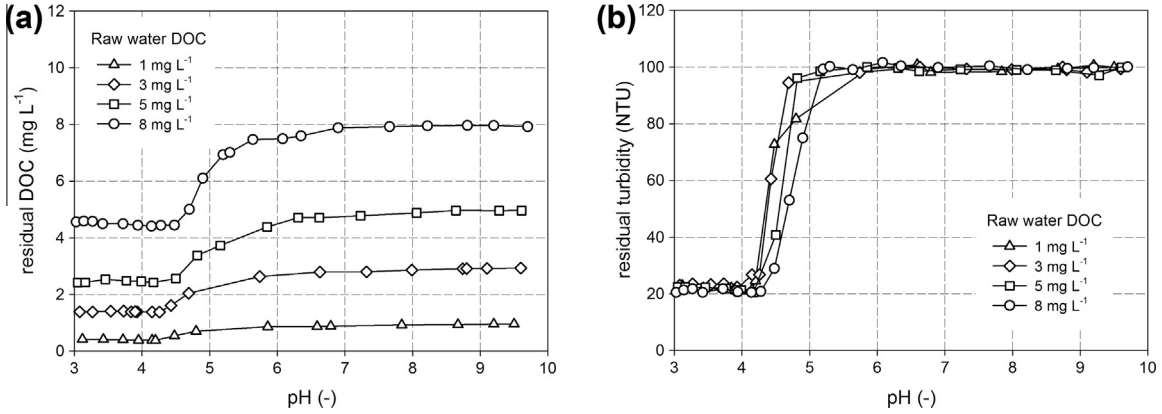

Fig. 5. Coagulation tests with COM peptides/proteins (initial DOC of 1, 3, 5 and 8 mg L^{-1}) and kaolin – dependence of residual DOC (a) and turbidity (b) on a pH value.

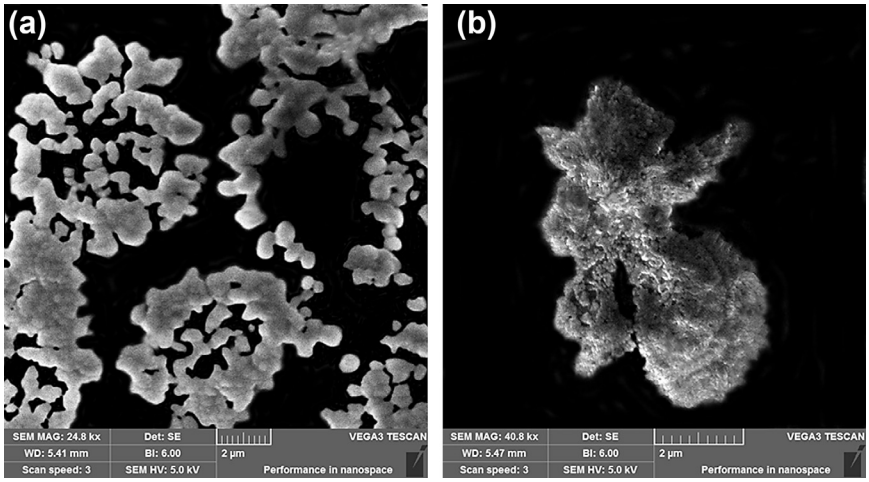

Fig. 6. SEM micrographs of aggregates formed through the interaction between COM peptides/proteins and kaolin particles (without Al/Fe) – complete (a) and detail (b) picture.

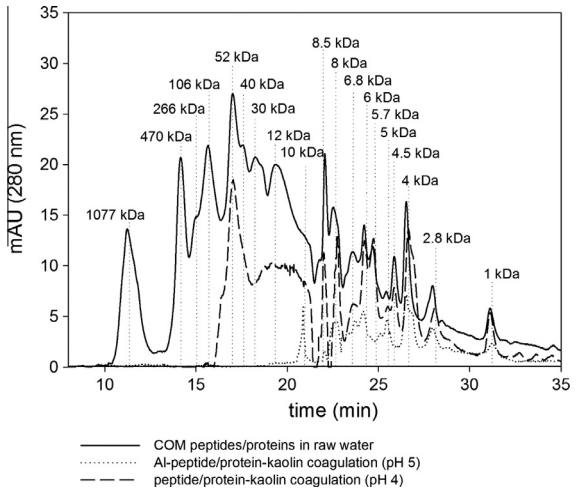

Fig. 7. Molecular weight distributions of COM peptides and proteins in raw water and residual peptides and proteins after coagulation test with: (1) Al, COM peptide/protein (initial DOC = 5 mg L^{-1}) and kaolin (the tests with Fe as a coagulant provided almost the same results; thus, only one of the 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.

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Adsorption of peptides produced by cyanobacterium *Microcystis aeruginosa* onto granular activated carbon

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ABSTRACT

The aim of this study was to investigate adsorption of peptides included in the cellular matter of cyanobacterium *Microcystis aeruginosa*, as they are difficult to remove during the coagulation/flocculation processes. In order to elucidate the effect of solution properties on peptide uptake, there were carried out equilibrium and kinetic experiments at different pH values and ionic strengths, using 2 types of activated carbon with different textural and charge characteristics – Picabiol 12×40 and Filtrasorb TL830.

The results showed that the peptide adsorption on both carbons increases with decreasing pH value. The highest adsorption capacity was reached at pH 5 for Picabiol 12×40 due to a high portion of mesopores in its structure and the electrostatic attraction between functionalities of the carbon and the peptides. It was demonstrated that increasing ionic strength can enhance adsorption of the peptides by screening the repulsive forces, or by strengthening the attractive ones in the adsorption system, all of that depending on the type of carbon used and pH applied. Among peptides, those with low molecular weight of 1.0–4.5 kDa were adsorbed preferentially. Formation of H-bonds and electrostatic interactions were confirmed to play an essential role during the adsorption of peptides onto activated carbon.

1. Introduction

Surface waters employed for drinking water production contain a variety of natural organic matter (NOM). NOM involves both humic compounds (e.g. humic and fulvic acids) and algal organic matter (AOM) that is produced by phytoplankton and provides a significant contribution to this heterogeneous mixture, mainly in the summer season [1]. AOM is released into the water by microorganisms' metabolism as extracellular organic matter (EOM), and during the cell decay as cellular organic matter (COM) [2]. Composition of AOM changes in dependence on the species, growth phase and life conditions. AOM usually comprises organic nitrogen compounds and macromolecules such as peptides, proteins, mono-/oligo- and polysaccharides, amino sugars and other organic acids [3,4], of which proteinaceous and polysaccharide substances form the majority [5,6]. In recent years, there has been observed an increasing concentration of AOM as a result of

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excessive eutrophication and subsequent growth of algal blooms, which causes significant challenges in drinking water treatment [7].

High concentration of AOM may lead to low coagulation efficiency and consequently to increased consumption of coagulants [6,8–11], to membrane fouling [7,12] and to high production of disinfection by-products [4,13]. Another serious problem is a production of either taste and odour compounds [14] or dangerous toxins [15,16]. Low molecular weight (MW) fraction of AOM also reduces the efficiency of activated carbon adsorption for anthropogenic micropollutants [17].

Previous studies have shown that coagulation inhibitory effects are often related to proteinaceous COM [9,18]. Pivokonsky et al. [10] found out that especially low-MW COM peptides (<10 kDa) produced by *Microcystis aeruginosa* were removed less efficiently than the high-MW proteins (>10 kDa), and therefore such peptides represent residual dissolved organic matter remaining in treated water after the coagulation/flocculation processes. This COM fraction may also include high concentration (hundreds of $\mu g\,L^{-1}$) of toxic microcystin–LR [16,19] and thus serves as a potential precursor of carcinogenic trihalomethanes and haloacetic acids that are formed during the process of water disinfection [13,20]. Therefore, more advanced treatment, such as adsorption onto granular or powdered activated carbon (AC), is required for COM removal [15,20,21].

Activated carbon has been widely employed for removal of NOM with humic character from surface waters [22–25]. Nevertheless, studies focused on adsorption of AOM are limited [14,17] and systematic evaluation of adsorption effectiveness is necessary. These two groups of organic compounds, despite belonging to the large family of NOM, possess some different properties. Humic substances are polycyclic aromatic compounds with molecular weight ranging from a few hundred to more than 100,000 Daltons [1,26]. Due to the presence of prevailing carboxyl and hydroxyl groups, humic substances have strongly acidic character and carry negative surface charge in aqueous solution, which is given by the dissociation of these functional groups. They are generally classified as hydrophobic organic compounds [27]. On the other hand, AOM peptides (<10 kDa) are ampholytes, i.e. they carry significant positive and/or negative surface charge, and have a clearly defined isoelectric point (p*I*). Their charge is larger in comparison with humic substances and it may also be positive (e.g. $-NH_3^+$,$=NH_2^+$), depending on the solution pH [11]. The structure of peptides is more organised in comparison with humic substances, as it is clearly given by the sequence of amino acids connected by peptide bonds. Linear chains of peptides are often arranged into complex three-dimensional structures due to the interactions (e.g. hydrogen bonds) between functional groups on the peptide skeleton [28]. A large amount of polar groups on peptides can also enable interactions with water molecules and that is why the peptides are usually classified as a hydrophilic organic material. However, in case that some peptide is in a native folded state (near the p*I*), a large portion of the polar groups can form intramolecular hydrogen bonds that consequently contribute to peptide hydrophobicity [29].

In general, adsorption of NOM onto AC is a complex process affected by various factors like pore size distribution, surface structure and chemical properties of activated carbon [26,30] on one hand, and the nature of adsorbate (e.g. molecular size and conformation, functional groups, solubility) on the other hand [21,30]. The characteristics of a solution, such as pH value, ionic strength (IS) and chemical composition, are equally determinative as they affect the net surface charge of AC, the dissociation ability of NOM functional groups and consequently, the involved adsorption mechanisms [15,21].

Varied types of interactions can contribute to the adsorption of organic molecules onto AC, which has been reported in several studies [21–23,30–32]. Interactions between adsorbate and carbon are often controlled by non-specific dispersive interactions, such as van der Waals forces that are the universal attractive forces of short range acting between all kinds of particles. Other types of interactions can include hydrogen bondings and also interactions between hydrophobic parts of the adsorbing species and hydrophobic parts of the carbon skeleton (i.e. graphitic surface without any functionality) that are mainly driven by the dislike of hydrophobic species to the water and not by their attraction to the surface [21]. However, the contribution of these interactions to the adsorption on the activated carbon remains questionable due to the fact that some studies demonstrated hydrophilic character of the clean graphite surface (representative of carbon skeleton) [33]. Other researchers have recently presented a new study on graphite and highly ordered pyrolytic graphite (HOPG) also indicating hydrophilicity of clean graphene surface [34].

In the case of the ionic adsorbate that can be dissociated or protonated in aqueous solution, electrostatic interactions (i.e. Coulombic interactions) can appear between specific charged sites on the pore surface of AC and charged functional groups of the adsorbate. Depending on solution pH, ionic strength, and charge densities in the adsorption system these interactions can be either attractive or repulsive [21].

This study focuses on the application of granular activated carbon (GAC) to adsorption of COM peptides produced by *M. aeruginosa*, a cyanobacterium commonly dominating algal blooms. The main objective of this study was to evaluate the adsorption capacity of two types of GAC for the peptides under various solution conditions (pH and ionic strength) and to make a proposal of possible interactions and mechanisms that can occur during the adsorption of the peptides onto these two types of GAC.

2. Experimental

2.1. Granular activated carbon

Two types of granular activated carbon with different chemical and structural properties, Filtrasorb TL830 (abbreviated hereafter as FTL; Chemviron Carbon, Belgium) and Picabiol 12×40 (abbreviated hereafter as PIC; Pica Carbon, France), were used in this study. Both GACs are commercially available and designed for drinking water treatment. Based on information provided by the manufacturers, these adsorbents are suitable for the adsorption of natural organic substances, including microcystins, and they also have a substantial selectivity for the removal of disinfection by-product precur-

sors and of taste and odour compounds in the presence of high concentrations of NOM. Prior to the experiments, GAC samples were pulverised, sieved to achieve a uniform particle diameter between 0.3 and 0.4 mm, and then extracted in Soxhlet apparatus to remove the finest ash particles. Both GACs were dried in an oven at 110 °C for 24 h, then cooled and stored in a desiccator prior to use in all adsorption experiments.

2.1.1. Surface area and pore size distribution analysis

Nitrogen adsorption/desorption isotherm experiments at 77 K (−196.15 °C) were carried out with the volumetric instrument ASAP2020 (Micromeritics, USA) to determine structural properties of selected GACs as described in our previous study [17]. Before the analysis the adsorbents were dried and degassed for 24 h at 105 °C and 0.1 Pa. The specific surface area (S_{BET}) was evaluated from BET equation, the micropore volume (V_{micro}) and the mesopore surface area (S_{meso}) were obtained by the *t*-plot method with Lecloux–Pirard master isotherm. The total pore volume (V_{total}) was calculated from the adsorbed volume of N_2 near the saturation point ($p/p_{0=}0.995$). Total meso- and macropore volumes were determined by subtracting micropore volume from the total pore volume. Pore size distribution of GAC samples was determined from the nitrogen isotherm by the BJH method. The microporosity of adsorbents (V_{micro}/V_{total}) was expressed as a percentage ratio of micropore volume relative to the total pore volume.

2.1.2. Surface charge determination

Surface charge of GAC was determined by potentiometric titration using an Orion 960 Autotitrator (Thermo Scientific, USA) according to the study of Álvarez-Merino et al. [35]. Carbon suspensions were prepared with 1 g of GAC and 400 mL of 0.01 M NaCl solution, equilibrated for 48 h at room temperature (22 ± 0.5 °C) and then titrated either with 0.1 M HCl up to pH 3 or with 0.1 M NaOH up to pH 11. The titration agent was added in 5-min intervals of the equilibration time, each addition of 0.05 mL. Equilibration and titration were performed under nitrogen atmosphere to eliminate the influence of atmospheric CO_2 and consequent lowering of the pH value. A blank titration was performed under the same conditions. The proton balance was obtained from the following equation [35]:

$$Q = \frac{1}{mS_{BET}} \times \left[V_0\{[H^+]_i - [OH^-]_i\} + V_t N_t - (V_0 + V_t)\{[H^+]_f - [OH^-]_f\} \right] \quad (1)$$

where Q is the surface charge [μmmol m^{-2}], *m* is the dose of GAC [g], S_{BET} is its specific surface area [$m^2\,g^{-1}$], V_0 and V_t [mL] represent initial volume of titration agent and volume at a specific time, respectively, N_t is a normality of titration agent [mmol L^{-1}] and subscripts *i* and *f* represent initial and final concentrations of H^+ and OH^- ions, respectively.

Positive values of Q indicate the presence of basic functional groups on GAC surface, whereas the dissociation of acidic functional groups leads to negative Q values. The pH value, at which the amount of positive and negative groups is equal and, thus, the absolute charge on the surface of GAC is zero, was estimated as the point of zero charge (pH_{pzc}).

2.2. Cultivation of Microcystis aeruginosa and preparation of COM

The cyanobacterium *M. aeruginosa*, Kuetzing (strain: Zap. 2006/2) was obtained from the Culture Collection of Autotrophic Organisms, Centre of Phycology, Institute of Botany, AS CR, Czech Republic. The cultivation of the cyanobacterium and the preparation of cellular organic matter (COM) samples were done according to the methodology described in detail in our previous studies [5,17]. The samples of COM, cleaned from the residual solids by filtration through a 0.22 μm mixed cellulose ester membrane filter (Millipore, USA), were concentrated and stored at −18 °C prior to subsequent use in the experiments [5,36].

2.3. Isolation of COM peptides

Peptides and proteins were isolated from COM by precipitation using $(NH_4)_2SO_4$, and their portion in COM was calculated according to the equation described elsewhere [5].

