Investigating the coagulation of non-proteinaceous algal organic matter: Optimizing coagulation performance and identification of removal mechanisms

Jana Naceradska¹, Katerina Novotna¹, Lenka Cermakova¹, Tomas Cajthaml², Martin Pivokonsky¹,*

¹. Institute of Hydrodynamics of the Czech Academy of Sciences, Pod Patankou 5, 166 12 Prague 6, Czech Republic
². Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, 142 20 Prague 4, Czech Republic

ABSTRACT

The removal of algal organic matter (AOM) is a growing concern for the water treatment industry worldwide. The current study investigates coagulation of non-proteinaceous AOM (AOM after protein separation), which has been minimally explored compared with proteinaceous fractions. Jar tests with either aluminum sulphate (alum) or polyaluminium chloride (PACl) were performed at doses of 0.2–3.0 mg Al per 1 mg of dissolved organic carbon in the pH range 3.0–10.5. Additionally, non-proteinaceous matter was characterized in terms of charge, molecular weight and carbohydrate content to assess the treatability of its different fractions. Results showed that only up to 25% of non-proteinaceous AOM can be removed by coagulation under optimized conditions. The optimal coagulation pH (6.6–8.0 for alum and 7.5–9.0 for PACl) and low surface charge of the removed fraction indicated that the prevailing coagulation mechanism was adsorption of non-proteinaceous matter onto aluminum hydroxide precipitates. The lowest residual Al concentrations were achieved in very narrow pH ranges, especially in the case of PACl. High-molecular weight saccharide-like organics were amenable to coagulation compared to low-molecular weight (<3 kDa) substances. Their high content in non-proteinaceous matter (about 67%) was the reason for its low removal. Comparison with our previous studies implies that proteinaceous and non-proteinaceous matter is coagulated under different conditions due to the employment of diverse coagulation mechanisms. The study suggests that further research should focus on the removal of low-molecular weight AOM, reluctant to coagulate, with other treatment processes to minimize its detrimental effect on water safety.

© 2018 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.
Introduction

The presence of seasonal algal blooms in drinking water sources creates serious challenges in drinking water production. Water quality can be severely impacted by the release of algal organic matter (AOM), which reacts with disinfectants to form potentially harmful disinfection by-products (DBPs, Goslan et al., 2017). AOM is released during the growth of algae due to metabolic processes as extracellular organic matter (EOM) or during cell lysis as cellular organic matter (COM) and comprises a wide range of compounds, with polysaccharides, proteins, lipids, and small molecules being the main components (Henderson et al., 2008; Pivokonsky et al., 2014; Villacorte et al., 2015).

Coagulation/flocculation is one of the most widely applied techniques of AOM treatment in drinking water production. The complexity of AOM composition complicates the understanding of coagulation pathways and consequently, AOM removal. Therefore, efforts have been made to divide AOM according to its properties and coagulate the separate fractions (Liu et al., 2018; Pivokonsky et al., 2012, 2015). In recent years, attention has been paid to the coagulation of the proteinaceous fraction of the COM of cyanobacterium Microcystis aeruginosa (Pivokonsky et al., 2009, 2015). These studies ascertained that peptides/proteins of M. aeruginosa were removed by coagulation at acidic pH values with efficiencies of 60%–85% by both ferric and aluminum coagulants. They also demonstrated that peptides/proteins may inhibit coagulation by the formation of complexes with coagulant metals, but this can be avoided by consistent optimization of the coagulation pH value (Pivokonsky et al., 2009, 2015). By contrast, little work has been done on the coagulation of non-proteinaceous AOM. Pivokonsky et al. (2009), who coagulated the COM of M. aeruginosa by either ferric or aluminum sulphate, demonstrated higher removal efficiencies for proteinaceous (74% and 50%, respectively) than non-proteinaceous compounds (12% and 22%, respectively). Higher removal efficiencies for proteins (75% and 69%, respectively) than saccharides (30% and 40%, respectively) were removed by coagulation at acidic pH values with efficiencies of 60%–85% by ferric and aluminum coagulants. They also demonstrated that peptides/proteins may inhibit coagulation by the formation of complexes with coagulant metals, but this can be avoided by consistent optimization of the coagulation pH value (Pivokonsky et al., 2009, 2015). By contrast, little work has been done on the coagulation of non-proteinaceous AOM. Pivokonsky et al. (2009), who coagulated the COM of M. aeruginosa by either ferric or aluminum sulphate, demonstrated higher removal efficiencies for proteinaceous (74% and 50%, respectively) than non-proteinaceous compounds (12% and 22%, respectively). Higher removal efficiencies for proteins (75% and 69%, respectively) than saccharides (30% and 40%, respectively) were also achieved by Cui et al. (2016), who coagulated effluent organic matter from a wastewater treatment plant by either aluminum chloride (AlCl₃) or polyaluminium chloride (PACl). Aluminum chloride was, therefore, better for protein removal, while PACl was more effective in polysaccharide removal. Slightly higher COM removal for PACl than AlCl₃ was observed by Liu et al. (2018), who investigated the coagulation of COM produced by M. aeruginosa together with kaolin particles. Since these studies indicated that non-proteinaceous COM is generally disinclined to coagulate, attention should be paid to the limits of coagulation of non-proteinaceous COM and also the character of compounds not removed by coagulation. The properties of residual compounds will impact the setup of subsequent treatment technologies.