COM peptides (MW < 10 kDa), which were subsequently used as target adsorbates in all adsorption experiments, were isolated from the proteinaceous fraction of COM using a Solvent Resistant Stirred Cell (Millipore, USA) with ultrafiltration membrane PLAC 10,000 Da (Millipore, USA) and purified using ultrafiltration membrane PLAC 1000 Da (Millipore, USA). The stirred cell was operated at 60 rpm with the constant nitrogen pressure of 1 bar.

2.4. Characterisation of COM peptides

2.4.1. DOC analysis

Concentrations of COM peptides before and after adsorption experiments were quantified by the measurement of dissolved organic carbon (DOC) using a Shimadzu TOC-V_{CPH} (Shimadzu Corporation, Japan) [17].

2.4.2. Molecular weight fractionation

Distribution of molecular weights of isolated peptides was performed by high performance size exclusion chromatography (HPSEC) as described elsewhere [17]. The peptides were fractionated using two Agilent Bio SEC-5 100 Å columns (7.8 × 300 mm, 5 μm) (Agilent Technologies, USA) connected in series (separation range 100–100,000 Da). The HPLC system Agilent 1100 Series (Agilent Technologies, USA) coupled with a diode array detector (DAD) operated at 280 nm was used for the detection of the peptides. The system was calibrated using peptide and protein SEC standards (Sigma–Aldrich, USA) of MWs from 224 Da to 12 kDa. The apparent MWs of the peptides were calculated using semi-log calibration curve ($R^2 = 0.98$). Data analysis was performed using Agilent Technologies Chemstation software. Reproducibility of the MW distribution was very good with MW deviations of less than 3% from repeated measurements.

2.4.3. Determination of isoelectric points and charge

Isoelectric focusing (IEF) was used for determining the isoelectric points (pI_s) of the peptides. The measurements were performed with a Multiphor II electrophoresis system

(Pharmacia, Sweden) according to the methodology described elsewhere [17].

The charge behaviour of peptides depending on pH value was determined by potentiometric titration method [11] using an Orion 960 Autotitrator (Thermo Scientific, USA). The samples of COM peptides (DOC = 600 mg L^{-1}) were prepared in 200 mL of 0.01 M NaCl and equilibrated at room temperature (22 ± 0.5 °C) for 30 min. The pH values of peptide solutions were adjusted to 11.5 using 1 M NaOH and the samples were then titrated to pH 1.5 using 0.1 M HCl. The equilibration and titration were carried out under N_2 atmosphere to prevent the further dissolution of CO_2. The blank titrations were performed under the same conditions. The difference in the amount of added protons between the peptide and the blank titrations was attributed to the functional groups present in COM peptides which are able to dissociate. The points of titration curves with the minimum rate of change in pH value with added H^+ represent dissociation constants of peptide functional groups. Equivalence points, characterised by the maximum rate of change in pH value with added H^+, indicate the pH value where the influence of one functional group ends and of another one begins [28].

2.5. Analysis of microcystins

The analysis of microcystins, specifically the sum of MC–LR, –RR and –YR, was performed following the standard operation procedure adapted from EPA Method 1694 [37]. The microcystins were determined in water samples by high performance liquid chromatography combined with triple quadrupole mass spectrometry (LC/MS/MS) in the positive electrospray ionization (ESI+) mode. An Agilent 6410 Triple Quadrupole LC/MS/MS system (Agilent Technologies, USA) and column Zorbax Eclipse XDB-C18 (4.6 × 100 mm × 3.5 μm) (Agilent Technologies, USA) were used for the analysis. The chromatographic separation was performed using gradient elution combining mobile phase A (water) and mobile phase B (methanol), both containing 0.1% (v/v) formic acid and 5 mM sodium formate, at a flow rate of 300 μl min^{-1} as follows: 3–30% B for 0.3 min, 30–35% B for 6 min, 35–95% B for 3 min. Concentration of microcystins was evaluated using a combination of isotope dilution (Isoproturon D6; Sigma Aldrich, USA) and internal standard quantitative techniques. The quantification limit of the method was 100 ng L^{-1}.

2.6. Equilibrium adsorption experiments and data modeling

The equilibrium isotherm experiments with COM peptides were performed at different pH values and ionic strengths to determine the effect of solution properties on the adsorption process. The test solutions (250 mL), having a target concentration of COM peptides between 1 and 150 mg L^{-1} DOC, were prepared by diluting COM peptides in ultra-pure water with the total alkalinity adjusted to 1.5 mmol L^{-1} by 0.125 M $NaHCO_3$ to simulate the typical alkalinity values of surface/drinking water. The pH value of the solutions was adjusted either by 0.1 M HCl or 0.1 M NaOH to reach the desired values of pH 5, 7 and 8. Furthermore, 50 mg L^{-1} of sodium azide was added to all solutions to eliminate biological activity and decomposition of the peptides. For the experiments under different ionic strengths (IS), there were prepared solutions in a 0.01 M NaCl background electrolyte and in a 0.3 M NaCl background electrolyte in the same manner as was described above. The electrical conductivity (EC) of water samples was measured using a S230 SevenCompact™ conductivity meter (Mettler-Toledo, Switzerland) equipped with conductivity probe InLab 731-ISM (Mettler-Toledo, Switzerland) with measurement range of 1–105 mS m^{-1}. The IS in solutions without background electrolyte ranged from 0.0035 to 0.0036 mol L^{-1}, and EC ranged from 3.0 to 3.3 mS m^{-1}, depending on pH value. The IS in solutions prepared in a 0.01 M NaCl background electrolyte ranged from 0.0135 to 0.0136 mol L^{-1}, and EC ranged from 81.4 to 82.4 mS m^{-1}, which corresponds to the values of drinking/surface water (e.g. 5–150 mS m^{-1} [38]). The solutions prepared in a 0.3 M NaCl background electrolyte had IS of 0.3035–0.3036 mol L^{-1} and EC of 348.0–349.2 mS m^{-1}, i.e. values higher than those of drinking water. They were chosen in order to improve the description of the adsorption process and mechanisms and also to elucidate the influence of IS onto adsorption. Since the addition of reagents other than NaCl (e.g. NaN_3, HCl, NaOH) had minimal influence on the increase in ionic strength, the solutions without NaCl will be simplified hereafter as "ultra-pure water" and the solutions with NaCl as "0.01 M NaCl" (low ionic strength) and "0.3 M NaCl" (high ionic strength). Moreover, all test solutions described above were prepared from ultra-water and contained only monovalent ions, and therefore can be classified as soft in terms of typical surface water hardness.

Carbons PIC or FTL (400 mg L^{-1}) were then mixed with each solution in glass screw-capped bottles and agitated on a magnetic stirrer (200 rpm) at room temperature (22 ± 0.5 °C) for 48 h, a time interval pre-determined to be sufficient to reach adsorption equilibrium. After the equilibrium was reached, the solutions were filtered through a 0.22 μm mixed cellulose ester membrane filter (Millipore, USA) to separate GAC particles and DOC samples were taken to determine the residual concentration of COM peptides in the solution. All analyses were carried out in triplicate with errors of measured DOC less than 3%. Bottles without any adsorbent served as blanks to monitor the loss of adsorbate during the adsorption experiments, which was found to be negligible in blank samples. Analyses of apparent MWs of peptides remaining in the solutions after the adsorption were performed by HPSEC as described in our previous study [17]. To eliminate possible effect of different peptide initial concentration on HPSEC results [10], only the samples with constant initial concentration of 10 mg L^{-1} DOC and GAC dose 1–400 mg L^{-1} were analysed.

The amount of COM peptides adsorbed onto GAC was calculated using the following mass balance equation [14]:c

$$q_e = (C_0 - C_e)\frac{V}{m} \quad (2)$$

where q_e is the amount of COM peptides adsorbed per unit mass of GAC at equilibrium [mg g^{-1}], C_0 and C_e are the initial and equilibrium concentrations of COM peptides in the solution [mg L^{-1}], respectively, V is the volume of the solution [L] and m is the mass of the adsorbent [g].

The data obtained from the adsorption isotherm experiments were fitted to the Langmuir (3) and Freundlich (4) models given by the adapted equations as follows [31]:

$$q_e = \frac{a_m b C_e}{1 + bC_e} \quad (3)$$

and

$$q_e = K_f C_e^{1/n} \quad (4)$$

where q_e [mg g^{-1}] and C_e [mg L^{-1}] represent adsorbate uptake and solution concentration at equilibrium, respectively. Parameters a_m [mg g^{-1}] and K_f [(mg g^{-1})(L mg^{-1})$^{1/n}$] are reflective to adsorption capacity; constants b [L mg^{-1}] and $1/n$ represent the surface affinity and the heterogeneity of surface site energy distribution, respectively.

2.7. Kinetic adsorption experiments and data modeling

The kinetic experiments were performed with COM peptides (initial concentration 10 mg L^{-1} DOC) and overnight pre-wetted GAC PIC and FTL (dose 400 mg L^{-1}) at pH 5, 7 and 8. A series of 16 identical test solutions (200 mL) was prepared by diluting the peptides in ultra-pure water with total alkalinity adjusted to 1.5 mmol L^{-1} by 0.125 M $NaHCO_3$ and pH adjusted to desirable values by 0.1 M HCl and 0.1 M NaOH. The samples were shaken by a magnetic stirrer (200 rpm) at room temperature (22 ± 0.5 °C). The concentration of residual peptides, measured as DOC content, was analysed after sampling at different time intervals within 168 h (0, 10, 30 min, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24, 48 and 168 h). The amount of peptides adsorbed at each time interval per unit mass of GAC, q_t [mg g^{-1}], was calculated as follows [14]:

$$q_t = C_0 - C_t \frac{V}{m} \quad (5)$$

where C_0 is the initial solution concentration of COM peptides [mg L^{-1}], C_t is solution concentration of peptides at specific time t [h], V is the volume of the solution [L] and m is the mass of the adsorbent [g].

Two empirical kinetic models, pseudo first-order (6) and pseudo second-order (7), were used to describe peptide adsorption kinetics. The models are given by following equations [14]:

$$q_t = q_e(1 - \exp(-k_1 t)) \quad (6)$$

and

$$q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \quad (7)$$

where q_t and q_e represent uptake of COM peptides [mg g^{-1}] at specific time t and at equilibrium, respectively, k_1 [h^{-1}], k_2 [g/(mg h)] are rate constants, and t is the specific time of sampling [h].

3. Results and discussion

3.1. GAC characterisation

The main characteristics of adsorbents are summarised in Table 1. The external porous structure of GAC is illustratively displayed on the micrographs from scanning electron microscopy (SEM) in Fig. 1. Deep in GAC internal structure, large macropores (diameter > 50 nm) branch into smaller mesopores (diameter 2–50 nm) and micropores (diameter < 2 nm) [30].

Table 1 – Textural and surface charge characteristics of GAC PIC and FTL.

	PIC	FTL
General characteristics		
Precursor	Vegetal material	Bituminous coal
Activation agent	Phosphoric acid	Steam
Form	Granular	Granular
Textural properties		
S_{BET} ($m^2 g^{-1}$)	1668	1039
S_{meso} ($m^2 g^{-1}$)	770	421
V_{total} ($cm^3 g^{-1}$)	1.20	0.63
$V_{meso+macro}$ ($cm^3 g^{-1}$)	0.75	0.33
V_{micro} ($cm^3 g^{-1}$)	0.45	0.30
(V_{micro}/V_{total})·100 (%)	37	48
Surface charge		
pH_{pzc}	3.5	8.6

The N_2 adsorption isotherm analysis and pore size distribution in Fig. 2 demonstrate well the relatively different textural properties of both GACs used.

PIC had higher specific surface area (S_{BET}), mesopore surface (S_{meso}) and nearly double the total pore volume (V_{total}) compared with FTL. There was also a significant difference in micropore volumes (V_{micro}) of the adsorbents; it was 0.45 $cm^3 g^{-1}$ for PIC and 0.30 $cm^3 g^{-1}$ for FTL. Consequently, in terms of microporosity (V_{micro}/V_{total}), FTL reached a higher value (48%) compared with PIC (37%). The textural dissimilarities of the adsorbents are generally dependent on the type of raw precursor used for their manufacture (vegetal material in the case of PIC vs. bituminous coal in the case of FTL).

The characteristically high value of the specific surface area predetermines GAC to be an excellent adsorbent for many organic compounds, but, in the case of algal organic matter (AOM) and related toxins, the total available surface area might not be determinative [30]. The adsorption capacity is given by the accessibility of the adsorbate molecules to the inner surface of the GAC, which depends on their size. The compounds are also preferentially adsorbed into pores of similar size to the adsorbate because of a greater number of contact points between the molecule and the carbon pores. Therefore, the pore size distribution of GAC in relation to molecular weight of the adsorbate will be an important parameter affecting the removal of adsorbate from the solution [21,24]. As the algal and natural organic matters contain relatively large molecular structures, from a few hundred Da to more than 10,000 Da [10,26], previous studies pointed out that mesopores and transport pores of the carbon influence positively the adsorption capacity and kinetics due to the higher intraparticle diffusion rate [16,39]. Some studies also demonstrated a benefit of increase in carbon supermicroporosity (diameter 0.8–2 nm) because a wide range of dissolved organic matter (DOM) can be adsorbed within these pores [25,40]. Another study on adsorption of four microcystin variants (985–1024 Da) and three model NOM compounds

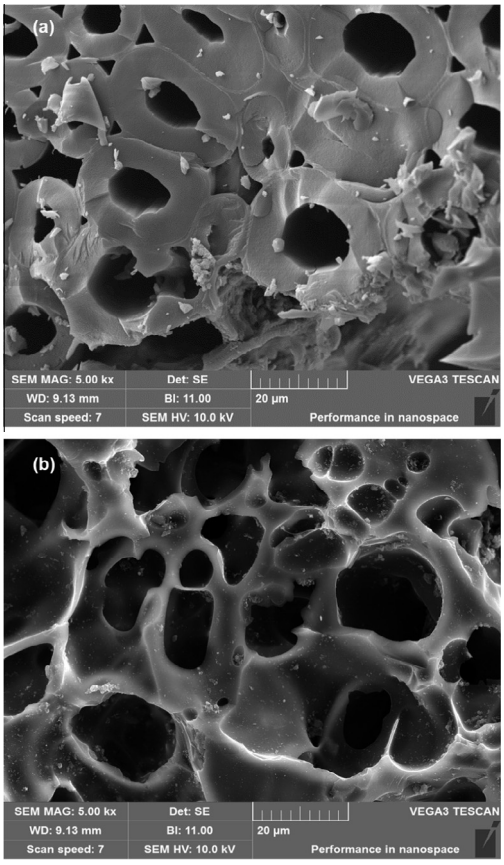

Fig. 1 – SEM micrographs of external surface structure of vegetal-based GAC PIC (a) and coal-based GAC FTL (b) scanned by Vega 3 (Tescan, Czech Republic).

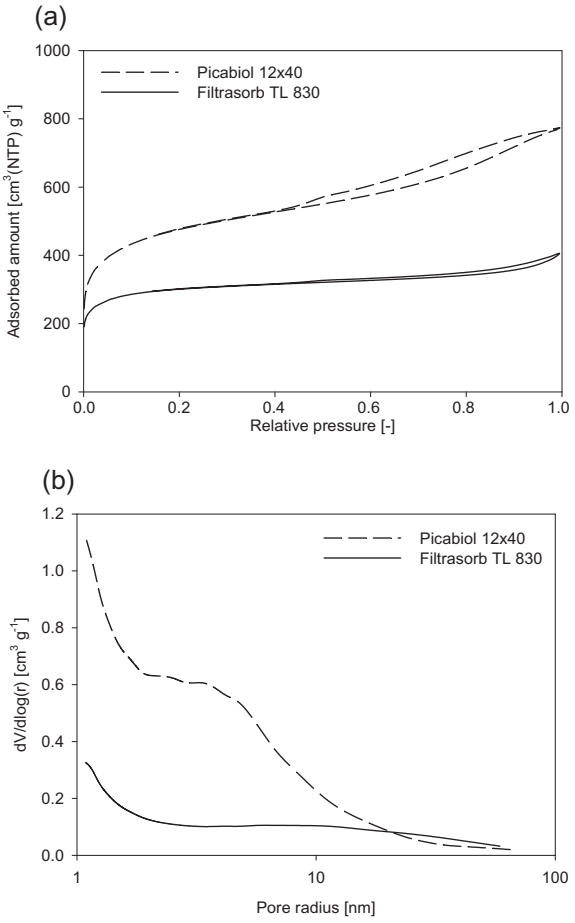

Fig. 2 – Nitrogen adsorption–desorption isotherms (77 K) (a) and pore-size distributions of GAC PIC and FTL evaluated from adsorption branches of sorption isotherms (b).

(138–11,000 Da) described the advantageous application of powdered activated carbon with high mesopore and secondary micropore volume for the uptake of the studied compounds. Based on the previous findings mentioned above, it was concluded that only pores with diameter no smaller than 0.8 nm, i.e. supermicropores and mesopores, might be accessible to the COM peptides with MW < 10 kDa. Unfortunately, the method applied for pore size distribution analysis of adsorbents [17] only enables to specify the mesopore surface area (S_{meso}) and the micropore volume (V_{micro}). The exact contribution of secondary micropores to the total pore volume cannot be determined in this way. The differences between both adsorbents are apparent from Table 1 and Fig 2.

Potentiometric titration curves for PIC and FTL are depicted in Fig. 3. The results demonstrated that both GACs had different values of pH_{pzc} and that their net surface charge (Q) strongly depends on the solution pH. In general, at a solution pH lower than pH_{pzc}, a net surface charge of GAC is positive while it is negative at a higher solution pH [21].

PIC had the pH_{pzc} of 3.5 and therefore carried a net negative surface charge during all adsorption tests. Moreover, the amount of negative charge gradually increased from pH 5 to pH 8, as it is evident from Fig. 3. The pH_{pzc} of FTL occurred at 8.6, which implies that this GAC bore a positive charge on its surface in all adsorption experiments. The positive charge of FTL decreased as pH value changed from 5 to 8.

The net positive charge is usually assigned to the basic oxygen complexes (e.g. pyrone, chromene, quinone groups), nitrogen-containing functional groups, inorganic impurities (metal oxides) on the carbon surface and/or to the existence of delocalized π-electron system of carbon basal planes, which accept protons from the aqueous solution [21,30]. On the other hand, the dissociation of acidic oxygen groups (e.g. carboxyl, phenol or lactone) coupled with the proton release to the solution, is responsible for a net negative charge of GAC [21].

3.2. *Characterisation of COM peptides*

Cellular organic matter of cyanobacterium *M. aeruginosa* was found to comprise 63% of protein material and 37% of non-protein material, which corresponds to the observations in our previous studies [10,17]. Fig. 4 depicts the peptides of MWs of approximately 1.0, 1.9, 2.8, 4.0, 4.5, 5.5, 6.0, 8.3 and 9.5 kDa which were identified by HPSEC. These peptides accounted for approximately 21% of COM protein fraction and it is very likely that they also include cyanobacterial microcystins. According to the data published in literature, the peak of MW of 1.0 kDa can probably be assigned to some of these toxic heptapeptides [16].

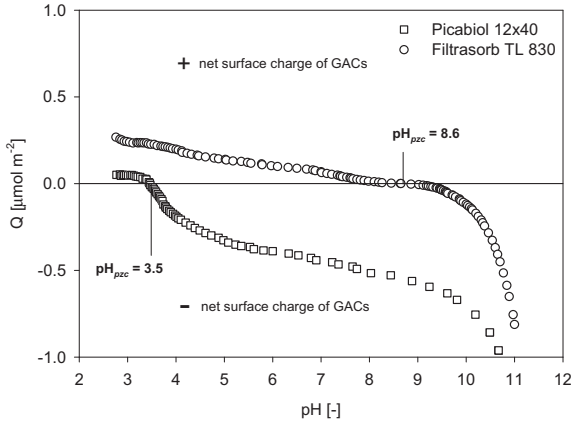

Fig. 3 – Dependence of the net surface charge (Q) of GAC PIC and FTL on experimental pH and estimated values of pH$_{pzc}$ in a 0.01 M NaCl background electrolyte.

The charge properties of isolated peptides were quantified both by potentiometric titration and by determination of p*I* values which were found to be 5.2, 5.3, 5.5, 5.8, 6.1, 6.2, 7.2, 7.8 and 8.0. The number of p*I* measured by IEF corresponded to the number of peptides identified by HPSEC. Peptides have an amphoteric character originating from protonation and deprotonation of ionizable functional groups. These groups, such as —OH, —COOH, —SH, $=NH_2^+$, $-NH_3^+$, etc., are able to accept or release protons according to the pH value. Fig. 5 expresses the amount of protons added to the solution as a function of pH value, which is considered to be equivalent to the number of dissociated peptide functional groups. Several equivalence points (pE_1, pE_2, pE_3) and dissociation constants (pK_1, pK_2, pK_3) corresponding to different COM peptide functional groups are apparent on the pH-titration curve. Dissociation constants pK_1 = 2.5 and pK_2 = 4.2 correspond to the carboxyl groups on the terminal part of the molecule (α-COOH) and on the side chain, respectively (e.g. β-COOH of aspartic acid with pK_a = 3.9, γ-COOH of D-glutamic acid with pK_a = 4.3) [28]. On the other hand, pK_3 = 9.8 could be assigned

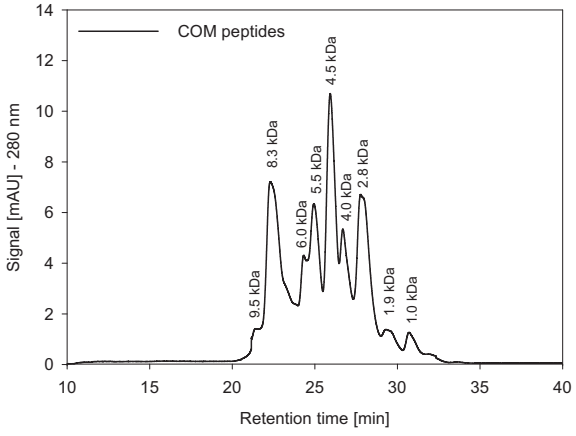

Fig. 4 – Molecular weight distribution of peptides isolated from COM produced by cyanobacterium *Microcystis aeruginosa*.

to groups, such as —OH, —SH, $=NH_2^+$, $-NH_3^+$, which have dissociation constants in alkaline pH (e.g. —SH with pK_a = 8.3, $-NH_3^+$ with pK_a = 9.2 or —OH with pK_a = 10.1) [28]. The equivalence point pE_2 corresponds to the total isoelectric point of the peptide mixture (pI_T), where the amounts of negatively and positively charged groups are considered to be equal.

3.3. *Equilibrium adsorption of COM peptides*

Driving forces involved in the adsorption of organic molecules onto GAC are usually on van der Waals basis and they are always attractive [21]. Moreover, the presence of H-bonds has been identified as an important property that affects adsorption of AOM onto AC [17]. In the case of peptides and proteins, which can act as polyelectrolytes during adsorption, electrostatic interactions were also found to play an essential role [15,32].

Adsorption isotherms displayed in Fig. 6 quantify adsorption capacity of both GACs for COM peptides at different pH values. The Freundlich and Langmuir models were applied to the experimental data. Coefficients of determination (R^2) suggest that the experimental data were slightly better represented by Langmuir model which is therefore included in Fig. 6. This was mainly evident at pH 8, when the isotherms stabilised after an initial increase, which is interpreted as saturation (Langmuir model: R^2 = 0.994 for FTL, R^2 = 0.988 for PIC; Freundlich model: FTL R^2 = 0.912 for FTL, R^2 = 0.936 for PIC). Parameters of both models are summarised in Table 2 and will be discussed in detail in Section 3.4.

The amount of adsorbed COM peptides was highest at pH 5 and it decreased with an increase of pH value for both GACs. Higher adsorption of COM peptides was observed on PIC compared with FTL. Similar results of higher adsorption of DOM, AOM components and cyanobacterial metabolites at low pH values have been ascertained by other studies [17,24,36]. As the solution chemistry and experimental conditions were

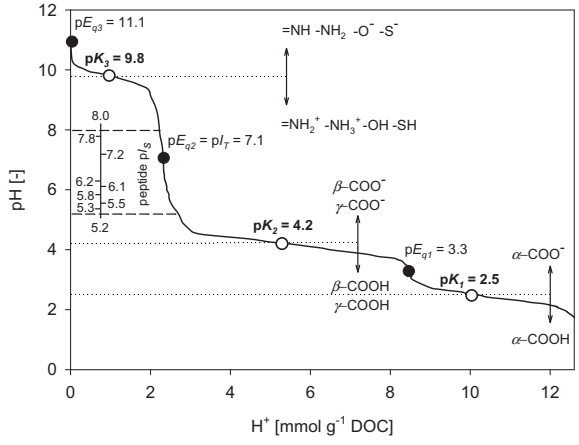

Fig. 5 – pH-titration curve of COM peptides with dissociation constants (pK_1, pK_2, pK_3) and equivalence points (pEq_1, pEq_2, pEq_3) related to the effect of different functional groups. pI_T represents the total isoelectric point of the peptide mixture. The isoelectric points (p*I*s) of single peptides were determined by IEF method.

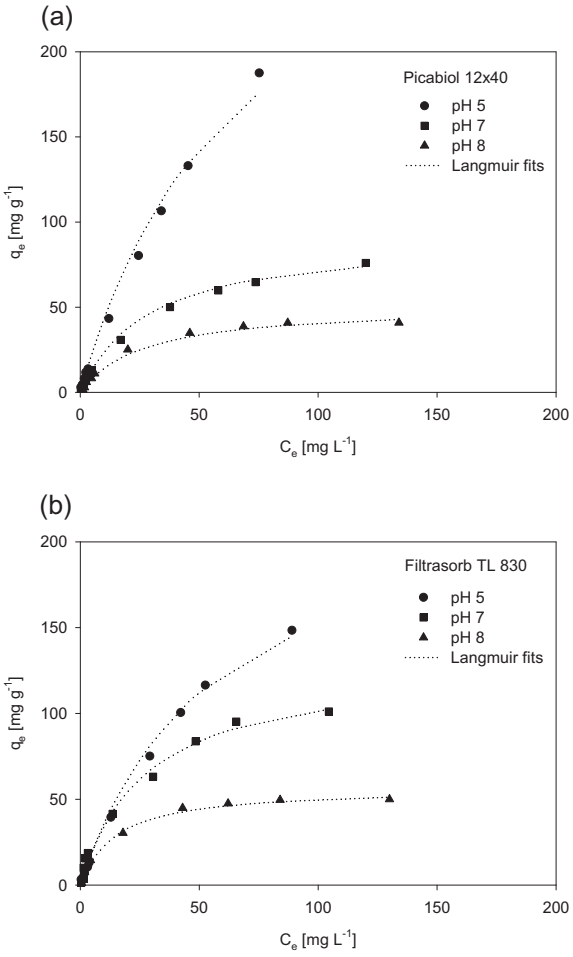

Fig. 6 – Adsorption isotherms of COM peptides at pH 5, 7 and 8 on PIC (a) and FTL (b). C_e and q_e represent equilibrium solution concentration and uptake of COM peptides, respectively. Langmuir fits were calculated from measured data according to Eq. (3).

the same for both carbons at pH 5, the higher uptake of peptides on PIC compared with FTL can be attributed to higher available pore volume and accessible surface area of PIC (Table 1). The broad pore size distribution of GAC with a high portion of mesopores and larger micropores was reported to facilitate the uptake of DOM and algal products in many cases [16,25,39,40]. However, a higher solution pH value resulted in a decreasing amount of adsorbed peptides, mainly for negatively charged PIC. As the pH value increased to 7 and 8, more COM peptides were removed by FTL than by PIC and the adsorption efficiency decreased in the following order: $PIC_{pH\ 5} > FTL_{pH\ 5} > FTL_{pH\ 7} > PIC_{pH\ 7} > FTL_{pH\ 8} > PIC_{pH\ 8}$. The impact of solution pH on adsorption of peptides is described in detail below.

The results of charge characterisation (Fig. 5) demonstrated that COM peptides carried both positively (=NH_2^+, —NH_3^+) and negatively charged groups (—COO^-) at pH 5–8. These groups could participate in electrostatic interactions with charged groups on carbon surface during the adsorption. Other peptide acidic functional groups, for instance —OH, exist predominantly in a protonated form under the applied experimental conditions and thus contributed to the formation of hydrogen bonds both with the aqueous solution and the functional groups on the surface of GACs.

At pH 5 the net negative charge predominates on the surface of PIC and strong electrostatic attractive interactions between carbon surface and positively charged functional groups of peptides (e.g. =NH_2^+, α-NH_3^+, ε-NH_3^+) enhanced the adsorption. Similar attractive interactions were identified in the case of lysozyme adsorption on modified polystyrene amphoteric surface with basic and acidic sites [41]. The presence of positive functional groups in protein/peptide structure is one of the main differences from NOM of humic character. The charge present on humic substances in natural waters is negative and is predominantly attributed to carboxylic and phenolic functionalities [22,23].

As the pH value increased, adsorption capacity of PIC fell rapidly and the total lowest peptide uptake was achieved at pH 8. Under these conditions the negative charge on PIC increased due to the high amount of dissociated acid functional groups and thus controlled the surface of PIC completely. According to the results of peptide charge characterisation (Fig. 5), it is very likely that at pH 7 and pH 8 the negative charge also increased in the peptide mixture due to the complete dissociation of carboxyl groups (e.g. α-COOH with $pK_1 = 2.5$, β-, γ-COOH with $pK_2 = 4.2$) and due to the gradual dissociation of some other groups than carboxyl (e.g. —S^- with $pK_a = 8.3$) [28]. Consequently, the electrostatic repulsion built up between PIC and the dissociated —COO^- and —S^- groups of the peptides and hindered the adsorption. In addition, some peptide molecules might become unfolded due to the high amount of negatively charged functionalities and the whole peptide mixture was thus less compact and more separated. This intermolecular repulsion could then contribute to poor removal at alkaline pH. There has previously been reported deprotonation of carboxylic and phenolic functional groups that leads to a higher charge density in the adsorbed NOM and consequently lower adsorption on activated carbon [22,23]. Similar results were observed by Gorham et al. [42] during the adsorption of NOM macromolecules on highly ordered pyrolytic graphene that represents the dominant surface features of AC. With increasing intermolecular repulsion between adsorbed macromolecules, their adsorption decreased.

As for FTL, the positive charge prevailed on its surface at pH 5 (see Fig. 3) and adsorption was favoured by electrostatic attractive interactions between FTL surface and —COO^- groups of the peptides. The increase in pH value to 7 and 8 diminished the positive charge of FTL, and the adsorption capacity gradually reached lower values due to the weaker effect of electrostatic attraction. The lowest uptake of COM peptides on FTL was reached at pH 8, where this GAC has very small positive net charge, see Fig. 3. However, the adsorption capacity of FTL at pH 7 and 8 was higher than the one of PIC. Taking into account that FTL has smaller total pore volume and accessible surface area of mesopores for COM peptides than PIC does (Table 1), a higher uptake of peptides can be attributed to the formation of hydrogen bonds between protonated FTL surface sites and protonated functional groups of peptides. This type of interactions has previously been identified to participate in the adsorption of low-MW peptides

Table 2 – Parameters of Freundlich and Langmuir isotherm models for COM peptide adsorption onto GAC PIC and FTL (Section A – ultra-pure water without NaCl, Section B – ultra-pure water with 0.01 M and 0.3 M NaCl).

	GAC	pH	Freundlich			Langmuir		
			K_f	1/n	R^2	a_m	b	R^2
Section A								
Ultra-pure water	PIC	5	5.67	0.82	0.996	335	0.015	0.988
		7	4.16	0.65	0.987	91	0.035	0.978
		8	2.49	0.65	0.936	50	0.043	0.988
	FTL	5	5.20	0.77	0.987	236	0.018	0.956
		7	5.17	0.72	0.935	129	0.037	0.999
		8	3.95	0.61	0.912	57	0.070	0.994
Section B								
0.01 M NaCl	PIC	5	5.79	0.83	0.999	342	0.016	0.979
		7	4.21	0.66	0.987	94	0.037	0.985
		8	2.82	0.66	0.902	55	0.045	0.976
	FTL	5	5.22	0.81	0.988	239	0.020	0.965
		7	4.31	0.69	0.955	97	0.040	0.994
		8	4.13	0.65	0.957	75	0.051	0.997
0.3 M NaCl	PIC	5	5.84	0.84	0.998	375	0.014	0.991
		7	4.43	0.72	0.959	110	0.039	0.987
		8	4.05	0.62	0.931	65	0.054	0.986
	FTL	5	5.33	0.82	0.989	249	0.020	0.968
		7	3.96	0.62	0.908	69	0.046	0.976
		8	4.23	0.67	0.939	82	0.048	0.987

Units: K_f [$(mg\ g^{-1})(L\ mg^{-1})^{1/n}$], n [-], R [-], a_m [$mg\ g^{-1}$], b [$L\ mg^{-1}$].

on granular activated carbons [17]. A similar concept of protein-surface interactions were also described by Yoon et al. [32] for bovine serum albumin and polymeric functionalised microspheres.

3.4. *The effect of solution ionic strength on equilibrium adsorption*

Solution ionic strength has been found to be another key factor that affects adsorption of organic molecules onto activated carbon. Through the influence on electrostatic interactions, the adsorption capacity of GAC can be either enhanced or reduced by the increase of the ionic strength in dependence on solution properties [21].

Fig. 7 depicts the results of adsorption tests at different IS (0.01 M, 0.3 M NaCl) and at pH 5, 7 and 8. The corresponding parameters of Freundlich and Langmuir isotherm models are reported in Table 2 (Section B). Although both models allow the comparable description of IS effect on peptide uptake (see R^2 in Table 2), there are some differences that should be taken into account. The application of Freundlich model is more difficult as the capacity parameter K_f and the heterogeneity parameter of the surface site energy $1/n$ affect simultaneously both the size and the rate of adsorption change. In the case of Langmuir model, it is possible to deduce the change of peptide uptake only from one or the other parameter. Parameter a_m corresponds to the maximum adsorbate uptake at monolayer coverage, while the parameter b represents the surface affinity and reflects the rate of uptake change. Based on these considerations, the change of peptide adsorption at different ionic strengths (i.e. in ultra-pure water, 0.01 M NaCl and 0.3 M NaCl) for the same pH value was evaluated only by the Standard Deviation (SD) and by the Coefficient of Variation (CV) of Langmuir parameters (Table 2).

The effect of IS on the adsorption of COM peptides changed in dependence on the type of GAC and on the solution pH. In the case of PIC, the increasing IS led to higher adsorption of peptides at all pH values in comparison with the tests in ultra-pure water without ionic strength adjusted by NaCl as described in Section 3.3. Moreover, a higher amount of peptides was removed as IS increased from 0.01 M to 0.3 M NaCl. Adsorption of COM peptides was presumably enhanced due to the screening of negative charge of PIC by added salt, which prevented electrostatic repulsion between GAC surface and dissociated functional groups of the peptides (e.g. $-COO^-$). The enhanced screening effect was most pronounced at pH 8 where PIC bears the largest net negative charge on its surface as was well reflected by the highest variation of the parameter a_m (SD = 6, CV = 11%) and b (SD = 0.005, CV = 10%) in the three different ionic matrices. The effect of IS on peptide uptake was less pronounced at pH 7 (SD = 8, CV = 8% for a_m and SD = 0.002, CV = 4% for b) and even lesser at pH 5 (SD = 17, CV = 5% for a_m and SD = 0.001, CV = 5% for b). A similar mechanism of adsorption enhanced regime for humic NOM was previously described in literature [23]. Another study of Newcombe and Drikas [22] examined adsorption of NOM (500–3000 Da) concentrated from raw surface water onto two activated carbons at high and low ionic strength over a range of pH values (3–9). It was found that at high adsorbed amount of NOM an increase in ionic strength improved the adsorption onto both activated carbons at all pH values. The increase was more pronounced at high pH as the electrostatic repulsion forces in the solution were higher in that pH region

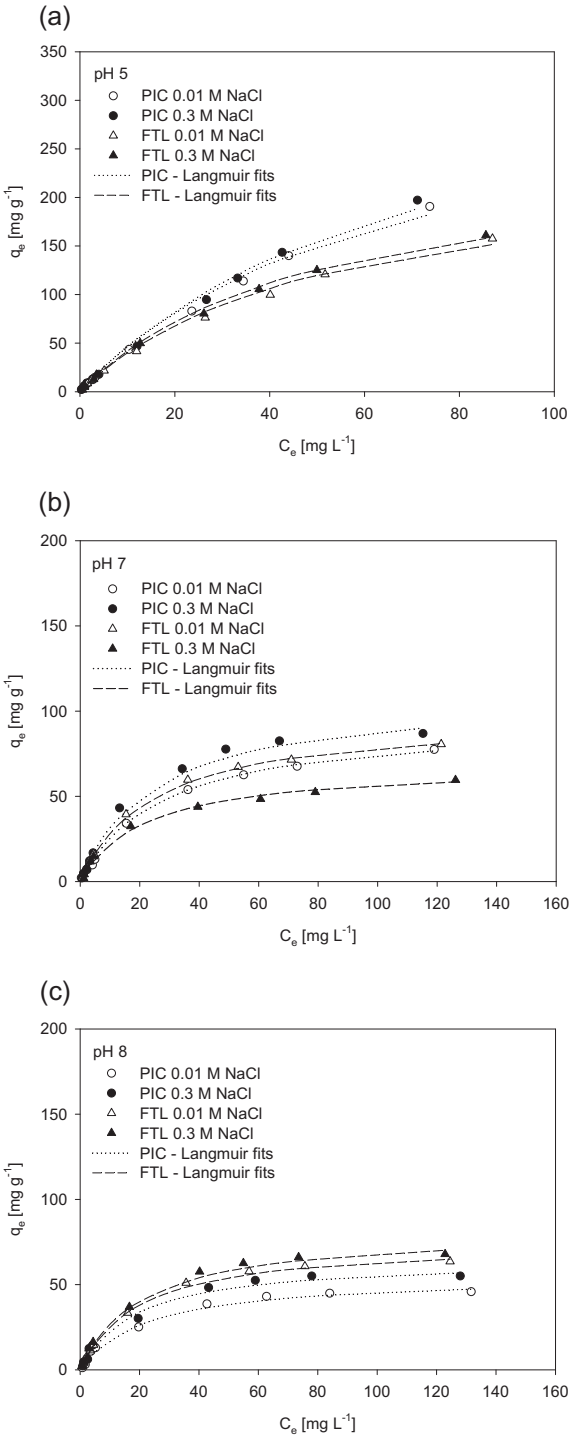

Fig. 7 – Adsorption isotherms of COM peptides at low (0.01 M NaCl) and high (0.3 M NaCl) ionic strengths on PIC and FTL at pH 5 (a), pH 7 (b) and pH 8 (c). C_e and q_e represent equilibrium solution concentration and uptake of COM peptides, respectively. Langmuir fits were calculated from measured data according to Eq. (3).

and thus were effectively screened by added salt. Some adsorption studies also confirmed that higher IS resulted in higher adsorbate uptake due to the changes in its chemical and structural properties. Particularly at higher adsorbed amounts, when peptide molecules are in close proximity, the intramolecular repulsion between functional groups can be reduced by added salt with subsequent molecule shrinkage and facilitating the adsorption [15,22].

The overall peptide uptake by FTL at pH 5 and pH 8 (Fig. 7a, c) was higher during the tests in 0.01 M and 0.3 M NaCl electrolytes compared with the tests in ultra-pure water (Section 3.3). However, this profitable effect of higher IS significantly varied depending on pH value. The added salt did not appear to have a noticeable impact on the peptide uptake at pH 5, where the adsorption was generally enhanced by the strong electrostatic interactions between positively charged FTL surface and dissociated functional groups of peptides (e.g. —COO^-). This was well documented by the small variation of Langmuir model parameters a_m (SD = 6, CV = 2%) and b (SD = 0.001, CV = 5%) in the three different ionic matrices. On the other hand, the effect of IS was much more evident at pH 8 (SDa_m = 11, CVa_m = 15%; SDb = 0.01, CVb = 17%). At this pH value, FTL was very close to its pH_{pzc} = 8.6 and bore a small amount of positive surface charge. The added salt probably helped to strengthen the attractive electrostatic interactions between carbon surface and oppositely charged functional groups of peptides (e.g. dissociated α-, β-, γ-COO^-). As the uptake of peptides was high during all the tests and a large amount of dissociated functional groups was present in peptide mixture, the molecular size reduction described above in the case of PIC could be another reason [22] for the increased adsorption at pH 8.

The effect of increasing IS was wholly reversed at pH 7, as is evident from Fig. 7 b. The uptake of peptides by FTL decreased by 20.5% in 0.01 M NaCl and by 41.4% in 0.3 M NaCl compared with the tests without NaCl (Section 3.3). This effect was also reflected by the significant variation of parameters a_m (SD = 25, CV = 25%) and b (SD = 0.004, CV = 9%) in the three different ionic matrices. The added salt probably screened electrostatic attractive forces between positively charged carbon surface and dissociated functional groups of the peptides and restricted the adsorption. This was particularly apparent in the case of the test at high IS of 0.3 M NaCl. A similar concept of adsorption reduced regime was described in literature [22]. Campinas and Rosa [15] studied the effect of increased ionic strength on the adsorption of a tannic acid (a model NOM compound) onto powdered activated carbon. It was proposed that an increase in ionic strength from 2.5 mM KCl to 10 mM KCl caused a decrease in adsorption through the shielding effect and consequent diminishing the electrostatic attraction. Another reason why increasing IS led to diminution in peptide adsorption onto FTL at pH 7, but enhanced it at pH 5 and 8, could be associated with the fact that at pH 7, the mixture of peptides is very close to its overall isoelectric point (pI_T = 7.1). It can be assumed that the amount of positive and negative charge in peptide mixture is in equilibrium at pH close to pI_T, and that the mixture is in a compact form due to strong intramolecular forces [28,42]. The addition of NaCl could change the ratio of charges and thus could weaken interactions within the peptide molecules and between peptides themselves. The mixture of peptides might hence be less compact and more difficult to be adsorbed.

3.5. Adsorption kinetics of COM peptides

Adsorption kinetics describes the rate of adsorbate uptake from the solution and the concentration distribution of the adsorbate at solid–liquid interface [31]. The empirical pseudo first-order and pseudo second-order models were used to describe the adsorption kinetic behaviour of COM peptides over time. The experimental and model data at pH 5, 7 and 8 are shown in Fig. 8. As seen from the corresponding model parameters summarised in Table 3, the pseudo second-order model was more appropriate to describe the peptide adsorption kinetics (R^2 0.973–0.995) and was therefore included in Fig. 8.

The uptake of peptides increased with time until the equilibrium was reached. The maximum amount adsorbed was achieved within 48 h, a time interval applied also for isotherm experiments. Although the values of q_e calculated from the two kinetic models were smaller than the corresponding experimental values $q_{e\ exp.}$ (see Table 3), the trend of increasing uptake of peptides at lower pH values was kept just as in the case of isotherm experiments (Section 3.3). For PIC, the increase of q_e compared with pH 8 was observed to be 58–60% (pH 5) and 12–13% (pH 7), depending on the kinetic model used. In the case of FTL, the increase of q_e was 25–27% at pH 5 and 9–10% at pH 7. The changes in q_e values calculated according to the first-order kinetic model were higher in all cases. The highest values of both pseudo first-order and pseudo second-order adsorption rate constants were estimated at pH 5 for both adsorbents; specifically $k_1 = 8.09\ h^{-1}$ and $k_2 = 1.07$ g (mg h^{-1}) for PIC and $k_1 = 7.61\ h^{-1}$ and $k_2 = 1.21$ g (mg h^{-1}) for FTL. It is well-known that the size and configuration of the adsorbate molecule affect the overall adsorption rate. The smaller the molecule or the more compact, the higher is the rate of adsorption [31]. Therefore, the high adsorption capacities and preferential uptake of the peptides at acidic pH can partly be attributed to the peptide suitable conformation.

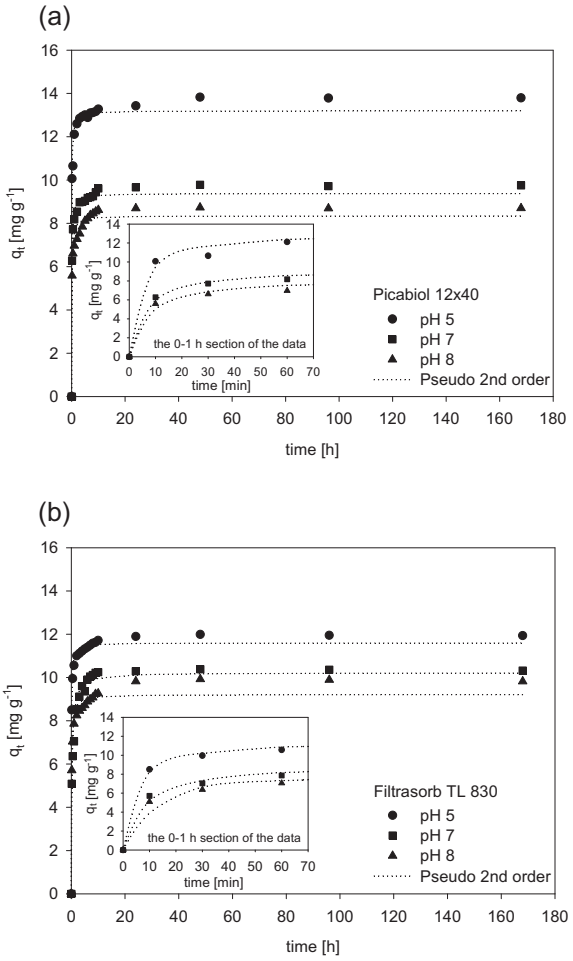

Fig. 8 – Adsorption kinetics of COM peptides on PIC (a) and FTL (b) at pH 5, 7 and 8. The value q_t represents the uptake of COM peptides at a specific time (t). Pseudo 2nd order kinetic fits were calculated from measured data according to Eq. (7).

3.6. Molecular weight distribution of COM peptides after adsorption

Fig. 9 depicts MW distributions of cellular organic matter (COM) peptides remaining in the solutions after the adsorption onto different doses of GAC PIC and FTL. The graphical results of SEC analyses affirmed the trend of deteriorating removal of COM peptides with rise in pH value from 5 to 7 and 8 as described in Section 3.3. Since the chromatograms at pH 7 and 8 were practically identical, the results measured at pH 5 and pH 7 were chosen as illustrative examples.

Generally, SEC analyses of the samples after the adsorption demonstrated that compounds of MW from the entire studied range (1.0–9.5 kDa) were to some extent removed. This may be attributed to the appropriate pore size distribution of both GACs with respect to the MW distribution of COM peptides [26,40]. However, the mutual comparison of chromatograms for PIC and FTL proved small differences, which were presumably caused by both the dissimilarities of adsorbents (Table 1) and conformation changes in peptide molecules.

At pH 5, COM peptides with MWs of 1.0, 2.8, 4.0, 4.5 and 5.5 kDa were removed by PIC proportionally to carbon dose, while the peptides with higher MWs of 8.3 kDa and 9.5 kDa were removed less efficiently even with the highest carbon dose. At higher pH values, the residual peaks for peptides with MWs 5.5–9.5 kDa were even higher, which corresponded to lower adsorption of COM peptides on PIC under these conditions (Section 3.3). This observation can partly be explained by the electrostatic repulsion between PIC (strong net negative charge) and COM peptides (e.g. —COO^-) at pH 7 and partly by the conformation changes in peptide structure at higher pH values and consequent size exclusion effect, which prevented these molecules from being adsorbed [26,40]. Similarly, the peptides with low MWs of 1.0–4.5 kDa were adsorbed almost completely on FTL compared with higher-MW peptides. Nevertheless, even at the highest FTL dose used (400 mg L^{-1}), a noticeably lower amount of the peptide with MW of 5.5 kDa was adsorbed. This finding might be related to the expanded conformation of this particular peptide under the specific conditions. Since FTL has lower amount of mesopores in its structure than PIC does (see

Table 3 – Parameters of pseudo first-order and pseudo second-order kinetic models for COM peptide adsorption onto GAC PIC and FTL at pH 5, 7 and 8.

GAC	pH	$q_{e\ exp.}$	Pseudo first-order			Pseudo second-order		
			q_e	k_1	R^2	q_e	k_2	R^2
PIC	5	13.44	13.01	8.09	0.963	13.30	1.07	0.990
	7	9.46	9.19	6.04	0.963	9.44	1.06	0.991
	8	8.52	8.15	5.80	0.929	8.41	1.04	0.977
FTL	5	11.69	11.42	7.61	0.978	11.66	1.21	0.995
	7	10.32	9.83	2.16	0.921	10.26	0.36	0.973
	8	9.44	9.03	4.65	0.941	9.34	0.77	0.980

Units: q_e and $q_{e\ exp.}$ [mg g^{-1}], k_1 [h^{-1}], k_2 [g $(mg\ h)^{-1}$], R [-].

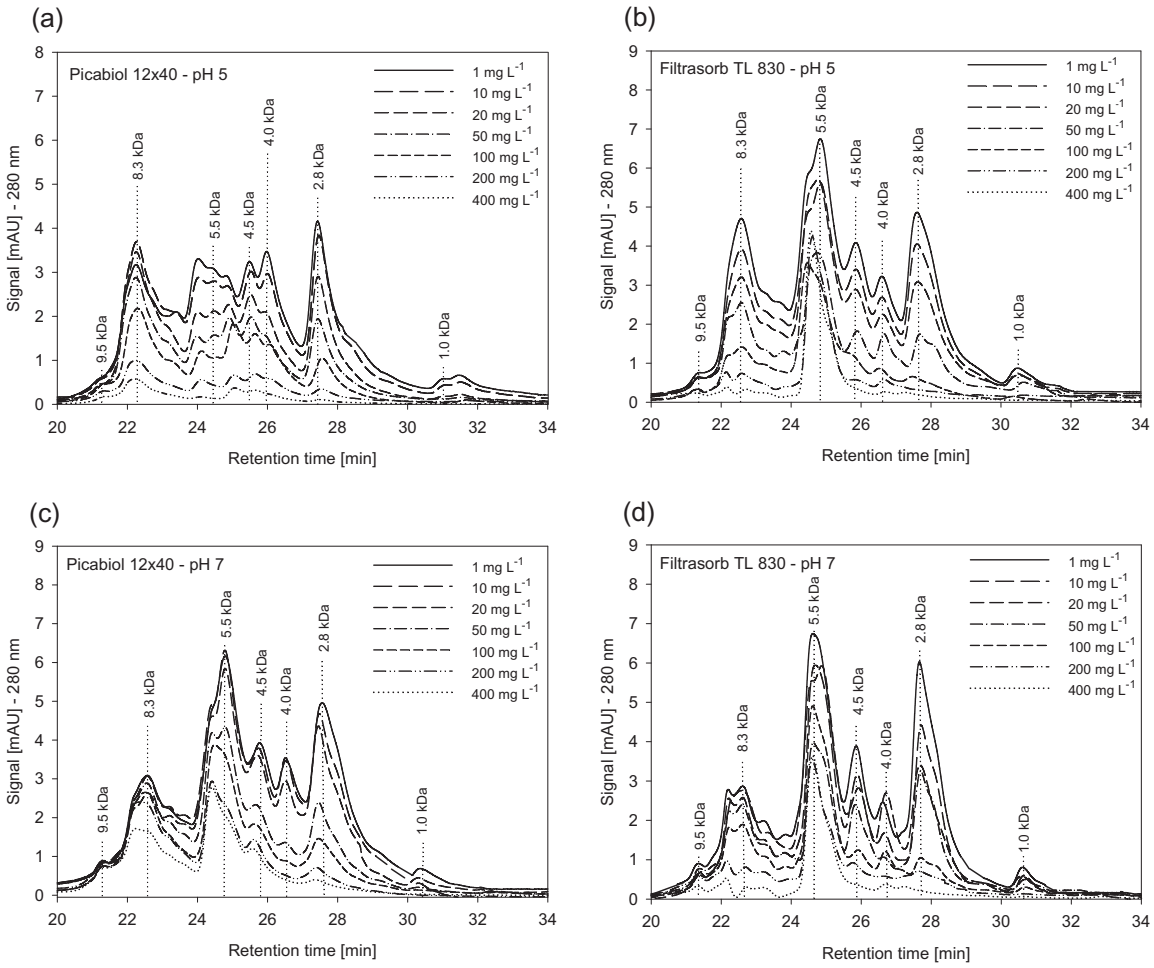

Fig. 9 – Molecular weight distributions of COM peptides remaining in the solution after adsorption on PIC (a), (c) and FTL (b), (d) at pH 5 and pH 7.

Table 1), this phenomenon could be more pronounced. Adsorption tests were carried out with a mixture of peptides and the applied methods did not allow the isolation of single peptides to clarify why the 5.5 fraction would expand its conformation, while the other size fractions do not. Unfortunately, the results are not sufficient to provide a definite interpretation for this phenomenon.

Although the study does not directly address the competitive adsorption issue of particular MW fractions of COM peptides and their contribution to the total DOC removal, the overall results of all analyses demonstrated that among COM peptides, the compounds with lower MWs were removed preferentially compared to the higher-MW ones. This observation is consistent with the results of other authors

who studied adsorption of different NOM and AOM fractions onto AC [17,24,26].

The LC/MS/MS analysis of the solutions after experiments confirmed that activated carbon adsorption can be a very suitable technique for microcystins uptake from water [30]. As is evident from Fig. 9, the peak of 1.0 kDa assigned to microcystins, was not detected in any experimental solution after adsorption with a carbon dose of 100 mg L^{-1} and higher. The residual solution concentration of microcystins remaining after the treatment was very low in all cases. At initial concentration of microcystins of 11.54 μg L^{-1} (sum of MC-LR, -RR and -YR at initial concentration of peptides 10 mg L^{-1} DOC and GAC dose 400 mg L^{-1}), it was 0.31 and 0.2 μg L^{-1} for PIC and 0.5 and 0.6 μg L^{-1} for FTL at pH 5 and 7, respectively. Higher adsorption efficiency of PIC (97.3–98%) compared to FTL (94.8–96%) for microcystins can be due to the higher amount of mesopores in its structure, which was documented by the results of other studies [16,39].

4. Conclusions

The present study evaluated the adsorption capacities of two granular activated carbons for the removal of undesirable COM peptides produced by *Microcystis aeruginosa* from aqueous solutions. Emphasis was placed on the charge characterisation of both GACs and peptides, as well as on identifying the influence of solution pH and ionic strength on the adsorption. The obtained results support the observed trend in our previous study that decreasing solution pH is probably an effective approach for enhancing adsorption capacity of GAC for COM peptides. These results are also in accordance with previous observations made for other natural organic macromolecules in water. High adsorption capacities reached for both adsorbents at acidic conditions were given by the electrostatic attraction between oppositely charged surfaces of GACs and peptide functional groups. As pH value increased, the positive effect of electrostatic attractive forces diminished and resulted in a reduction in adsorbed amount of peptides onto FTL. A similar decrease of adsorption was observed also on PIC due to the strong electrostatic repulsion between dissociated functional groups of the GAC and the peptides, and/or was likely to be connected with conformation changes in peptide molecular dimensions.

The study also confirmed that adsorption of peptides depends on the solution ionic strength, the effect of which changed according to the used GAC. In the case of PIC, increased ionic strength led to higher adsorption of peptides at all applied pH values due to the screening of electrostatic repulsion between negative charges in adsorption system. Peptide uptake by FTL was also enhanced by increasing IS at pH 5 and pH 8. However, the effect of IS was completely reversed at pH 7, where the added salt screened electrostatic attractive forces between FTL surface and the dissociated functional groups of the peptides and in this way the salt induced the adsorption reduced regime.

On the basis of equilibrium and kinetic test results, the mechanisms of COM peptides adsorption onto GAC were discussed. The formation of hydrogen bonds and electrostatic attractive and/or repulsive interactions were confirmed to play an important role in COM peptide adsorption onto GAC. Among COM peptides remaining after the conventional treatment in water, the peptides with low molecular weight of 1.0–4.5 kDa were removed onto both GACs more effectively than the peptides with higher MW of 8.3 and 9.5 kDa.

Acknowledgements

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

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Contents

1. Introduction

Natural organic matter (NOM) is a ubiquitous component of aquatic environmental systems. It is a heterogenous mixture of particulate and dissolved organic compounds that originate from allochthonous input dominated by humic substances derived from vegetative debris from terrestrial sources within a catchment and autochthonous input including mainly compounds derived from algae and cyanobacteria, i.e. algogenic organic matter (AOM). With respect to increasing eutrophication of aquatic environments followed by a phytoplankton population increase, the AOM may comprise a substantial proportion of NOM in surface waters and play 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).

AOM is a spectrum of organic compounds comprising saccharides and polysaccharides, nitrogen-containing compounds (amino acids, peptides, proteins, nucleic acids), various organic acids (e.g. glycolic acid), lipids and fatty acids (Hellebust 1974; Fogg 1983; Leenheer and Croue 2003; Huang et al. 2007c). AOM may also contain harmful cyanobacterial toxins (Harada 2004) and undesirable odour and taste compounds (Huang et al. 2007c; Li et al. 2012). AOM comprises both extracellular and cellular substances. Extracellular organic matter (EOM) is produced by algae as a by-product of photosynthesis and secondary metabolism. Cellular organic matter (COM) is released on cell lysis, mainly during the stationary and decline phases (Nguyen et al. 2005; Pivokonsky et al. 2006). COM can also be released when water treatment processes induce cell rupture, for example, during pre-oxidation (Ma and Liu 2002; Ma et al. 2012b; Coral et al. 2013; Pranowo et al. 2013; Wang et al. 2013).

The AOM composition 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; Henderson et al. 2008b; Leloup et al. 2013; Pivokonsky et al. 2014). Furthermore, the amount and character of organics released by algal cells is influenced by factors, such as nutrient status (Myklestad 1995; Wilhelm 1995; Huang et al. 2007c; Huang et al. 2012; Kong et al. 2014) or the presence of toxic substances (Mohamed 2008).

When algal populations increase in reservoirs supplying drinking water treatment plants (WTPs), water treatment technology has to deal not only with increased cell concentration but also with AOM. The highest concentrations of dissolved AOM are present in surface water during the decline phase of an algal bloom, when COM is released due to cell lysis. This sudden release of high concentrations of COM into WTP source water may instigate serious problems in drinking water treatment processes and may even lead to the collapse of a WTP and/or to 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. 2013b), decreased adsorption efficiency for micropollutants and low molecular weight compounds onto activated carbon (Hnatukova et al. 2011; Kopecka et al. 2014), occurrence of cyanobacterial toxins (Harada 2004) and odour and taste compounds (Huang et al. 2007c; Li et al. 2012), and disinfection by-product formation (Nguyen et al. 2005; Fang et al. 2010; Yang et al. 2011; Li et al. 2012; Wert and Rosario-Ortiz 2013).

This review aims to evaluate the performance of water treatment technologies, including both conventional coagulation and advanced techniques, such as membrane filtration and adsorption onto activated carbon, in terms of the impact of AOM on treatment performance and its associated

removal. The review addresses the main factors influencing the effectiveness of constituent treatment processes and examines the removal mechanisms. The AOM treatability with respect to its properties as well as its potential to form disinfection by-products (DBPs) is also discussed. Finally, the review suggests strategies to control and optimize the removal processes in order to achieve maximum efficiency and prevent the formation of DBPs.

2. AOM properties from a perspective of water treatment

Several studies characterising AOM have been conducted (Hoyer et al. 1985; Nguyen et al. 2005; Pivokonsky et al. 2006; Henderson et al. 2008b; Fang et al. 2010; Huang et al. 2012; Li et al. 2012; Chon et al. 2013; Leloup et al. 2013; Pivokonsky et al. 2014; Zhou et al. 2014a). These have found that AOM differs from NOM of humic character in several aspects that are important from the perspective of water treatment, such as hydrophobicity, SUVA value or molecular weight (MW) distribution.

Specifically, it was shown that AOM predominantly comprises hydrophilic (HPI) compounds. In general, the 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 the HPO fraction includes hydrocarbons, high-MW alkyl amines, high-MW alkyl carboxylic (fatty) acids and aromatic acids, phenols and humic substances (Edzwald 1993; Penru et al. 2013). Both EOM and COM also contain transphilic fraction (TPI) which accounts for a small proportion. It was demonstrated that all the three AOM fractions (HPI, HPO and TPI) contain both saccharide-like and protein-like substances (Qu et al. 2012b; Zhou et al. 2014a). The hydrophilic (HPI) fraction usually constitutes more than 60 % of EOM (Henderson et al. 2008b; Li et al. 2011; Huang et al. 2012; Li et al. 2012; Qu et al. 2012b; Leloup et al. 2013; Pivokonsky et al. 2014; Zhou et al. 2014a) depending on the species, growth phase and method of EOM isolation. Li et al. (2011) used 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. COM contains an even larger portion of hydrophilic compounds than EOM does. 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, i.e. about 10 % (Li et al. 2012; Pivokonsky et al. 2014; Zhou et al. 2014a) (Figure 1).

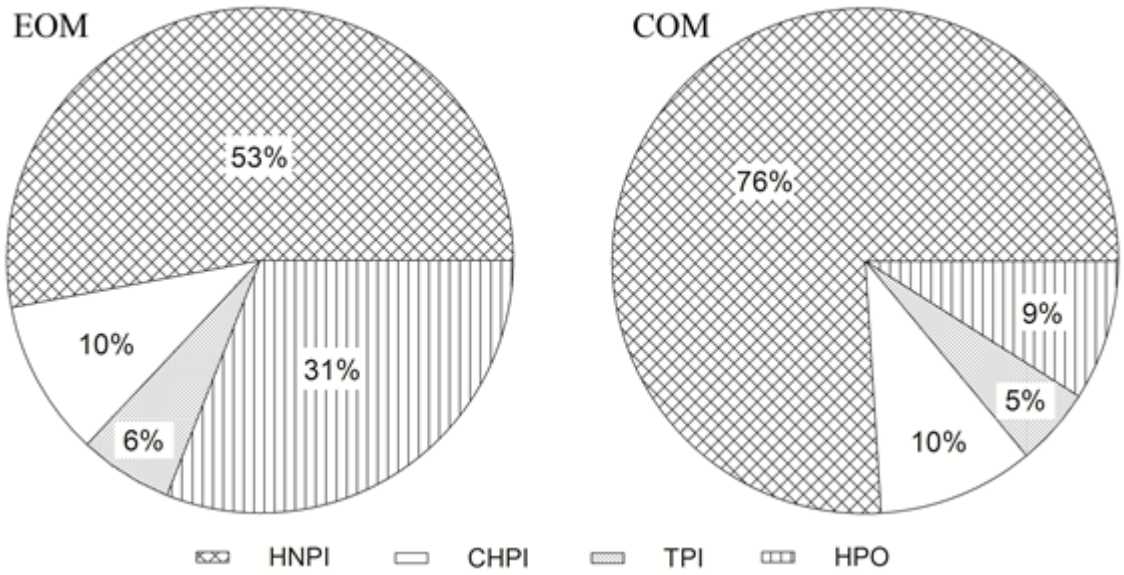

Figure 1. Comparison of the relative proportions of hydrophobic, hydrophilic (charged and neutral) and transphilic fractions in EOM and COM.

In accordance with the low hydrophobicity of AOM, both EOM and COM exhibit very low values of specific UV absorbance (SUVA) which reflects the content of aromatic structures and conjugated double bonds in organic matter and correlates with the hydrophobicity of organic molecules (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 hydrophilic 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, while the SUVA of stationary phase EOM ranged between 0.3-1 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 had a lower SUVA than EOM did, which is likely to be connected with very low COM hydrophobicity. On the contrary, Li et al (2012) reported that EOM of *M. aeruginosa* had a lower SUVA (0.72 m^{-1} mg^{-1} L) than COM did (0.91 m^{-1} mg^{-1} L). This difference can be given by the fact that Li et al. (2012) determined the SUVA in EOM/COM samples derived from cells harvested during exponential growth phase while Fang et al. (2010) and Pivokonsky et al. (2014) used samples from stationary growth phase.

As for molecular weight (MW) distribution, AOM was shown to comprise compounds with MWs ranging from several hundreds of daltons to hundreds of kilodaltons (Chrost and Faust 1983; Pivokonsky et al. 2006; Henderson et al. 2008b; Fang et al. 2010; Huang et al. 2012; Li et al. 2012; Wang et al. 2013; Zhang et al. 2013b; Pivokonsky et al. 2014). Low-MW fractions include intermediate products of metabolism, i.e. 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) like 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). Their concentration in EOM rises throughout the culture growth and is 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 most investigated for polysaccharides. 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. 2007c).

The content of saccharide-like and protein-like substances in AOM has also been investigated. Maksimova et al. (2004) reported that extracellular polysaccharides of green alga *Chlorella pyrenoidosa* S-39 comprised 20-40% of its EOM. The recent study of Pivokonsky et al. (2014) showed that the portions of non-peptide 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. Similarly, Lewin (1956) stated that 25 % of EOM produced by *Chlamydomonas mexicana* was formed by polysaccharides. Protein-like substances comprise up to 42 % of EOM depending on the species and age of culture. The quantity of peptides and proteins in EOM as well as their diversity increase with the age of algal culture, which is caused probably by the release of cellular organics into water. Moreover, COM contains a higher portion of peptides and proteins than EOM does (Pivokonsky et al. 2006; Henderson et al. 2008b; Fang et al. 2010; Huang et al. 2012; Pivokonsky et al. 2014). 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). With regard to a high portion of protein-like compounds in AOM and especially in COM, some studies focused on the removal of these compounds during water treatment (Takaara et al. 2007; Pivokonsky et al. 2012; Safarikova et al. 2013; Kopecka et al. 2014).

Another important AOM property from the perspective of water treatment is its charge. 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; Bernhardt et al. 1986; Bernhardt and Clasen 1991; Henderson et al. 2008b), attributed to the presence of various functional groups in EOM molecules. It can be concluded that this also applies to COM since it comprises the same or similar compounds as EOM, though in different amounts. The negative charge density of EOM, determined by the addition of cationic polyelectrolyte needed to reach the point of neutralisation, 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} of C at exponential growth phase to 0.1 meq g^{-1} of C at stationary growth phase, while that of *Chlorella vulgaris* EOM at pH 7 increased from 0.9 meq g^{-1} of C at exponential growth phase to 3.2 meq g^{-1} of C at 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 that can be attributed to the ionisation of carboxylic groups (-COOH) present in charged polysaccharides and proteins.

Decrease in electrophoretic mobility at alkaline pH values is caused by 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 able to bear positive or negative charge or both depending on pH value. This is due to their functional groups such as, –OH, –COOH, –SH, $–NH_3^+$, $=NH_2^+$, which are able to release or accept proton depending on the pH conditions (Creighton 1993). The charge of a protein-like compound is defined by its isoelectric point, i.e. pH value where the negative and positive charges of the molecule are equal. Pivokonsky et al. (2014) ascertained that peptides and proteins of the cyanobacterium *M. aeruginosa* bear a noticeable amount of ionisable functional groups on their surfaces. The isoelectric point of those peptides and proteins ranged between 4.8 and 8.1. 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 hydrolysing 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 charge conditions in the system, as coagulants and organic substances usually carry a charge that is dependent on 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) and is 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 does. 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 co-workers (Bernhardt et al. 1985; Bernhardt et al. 1986; Bernhardt et al. 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 non-ionic and anionic polyelectrolytes which can be characterized as neutral and acidic polysaccharides (polyuronic acids) and non-saccharide macromolecular compounds, such as proteins. Since EOM bear a negative charge (as described in section 3) it is removable by ferric coagulants in the pH range, where Fe forms hydrolytic products bearing a positive charge, i.e. at pH

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. (Henderson et al. 2010) EOM of *C. vulgaris*, *M. aeruginosa* and *Asterionella formosa* was relatively treatable by coagulation using aluminium sulphate 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 aluminium 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 aluminium based coagulants are too soluble and ferric ones are hence 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 was able to be coagulated by ferric salt at pH values below 6 (Bernhardt et al. 1986).

The coagulation of peptides and proteins was described in detail in the studies of Pivokonsky and co-workers (Pivokonsky et al. 2009; Pivokonsky et al. 2012; Safarikova et al. 2013). They 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 charge in the system, with the highest efficiency (between 60 and 85 % depending on 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 to 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 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.

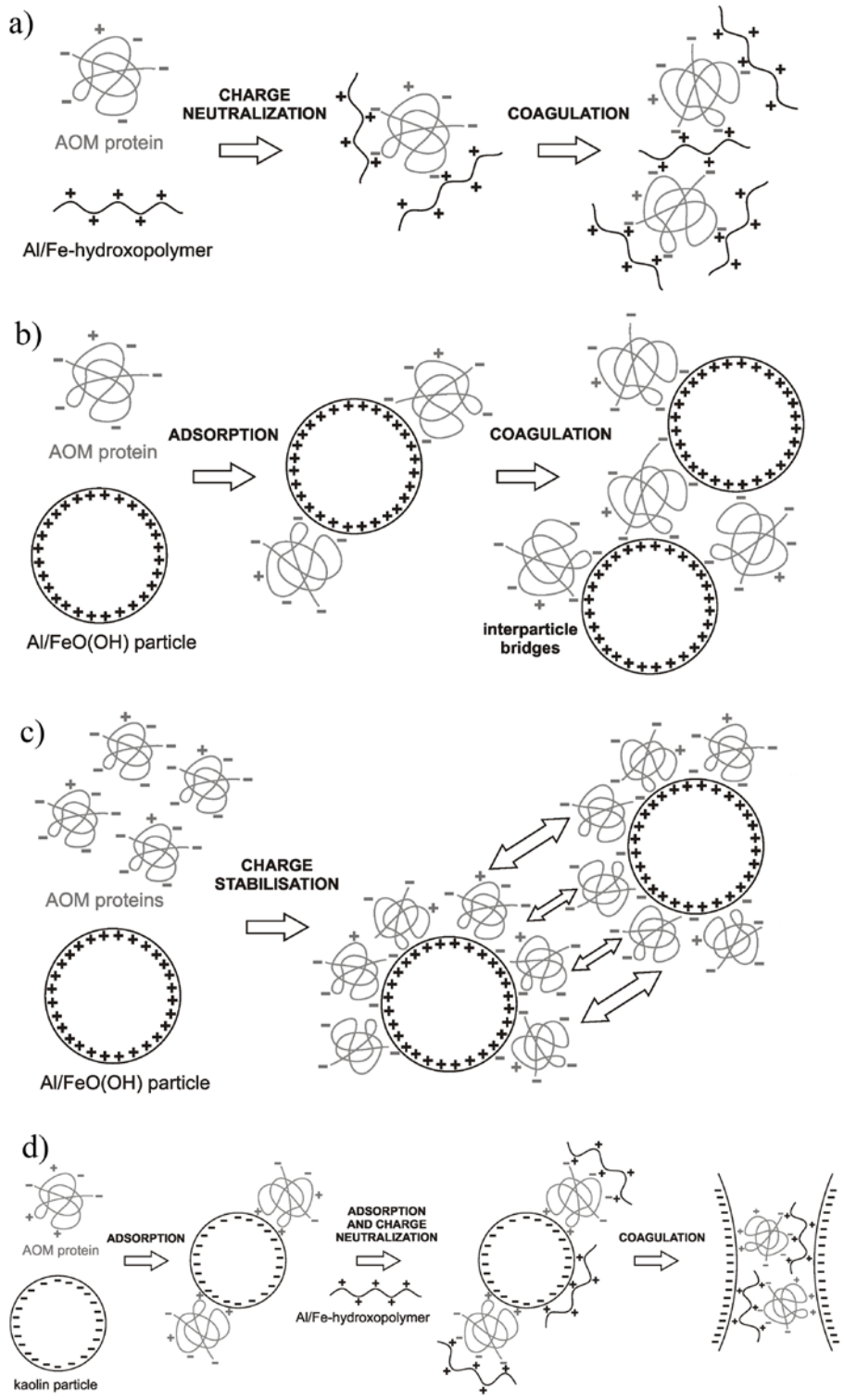

Figure 2. Mechanisms of coagulation: (a) electrostatic interactions between negatively charged acid functional groups (-COO^-) of peptides/proteins and positively charged Al/Fe-hydroxopolymers, leading to the coagulation (pH 4-6); (b) adsorption of peptides/proteins onto Al/Fe-oxide-hydroxide particles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

However, this process is highly dependent on the coagulant/DOC concentration ratio (Bernhardt et al. 1985; Bernhardt et al. 1991; Pivokonsky et al. 2012). When the ratio is high, peptides/proteins attach to the surface of Fe-oxide-hydroxide particles, which results in the development of negatively charged areas on the surface of iron colloidal particles. These negatively charged areas can interact with 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. This is followed by an increase in the negative charge density as peptides/proteins bear a significant amount of negative charge at pH 6-8 and correspondingly by 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 which 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. (Pivokonsky et al. 2014) observed that coagulation of peptides and proteins of *M. aerugiosa* become ineffective at pH 6-8 when Fe dose was 2.3 (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.

HPSEC analyses performed after coagulation showed that high-MW protein compounds are much more effectively removed by coagulation than low-MW (Pivokonsky et al. 2012; Safarikova et al. 2013). This is consistent with 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. (2009) showed that protein-like substances (of MW > 1 kDa) of *M. aeruginosa* COM were coagulated more easily than the non-protein ones. This was probably due to the high portion of low-MW organics (<3 kDa) in non-protein matter, which comprises 18 % of *M. aeruginosa* COM (Pivokonsky et al., 2014).

3.1. Impact of AOM on coagulation of other substances

Several studies dealt with the influence of AOM onto coagulation of particles present in raw water (Bernhardt et al. 1985; Bernhardt et al. 1986; Henderson et al. 2010; Ma et al. 2012a; Ma et al. 2012b; Zhang et al. 2012a; Pranowo et al. 2013; Safarikova et al. 2013; Wang et al. 2013). Some of them (Henderson et al. 2010; Ma et al. 2012b; Pranowo et al. 2013; Wang et al. 2013) observed a positive influence of high-MW EOM as well as COM that was released after pre-oxidation on the coagulation of algae cells. Wang et al. (2013) found that the pre-oxidation of *M. aeruginosa* cells by low concentrations of $KMnO_4$ led to the secretion of high-MW compounds with molecular weight above 200 kDa. These molecules easily combine with polyaluminium chloride (coagulant), aiding coagulation and improving the efficiency of algae removal, possibly through adsorptive bridging. On the other hand, when higher concentrations of pre-oxidant are applied, the significant release of COM accompanied by its degradation into low-MW compounds cause reduction in AOM removal efficiency and a concurrent increase in coagulant demand (Ma et al. 2012a; Ma et al. 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 like 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. A study by Paralkar and Edzwald (1996) showed that EOM can also interact with positively charge surfaces. They ascertained that EOM adsorbed onto positively charged latex particles and neutralized its charge during an experiment conducted at pH 7. They also found that EOM from *Cyclotella*, which contained a high percentage of high-MW material (about 90 % of compounds >30 kDa), induced the coagulation of latex particles. A similar process was observed for COM peptides/proteins by Safarikova et al. (2013), suggesting that this was the result of electrostatic interactions between the negatively charged kaolin surface and positively charged peptide/protein functional groups. This can result in two different features, depending on charge properties of peptides/proteins, and thus on pH value. First, when the peptides/proteins attached to kaolin surface bear enough negatively charged $–COO^-$ groups, charge stabilization of kaolin particles by peptides/proteins occurs. Second, when the amount of negative charge of peptides/proteins is reduced by the protonation of $–COO^-$ to –COOH groups, the repulsion between peptide/protein molecules occurs to a lesser extent and can be overcome by attractive forces. Coagulation by this mechanism was observed at pH < 4.5, i.e. at pH values close or lower than the dissociation constants of peptide/protein carboxyl groups (β-COOH of aspartic acid with p*K*a = 3.86 and γ-COOH of glutamic acid with p*K*a = 4.25) (Creighton 1993), even without the addition of coagulant (Safarikova et al. 2013). After the addition of coagulant, positively charged Al/Fe hydroxopolymers can interact with $–COO^-$ groups of peptides/proteins bound to kaolin particles and also with kaolin particles (Figure 2d). As for coagulation of AOM, this process is effective at pH values below neutral, i.e. 5-6.5 for aluminium 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. 2012b; Pivokonsky et al. 2012; Safarikova et al. 2013).

3.2. 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. 2004; Pivokonsky et al. 2006; Takaara et al. 2007; Takaara et al. 2010; Pivokonsky et al. 2012; Safarikova et al. 2013). A number of studies demonstrated that algal polysaccharides, especially anionic ones containing uronic acids, are able to bind metals (Bernhardt et al. 1985; Kaplan et al. 1987; 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 pH values because 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 lower 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. 2004; Pivokonsky et al. 2006; Takaara et al. 2007; Sano et al. 2011; Pivokonsky et al. 2012; 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 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. For that reason, metal-organic complexes tend to remain in solution after the coagulation process (Pivokonsky et al. 2006; Takaara et al. 2007).

Since coagulation has been proved to be remarkably efficient in removing AOM (Bernhardt et al. 1985; Pivokonsky et al. 2009), especially high-MW organics, it is an essential pre-treatment process for other removal techniques such as membrane separation, adsorption onto activated carbon and reverse osmosis in AOM-containing waters (Edzwald and Haarhoff 2011; Myat et al. 2012). Moreover, the coagulation process offers advantages of low operating cost and of relatively simple operation.

4. Impact of AOM on membrane separation

In recent years there has been increased interest in the use of membrane processes (micro-, ultra- and sometimes nano-filtration [MF, UF and NF, respectively]) to be utilised for treatment of water that may have been impacted by algae or cyanobacteria blooms and therefore by AOM (Liang et al. 2008; Zhang et al. 2012b; Zhou et al. 2014b). Algae can also disrupt desalination plants that use reverse osmosis (RO) processes (Caron et al. 2010; Edzwald and Haarhoff 2011). This, in conjunction with 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 fouling by algae and associated AOM. It is agreed that AOM interferes with membrane separation of algae, with many studies confirming that transmembrane pressure increases, or flux significantly declines, during algal blooms due to severe membrane fouling (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 also 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). AOM extracted from a cyanobacteria-rich bloom was characterised using high performance size exclusion chromatography with UV_{254}, fluorescence and DOC detectors (a highly advanced combination at that time) and Fourier-transform infra-red (FT-IR) spectroscopy. It was determined that while molecules with MW 1000-5000 Da, high negative charge and high SUVA were rejected by a NF membrane via electrostatic repulsion and size exclusion mechanisms, poor rejection and fouling were caused by fractions with greater hydrophilic character, more neutral charge and low SUVA. Importantly, the authors concluded that while concentration is important, similarly to coagulation, it is the character of the AOM that can be related to fouling potential. Specifically, protein- and polysaccharide-like substances, respectively, were found to be the major foulants in this study and in other studies that followed when investigating extracted EOM from a number of species (Chiou et al. 2010; Zhang et al. 2010a; Qu et al. 2012a; Villacorte et al. 2013). For example, Chiou et al (2010) demonstrated that both EOM concentration and composition may have influenced that fouling potential that was found to be in the order *Microcystis* sp.>*C. vulgaris*>*Chotadella* sp. with the suggestion that the high proportion of polysaccharides in *M. aeruginosa* were responsible for fouling. This outcome was supported by another study that examined transparent exopolymer particles (TEP), considered to comprise acidic polysaccharides, released by *C. vulgaris* (Discart et al. 2013) where it was determined that polysaccharide-rich colloidal and particulate TEP can spread across a membrane surface relatively evenly, eventually blocking membrane pores, supporting the concept that polysaccharides are major foulants contributed by EOM. 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 (Section 2). Hence, different outcomes might be anticipated if these experiments were repeated for COM.

A number of studies have been undertaken to further characterise 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 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. 2013c). Given the multitude of parameters that can be varied, large variation can be observed when attempting to relate fouling to hydrophobicity using XAD-resin fractionation (Table 1); however, while all fractions have been observed to contribute to an extent to membrane fouling, broadly, it is the HPI fraction, and in particular the NHPI fraction that has typically observed to be the major foulant. In contrast, one study showed that HPO dominated in the reversible layer but this might be expected as this was the only study examined in Table 1 that used a microfilter instead of a UF filter, and for this study it was still the HPI fraction that dominated in the irreversible layer. Consistent between studies was that TPI did not play a major role in fouling, it is considered that this is likely due to the fact that TPI comprises a relatively small portion of the EOM (Section 2). Size fractionation experiments of a similar type contribute to improving our understanding of foulant mechanisms. 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 100kDa membrane, suggesting that major foulants were very large molecules, for example biopolymers. Similarly, using Method B, Zhang et al (2013c) showed that the reversible foulant layer comprised a large proportion (>90%) of biopolymers >>20000Da which were not observed in the irreversible foulant layer that was dominated by a combination of high MW substances (~1000Da) and substances of 500 Da or less. A recent study showed, for a suspension of *Chlamydomonas*, that the material with the highest fouling potential passed through a 5 µm MF filter but was 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 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 (>>20,000 Da), considered to be proteins and polysaccharides, with irreversible fouling caused by mostly (1000 Da) and low (<350 Da) MW hydrophilic AOM restricting the pores via adsorptive fouling (Zhang et al. 2013c); and 3) Polysaccharides attaching to the membrane via intermolecular bridges leading to the formation of a cake-gel layer (Zhang et al. 2013a); 4) Deposition of smaller colloidal species rather than cake layer formation (Rickman et al., 2012). The differences in reported mechanisms are unsurprising given the complexities of the system and likely due to variable experimental design, including but not limited to AOM character and concentration, flux, membrane type and filtration time period.

Overall, there have only been a small number of studies undertaken investigating membrane fouling mechanisms of AOM and it is therefore not surprising that the literature is particularly inconclusive in this area (Table 1); in fact, similar contradictory findings have dominated membrane fouling studies in the wastewater field over the last 10 years due to complex inter-relationships between the design and operation of the membrane system and the presence and properties of the organics released by the biomass (Drews 2010). However, wastewater bacterial exudates, termed extracellular polymeric substances (EPS) and soluble microbial products (SMP), are analogous with AOM, and thus examination of this comparable literature can lend insight into fouling that might be expected due to the presence of AOM. A major review undertaken by Le-Clech et al. (2006) identified four key fouling mechanisms: cake filtration, the build-up 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. In consideration of these mechanisms with observations of AOM fouling studies previously reported (Qu et al. 2012b; Rickman et al. 2012; Zhang et al. 2013a; Zhang et al. 2013c), it seems that AOM has the potential to contribute to fouling according to each of the identified mechanisms with the dominant mechanism being a factor of: initial AOM concentration, AOM character, volume filtered and length of filtration test, membrane type and configuration and flux. The extent to which a particular mechanism is present will be a factor of the experimental design.

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	Reference
			Overall EOM	HPO	TPI	HPI			
						CHPI	NHPI		
Microcystis aeruginosa/ Stationary phase	A	Constant pressure; pH7; 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; 50mL, 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. 2013c)

*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

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 relationships between growth conditions and water quality and membrane fouling by EOM. Bable et al. (2002), investigated the impact of temperature and radiation (to reflect seasonal variability) on membrane resistance when filtering *Chlorella vulgaris* by observing the specific cake resistance. During optimal growth conditions (temperature of between 28-35 ˚C), resistance was minimised. However, under adverse conditions, for example, temperature ranges outside the optimal range or high solar radiation, higher resistance was observed; nutrient availability was also implicated. It was identified that during optimal growth conditions, cells deposited on the membrane were isolated and void spaces were present; however, during adverse growth conditions, a layer of mucilaginous, cell bound EOM was observable, minimising the presence of void spaces (Babel et al. 2002). The effect of nutrient conditions have also been studied for *M. aeruginosa* (Huang et al. 2012). It was found that membrane fouling by EOM extracted from cells grown under various nutrient conditions decreased in the order: 1N:10P>1N:2P>1N:1P>2N:1P. The EOM extracted from algae grown under 1N:10P conditions had a particularly low SUVA and much higher neutral HPI fraction relative to the other nutrient conditions tested. Further research in 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 closely controlled when extracting EOM for characterisation 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; Zhou et al. 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 decline increased as did irreversible fouling when examining the impact of increasing 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+}. Interestingly, no influence of the Ca^{2+} ion was observed by Lee et al. (2006a) although they suggest that this may be due to the focus of previous studies on high pressure membranes while their study investigated low-pressure systems. 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. It was also noted that irreversible fouling was increased at pH 5, attributed to a weakening in electrostatic repulsion. An increase in fouling at more acidic pH was also observed by Zhang et al. (2012b) for *M. aeruginosa*. Not only were electrostatic interactions implicated in this study, but it was observed that EOM concentration increased at this pH value suggesting cells were under stress which would also have contributed to increased membrane resistance. Finally, the presence of natural organic matter (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) organic matter (OM) and autochtonous (produced in situ, i.e. of microbial origin) OM and how these may impact membrane fouling. Lee et al. (2006a) observed little difference in flux decline between allochtonous OM and COM, although increased fouling was observed for OM extracted

from an autochtonous reservoir. In another study, similar flux decline was also observed for an organic matter sample extracted from a reservoir and a *M. aeruginosa* sample (note this sample comprised cells and EOM) (Kwon et al. 2005). Interestingly, flux decline increased significantly when the NOM and *M. aeruginosa* were introduced to the membrane simultaneously. Myat et al. (2014) showed via molecular dynamic suimulations 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 aggresive 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 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) optimising membrane operation, and/or c) optimising 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. To illustrate, on coagulation using alum coagulant prior to membrane filtration of a recycled water sample spiked with *M. aeruginosa*, flux decline was significantly reduced and, furthermore, the foulant layer was fully reversible at optimised coagulant doses (Goh et al. 2011). Similarly, Babel and Takizawa (Babel and Takizawa 2011) observed a 70% reduction in cake resistance for *Chlorella* sp. after coagulation. Interestingly, when Goh et al. (2011) removed their coagulated flocs through a 1.5 µm membrane prior to MF treatment, while little change was observed in initial flux decline, there was a significant increase in irreversible fouling, indicating that the flocs also provide a protective cake layer to prevent internal fouling. Since coagulants interact with the charged, hydrophobic and high MW AOM components, it is suggested that low MW, neutral organics likely remain in solution and their associated fouling potential must still be considered. In the study by Goh et al. (2011), it is likely that the cake layer was filtering out the residual neutral, low MW organics which would otherwise adsorb at the surface of the membrane via direct adsorption processes. Use of preozonation prior to UF treatment of *Chlorella* showed an increase in total membrane resistance for contact times of 3-10 minutes irrespective of membrane type (Hung and Liu 2006). On increasing the contact time to 30 minutes, while a decrease in resistance was observed, it still was not any less than that obtained without preozonation. 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 removal of EOM from the cell surface and reduced biomass load. The concurrent increase in fouling resistance was attributed to concurrent adsorption of the released polysaccharides. It may also be a result of release of COM on cell lysis as described by Henderson et al. (2008) and Plummer and Edzwald (2002). No studies have investigated the impact of preozonation on AOM without cells. Powdered activated carbon (PAC) treatment was investigated as part of a hybrid submerged UF system treating *M. aeruginosa* cells (Zhang et al. 2011b). Prior to the addition of PAC, severe membrane fouling was observed and no DOC removal was achieved. PAC addition reduced fouling to an extent and enabled

approximately 10% DOC removal and 40% microcystin-LR removal; however, it had no impact on the rejection of high MW, hydrophilic AOM. PAC dosing is discussed in more detail in Section 5. Magnetic ion-exchange (MIEX®) resin has been investigated as a pre-treatment to coagulation where it was shown to remove both low and high molecular weight, anionic OM, amounting to between about 25-60% of OM depending on its character without coagulation (Mergen et al. 2008; Liu et al. 2011c). When combining MIEX treatment with coagulation, between 60-80% removal of AOM has been observed in algae-laden reservoirs (Mergen et al. 2008; Liu et al. 2010). Interestingly, when examining removal by coagulation alone, Mergen et al. (2008) found little difference when including MIEX treatment, while Liu et al. (2011b) increased removal from only 20% to more than 60%. However, the degree of OM removal would depend on effective coagulation optimisation. Liu et al. (2011b) further evaluated the impact of MIEX treatment inclusion on membrane fouling by AOM, determining that fouling rate was diminished. This was attributed to removal of high MW, hydrophobic AOM. While minimal 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 like PAC and MIEX are, however, expensive and economic assessments would have to be conducted as to the overall effectiveness of one over another in combination with coagulation and this remains a knowledge gap.

Another strategy to control fouling is by more careful operation of the membrane separation process. At the first stage, 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 hydrophobic membranes are more susceptible to fouling by AOM, including faster flux decline and more adsorptive and irreversible fouling that hydrophilic membranes (Sun et al. 2013; Qu et al. 2014). Zhou et al. (2014b) found little difference with respect to flux decline between hydrophobic and hydrophilic membranes; but did observe that irreversible fouling was greater for the hydrophobic membrane. Babel and Takizawa (2010) tested two hydrophilic membranes with different protein binding capacities, including PVDF with a protein binding capacity of only 4 μg cm^{-2}, and found no difference in fouling observed. Flux and crossflow velocity, when employing cross-flow membrane systems, can also be carefully controlled to mitigate fouling (Pongpairoj et al. 2011; Wicaksana et al. 2012). 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, a maximum was reached and 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) have also been examined with promising results.

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 fouling potential of supernatant (culture medium plus any AOM), algae suspension and algae in DI water was compared (Zhang et al. 2010a). The most severe fouling was observed by the original algae suspension, then the cells in DI water. Only a small amount of fouling was observed for the culture medium, which contained proteins, polysaccharides and lipids; however, this fouling was irreversible. It was found 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 x 10^4 cells/L 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 activated carbon

The adsorption process using carbonaceous porous media, such as activated carbon (AC), 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). Unfortunately, the vast majority of recent adsorption studies dealing with the removal of NOM is 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) passed 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).

Various types of activated carbon are applied for the water purification, with granular activated carbon (GAC) and powdered activated carbon (PAC) being those most frequently used. GAC is employed continuously in different types of filters/adsorbers as a final polishing step after coagulation/flocculation and before disinfection. PAC is usually applied only for short periods of unexpected deterioration in raw water quality. In this case, it is dosed directly to the treated water, most often before or simultaneously with the dosing of coagulants. Activated carbon is also frequently used in combination with other treatment techniques. The study of Zhang et al. (2011b) evaluated the effect of PAC addition on an immersed ultrafiltration (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 released into water and rejected only a part of the high MW AOM through a sieving mechanism. The removal of organic matter below 1 kDa had to be enhanced by PAC adsorption. Campinas and Rosa (2010) also confirmed in the study on *M. aeruginosa* that the combination of PAC/UF system is a suitable treatment method for the control of cyanobacteria and their products. The addition of PAC to UF contributed particularly to the control of irreversible membrane fouling caused by autochthonous NOM derived from the cyanobacteria as AOM/EOM. PAC also enhanced the overall removal of AOM (combined EOM and COM), although it was apparently ineffective for the highly hydrophilic EOM compounds.

A technology which combines PAC adsorption with microfiltration (MF) membrane is already being employed in some experimental plants (Matsushita et al. 2008) for the removal of cyanobacteria *Anabaena planktonica*, *Anabaena smithii* and *Anabaena spp.* as well as the associated odour compounds. Pilot-scale experiments using a hybrid system of coagulation, ceramic MF and adsorption onto micro-ground PAC (median particle size 0.65 μm) proved that this type of system can simultaneously and effectively remove both intracellular and extracellular geosmin and the cyanobacteria cells from the water. Another study by Ho et al. (2012) provides insight into biological treatment options for optimum removal of cyanobacterial metabolites. This extensive review quotes biological GAC filters as the attractive treatment option since they offer the advantage of two removal mechanisms, adsorption and biodegradation.

Table 2. Adsorption studies dealing with algal organic matter

Microorganism	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 μm) and S-PAC (0.65 μm) (Futurama Chemical Industries Co., Ltd., Japan)	Matsushita et al. (2008)
Microcystis aeruginosa	EOM (extracellular matter excreted during growth) and AOM (total EOM and intracellular matter released during cell lysis)	laboratory-scale experiments with hollow-fibre cellulose acetate UF membrane/PAC	PAC Norit SA-UF (Cabot Norit Americas Inc., USA)	Campinas and Rosa (2010)
Microcystis aeruginosa	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	cyanobacteria cells, AOM, MC-LR, MC-RR, MC-YR	laboratory-scale experiments with a hollow-fibre 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	COM peptides < 10 kDa	laboratory batch and kinetic adsorption experiments	GAC Filtrasorb TL830 (Chemviron Carbon, Belgium), GAC Picabiol 12x40 (Jacobi Carbons AB, Sweden)	Kopecka et al. (2014)

Many other studies focus on the AC adsorption of cyanobacterial toxins, especially on the most commonly occurring microcystins or saxitoxins (Pendleton et al. 2001; Campinas and Rosa 2006; Huang et al. 2007b; Dixon et al. 2011; Ho et al. 2011). Even though 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

The character of algal products that are adsorbable by activated carbon is heterogeneous. Substances of various MW can be adsorbed depending on carbon pore size distribution, often with the preferential adsorption of lower MW compounds compared to higher MW ones. Hnatukova et al. (2011) detected COM peptides of *M. aeruginosa* of MW 700 – 1700 Da that were adsorbed proportionally to carbon doses, and were therefore identified as the most relevant components in restriction of GAC adsorption capacity for herbicides. Even with the highest carbon dose (100 mg L^{-1}) a negligible amount of the peptides with MW > 2300 Da was removed. Such peptides were supposed to cause pore blockage effect, since both GACs in the study were relatively microporous. Furthermore, among peptides of the same cyanobacterium remaining after the conventional treatment in water, the peptides with low MW of 1.0 - 4.5 kDa were removed onto microporous/ mesoporous GACs more effectively than the peptides with higher MW of 8.3 and 9.5 kDa (Kopecka et al. 2014).

MW profiles for the Myponga feedwater during the *Anabaena circinalis* bloom measured by Dixon et al. (2011) showed that coagulation in combination with PAC adsorption removed a greater amount of compounds in the region between 1500 and 3000 Da than the UF membrane alone. Moreover, the significant enhancement of coagulation of both the cells and intracellular metabolites in the presence of PAC has been reported in the study. In the case of water with a *Microcystis flos-aquae* bloom, PAC addition did not enhance the cell removal by coagulation, probably due to the high water turbidity and the different morphology of spherical *Microcystis* and filamentous *Anabaena* cells in the two tested waters.

Zhang et al. (2011b) found out that the addition of PAC to the immersed UF reactor enhanced the removal of DOC, UV_{254}, and microcystins, but it had little effect on the rejection of hydrophilic high-MW AOM such as carbohydrates and proteins. These results were in accordance with the study of Campinas and Rosa (2010) that confirmed PAC can increase hydrophobic AOM rejection (with subsequent benefits in disinfection by-products control), but it is apparently ineffective in adsorbing the highly hydrophilic EOM (mainly polysaccharides).

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), as well as both activated carbon (pore size distribution, surface chemistry) and adsorbate characteristics (molecular weight, conformation, functional groups, solubility) (Moreno-Castilla 2004; Newcombe 2006). Whether some factor will outweigh the others depends on particular experimental conditions. Zhang et al. (2011a) found out 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, authors attributed the relatively high adsorption capacities (dozens of mg g^{-1}) reached in laboratory-scale experiments to the irreversible chemisorption on the GAC, rather than to the formation of adsorption multilayer. Moreover, the pH effects on GAC adsorption of both odorants were also different. The dimethyl trisulfide adsorption was almost identical at pH 2-10 and then slightly increased at pH up to 12, while the capacity for *β*-cyclocitral was not significantly different at pH 2-13, probably due to the existence of this compound in the form of neutral molecules in acid, neutral and alkaline solutions.

Some pH effects on AOM adsorption were outlined also in the study of Hnatukova et al. (2011). Adsorption of COM peptides (expressed as DOC removal) was twice as high at pH 5 compared to pH 8.5. Moreover, higher adsorption of the peptides at pH 5 was seen on the tested carbon with higher available pore volume. Increased adsorption at pH 5 onto the carbons was explained by the formation of hydrogen bonds between protonated functional groups of the peptides and protonated surface groups of carbons which predominated on their surfaces at acidic pH value. On the other hand, at pH 8.5 where both the peptides and carbons were negatively charged, the repulsive electrostatic forces between them reduced the adsorption. The results of Kopecka et al. (2014) supported the trend that decreasing solution pH from 8 to 5 may be an effective approach for enhancing GAC adsorption capacity for COM peptides. Moreover, the study confirmed that a solution ionic strength (IS) is another key factor that controls the adsorption of AOM simultaneously with pH value. It often influences not only the adsorbent-adsorbate electrostatic interactions but also the interactions between AOM molecules through the shielding effect produced by the added salt.

6. AOM and disinfection by-product formation potential

Disinfectants, or oxidants, are used extensively in water treatment as a final process prior to distribution of treated water to supply. The extent to which disinfectants are applied can increase during algal blooms for two reasons: 1) Pre-oxidation 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 organic matter present in the system to produce potentially harmful disinfection by-products (DBPs), which is equally true for AOM. However, the type of DBPs that are formed will be driven to an extent by the molecular character of the AOM which differs quite significantly from that of allochtonous organic matter, as discussed in section 2. This section will examine AOM (EOM and COM) as precursor material for DBPs in chlor(am)ination processes.

6.1 AOM as a disinfection by-product 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 particularly, the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) on 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 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; hence reported only the average specific DBP. This suggests that while overall DOC concentration change with 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 specific C-DBP reactivity rather than growth phase or species. To illustrate, over a 3 day chlorination period, specific total THM reactivity for cyanobacteria and green algae species similarly varied from 12-17 µg mg C^{-1} (Fang et al. 2010; Yang et al. 2011) and 13-18 µg mg C^{-1} (Yang et al. 2011), respectively, irrespective of growth phase, increasing to 26-32 µg mg C^{-1} (Nguyen et al. 2005; Huang et al. 2009; Li et al. 2012) and 15-63 µg mg C^{-1}, 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 µg mg C^{-1} for *M. aeruginosa* (Fang et al. 2010); at 7 days, this is again significantly increased to 48-66 µg mg C^{-1} for cyanobacteria (Huang et al. 2009; Li et al. 2012) and 54-62 µg mg C^{-1} for green algae (Nguyen et al. 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 ranged from 15-48 µg mg C^{-1} and 27-43 µg mg C^{-1}, 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} while specific HAA reactivity was 68 µg mg C^{-1}. 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 C^{-1} and 7 µg mg C^{-1}, respectively.

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 mgC^{-1} 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 mgC^{-1} after 3 days of chlorination; interestingly, fulvic acid was more similar to AOM in this respect with specific HAA reactivity of 65 µg mgC^{-1}. 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).

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)

	Chlorination Conditions	Specific C-DBP Reactivity (μg mg C^{-1}) THM	HAA	References
Cyanobacteria				
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)	N.M.	(Nguyen et al. 2005)
		63±14 (TCM – 20L reactor)	34.8 (DCAA) 22.9 (TCAA) 2.3 (MCAA)	
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)
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.

6.1.2. Nitrogenous disinfection by-product formation

While N-DBPs including nitrosamines, haloacetonitriles (HANs), halonitromethanes (HNMs), haloacetamides (HAcAms) and cyanogen halides (CNX) are generally much lower in concentration that 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 to 290 µg N/L during algal blooms and even up to 1 mg/L (Pocernich and Litke 1997; Westerhoff and Mash 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). For example, a US drinking water treatment plant survey detected a shift in median concentrations of HANs, HNMs and CNX from 3 to 4 µg/L, 0.3 to 0.5 µg/L and 2.2-2.6 µg/L 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, while 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 cyanogen chloride (CNCl) formation potentials increased. Both studies measured the DOC/DON ratio as being in the order of around 4.5, approximately 4 to 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 to 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, molecular weight (MW) and also according to structural classes including proteins, lipids and fatty acids. For example, Zhou et al. (2014a) examined the reactivity of EOM and COM hydrophobic (HPO), transphilic (TPI) and hydrophilic (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 hydrophobic 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 TCM was produced (68 µg/mg 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 HPO fraction.

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

	Disinfection Conditions	**Specific N-DBP formation** (μg/mg N) **Nitrosamines**	**HANs**	**HNMs**	**CNCl**	**References**
Cyanobacteria						
Microcystis aeruginosa *(exponential phase)*	7 d, 25±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±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±1 °C, pH=7 Cl_2/DOC (w/w)= 3	N.M.	1.6 (DCAN)	0.06	0.12	(Fang et al. 2010)

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 C^{-1}; TCAA: 51.7 μg mg C^{-1}); however, the medium-MW fraction formed more HANs and HKs. The low-MW fraction was least reactive. The high-MW fraction was characterised 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 C^{-1}). In a separate study, the same authors further fractioned extracted algal proteins to isolate hydrophobic and hydrophilic proteins (Lui et al. 2011). It was determined that hydrophilic proteins had a specific THM reactivity of 85 μg mg C^{-1}, much greater than hydrophobic proteins which only formed 2.45 μg mg C^{-1}, whilst hydrophobic proteins were found to be more likely to form HAAs. Another study investigated fatty acids (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 C^{-1}, 2.0 μg mg C^{-1} and 8.7 μg mg C^{-1} of TCM, DCAA and TCAA, respectively, and, interestingly, for TCM and DCAA the formation potential increased with increasing number of double bonds in the fatty acid; 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 C^{-1}. The same study found that hydrophilic and hydrophobic proteins had a THM formation potential of 93.5 μg mg C^{-1} and 12 μg mg C^{-1}, respectively. Notably, the reactivity of fatty acids 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 6.1 (Table 3) may be the

result of hydrophilic proteins. While it might be expected that fatty acids and hydrophobic proteins may be removed to a large extent by coagulation-flocculation if performed and optimised, this is less likely for hydrophilic proteins and suggests that the presence of AOM in water sources could impact a water treatment plant's ability to meet THM guideline values. These studies also suggest that SUVA is not necessarily a good indicator to predict THM formation, especially in autochthonous sources.

7. Conclusions

Recent research has demonstrated that AOM can impact process performance at every stage of the treatment process and therefore requires consideration and evaluation when a drinking water source is impacted by an algal or cyanobacterial bloom. Specific conclusions of this review are listed as follows:

- AOM is predominantly hydrophilic (HPI) but highly variable with respect to MW, comprising carbohydrates, hydroxy acids, low-MW carboxylic acids, amino acids, amino sugars, peptides, low-MW alkyl alcohols, aldehydes and ketones. The SUVA is therefore low and decreases with the age of algal culture due to increasing release of largely hydrophilic high-MW and/or cellular organics. EOM as a whole is negatively charged throughout a wide range of pH values due to functional groups. COM is largely similar to EOM, although fraction proportions may vary.
- As EOM bear a negative charge it is removable by conventional metal coagulants. The mechanism of coagulation is highly dependent on the coagulant/DOC concentration ratio. Achieving effective coagulation is dependent on the ratio of charges in the system, higher AOM concentrations can be compensated by higher coagulant doses. Coagulant metal-AOM complexes can form leading to 1) inhibition of metal hydrolysis and contributing to a rise in coagulant demand and 2) blockage of negatively charged functional groups preventing the molecule from being coagulated through adsorption and charge neutralization mechanisms.
- AOM has the potential to contribute to reversible and irreversible fouling with the dominant mechanism being a factor of: initial AOM concentration, AOM character, volume filtered and length of filtration test, membrane type and configuration and flux. Interactions between terrestrial NOM and AOM could form a more aggresive foulant, particularly in the presence of divalent cations, and further research is required in this regard. Fouling can be controlled via pretreatment, avoidance of shear prior to membrane filtration, and selection of hydrophilic membranes. A combination of NaOH and NaOCl is currently agreed to be the best chemical cleaning strategy.
- PAC enhanced the overall removal of AOM (mixture of intra- and extracellular matter), although it was apparently ineffective for the highly hydrophilic AOM. Although the inclusion of AC in the water treatment works can assist the coagulation/flocculation based treatment of algae, the studies focused primarily on adsorption of AOM are still limited and systematic evaluation of adsorption effectiveness is necessary. Moreover, understanding adsorption of AOM on AC is of a major significance as many of drinking water treatment plants face up to the constantly increasing concentrations of AOM due to the eutrophication of surface waters.

- Overall, cyanobacteria and green algae formed more HAAs than THMs for a given DOC concentration and more N-DBPs than allochtonous NOM. It was determined that it is the length of disinfection time that impacts specific C-DBP reactivity rather than growth phase or species. AOM in water sources could impact a water treatment plant's ability to meet THM guideline values and it is suggested that SUVA is not a good indicator to predict THM formation for autochthonous OM sources.

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10. SUMMARY AND CONCLUSIONS

This dissertation thesis focused on the removability of algal organic matter (AOM), specifically of peptides and proteins contained in algal cellular organic matter, by coagulation. Cultivation of three representative algal species and characterisation of their AOM were carried out (Publication 1). Emphasis was put on characterisation of peptides and proteins of *M. aeruginosa* that were subsequently used in coagulation experiments (Publication 1, 2 and 4). The experiments contributed to the elucidation of the mechanisms of the interactions between peptides/proteins and coagulants and emphasized the importance of pH optimization (Publication 2 and 3). The interactions between peptides/proteins and kaolin particles and the influence of peptide/proteins on the coagulation of turbid waters were also described (Publication 4). Furthermore, adsorption onto activated carbon was found to be an effective treatment for the peptides that were difficult to remove by coagulation (Publication 5). Finally, the removability of AOM by different treatment techniques was discussed in Publication 6.

The following overall conclusions have been drawn:

1) The AOM of the three algal species (*Microcystis aeruginosa*, *Fragilaria crotonensis* and *Chlamydomonas geitleri*) is predominantly hydrophilic, with low SUVA and significant portion of peptide/protein material that is most noticeable in COM of *M. aeruginosa*. The AOM composition and characteristics are species-specific and change with the growth phase. EOM and COM show several differences. Specifically, a portion of peptides and proteins, protein quantity and diversity, a portion of hydrophilic fraction and of high-MW compounds are significantly bigger in COM than in EOM. Therefore, when developing the removal strategy for AOM, species, growth phase and the extent of COM release should be taken into consideration.

2) Coagulation of peptides and proteins is strongly dependent on pH value and on the charge properties of the involved particles/molecules. The highest coagulation efficiencies are achieved at acidic pH values (pH 4 – 6 for ferric coagulant) due to electrostatic interactions between positively charged Fe-hydroxopolymers and negatively charged functional groups of peptides/proteins. At low COM peptides and proteins/coagulant ratio (e.g. w/w DOC/Fe = 0.14), adsorption of peptides/proteins on the surface of colloidal Fe-oxide-hydroxide particles at pH values 6 – 8 may also be an important coagulation mechanism.

3) When COM peptides/proteins occur in turbid waters, optimum pH values for effective

treatment substantially change. At pH values suitable for the removal of kaolin (7 – 8.5 for Al and 6.4 – 8 for Fe coagulant), poor removal is achieved in the presence of peptides/proteins and the optimum pH for coagulation of peptide/protein-kaolin mixture decreases to pH 5 – 6.5 for Al and 4 – 6 for Fe. At these pH values, electrostatic interactions between amphoteric peptides/proteins, negatively charged kaolin particles and positively charged coagulant hydroxopolymers enable coagulation. 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).

4) Peptides/proteins of *M. aeruginosa* deteriorate the coagulation process by formation of dissolved complexes with coagulants (both Al and Fe dissolved species). However, this happens at quite narrow pH range (about 6.8 for Al and 6 for Fe) and can be avoided by optimization of pH conditions. Peptides and proteins of apparent MWs of approximately 1, 2.8, 6, 8, 8.5, 10 and 52 kDa are responsible for formation of complexes.

5) High-MW proteins (apparent MW > 10 kDa) are easily removable by coagulation, whereas low-MW peptides and proteins (< 10 kDa) are removed very poorly. Other treatment techniques need to be employed for their removal, particularly considering that they may include cyanobacterial toxins.

6) Low-MW peptides and proteins may be removed by adsorption onto activated carbon (AC). The highest removal rates are again achieved at acidic pH values (pH 5) due to electrostatic interactions between functional groups of peptides/proteins and AC. Peptides and proteins of lower apparent MW (< 4.5 kDa) are adsorbed onto AC to a greater extent than the larger ones.

The results show that coagulation is a useful technology for the treatment of algae-laden waters. Coagulation process effectively removes algae-derived organic substances of higher molecular weights. In the coagulation of turbid waters, polymers contained in algal organic matter can even act as polymer aids and enhance the removal of clay particles. However, in the presence of AOM in turbid waters, a decrease in the coagulation pH is a prerequisite for the efficient removal. For these reasons, coagulation is an important treatment step before technologies, such as adsorption onto activated carbon or membrane filtration, which are susceptible to the presence of biopolymers in source water. The tendency to deteriorate coagulation by the formation of complexes between algal organics and coagulants can be eliminated by consistent optimization of coagulation conditions.

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