On the basis of these considerations, this study aims to systematically investigate the coagulation of non-proteinaceous COM of green alga Chlorella vulgaris. The study focuses on COM, as these compounds present most of organic matter during the algal bloom decay and are known to disturb coagulation (Pivokonsky et al., 2016). The study quantifies the removal of non-proteinaceous COM after the consistent optimization of coagulation conditions and identifies the coagulation pathways. Special attention is paid to the influence of the pH value on coagulation because it governs the form of coagulants as well as the surface charge of coagulated impurities and thus coagulation mechanisms. In order to better understand the coagulation pathways and properties of residual organics after coagulation, characterization of non-proteinaceous COM before and after coagulation was performed. Two types of coagulants, traditional aluminum sulphate (alum) and polymeric coagulant polyaluminium chloride (PACl), were compared in this study as they have demonstrated diverse Al species distribution, coagulation mechanisms and efficiency. Finally, the coagulation of proteinaceous COM (investigated in our previous studies) and non-proteinaceous COM was compared in terms of efficiency, coagulation mechanisms and the character of residual organics. The outcomes may be of practical value in enhancing the removal of AOM and controlling its adverse effects on water quality.

1. Materials and methods

1.1. Cultivation of Chlorella vulgaris

Chlorella vulgaris was chosen for this study since it is a cosmopolitan bloom-forming alga, the COM of which contains large portion of non-proteinaceous compounds (Henderson et al., 2008; Safi et al., 2014). An inoculum of Chlorella vulgaris (strain CGALA 256) was obtained from Centre Algitech of the Institute of Microbiology, CAS, Czech Republic. The culture was grown in a photobioreactor in glass tubes situated in a water bath (30 °C) under continuous illumination with incident light intensity 100 μE m⁻² s⁻¹ (PAR sensor QSL-2101, Biospherical instruments Inc., USA) and feeding of air enriched with 2% CO₂ (V/V) at 15 L/hr per tube. Each tube contained 300 mL of mineral medium, having the initial composition (mg/L): 1100 (NH₄)₂CO, 238 KH₂PO₄, 204 MgSO₄ 7 H₂O, 40 Ca₃(PO₄)₂, 186 H₂BO₃, 88 CaCl₂, 0.832 H₃BO₃, 0.946 CuSO₄ 5 H₂O, 3.294 MnCl₂ 4 H₂O, 0.172 (NH₄)₆Mo₇O₂₄ 4 H₂O, 2.678 ZnSO₄ 7 H₂O, 0.616 CoSO₄ 7 H₂O, and 0.0014 (NH₄)VO₃. The pH value was adjusted to 6.5–7.0 using 1 mol/L KOH before inoculation from an agar plate (Brányiková et al., 2011).

1.2. Cell harvesting and COM extraction

Cells of C. vulgaris were harvested at the late stationary growth phase (20th day of cultivation) in order to acquire COM, the composition of which is similar to that of decline phase, which is most problematic from the perspective of coagulation performance. Moreover, carbohydrate production is favored at the stationary phase, while protein production prevails at the exponential phase (Lv et al., 2010). The cells were separated from the culture media by centrifugation (4000 r/min, 20 min) and then re-suspended in ultra-pure...
water. Dissolved COM was extracted using ultrasonication in an ice bath using an ultrasonic homogenizer (UP400S, Hielscher Ultrasonics, Germany) at 60% amplitude of ultrasonication (240 W) in pulse mode for 5 min, followed by filtration through a 0.45 μm membrane filter. Filtrates were stored at −60 °C. The acquired COM sample was considered a fraction comprising intracellular (IOM), surface-retained organic matter (SOM), and possibly also organics loosely bound to the cell surface, i.e. EOM.

1.3. Isolation of non-proteinaceous COM

In our previous studies (Pivokonsky et al., 2012, 2015), which investigated the coagulation of proteinaceous COM, we used a salting out method adapted from Dawson et al. (1986) to separate proteinaceous matter (PM) from non-proteinaceous matter (NM). This method is, however, not suitable for acquiring NM for coagulation tests as NM is burdened with very high concentrations of (NH4)2SO4. Approximately 17 g of (NH4)2SO4 per 1 g of dissolved organic carbon (DOC) were present in the residual non-proteinaceous fraction after the protein precipitation. We found that, for the amounts of organic matter needed for coagulation tests, (NH4)2SO4 is difficult to remove without a concurrent loss of NM (e.g., due to the sorption of organic compounds on dialysis membranes utilized for desalting). Therefore, PM was separated from NM by the thermal precipitation of proteins. This procedure assumes that most proteins denature predominantly at 40–80 °C and that denaturation is usually accompanied by aggregation and/or gelation (Bischof and He, 2005), while saccharide-like substances are generally stable at higher temperatures (Bothara and Singh, 2012). COM samples were, therefore, heated in a water bath (80 °C) for 30 min, and the precipitated proteins were then removed by filtration through a 0.45 μm membrane filter. PM and NM portions were expressed as DOC. NM was stored at −60 °C. The yields of PM and NM by thermal precipitation and salting out were compared. The protein content in NM after thermal precipitation was also controlled by additional salting out, which yielded no proteins, and by HPSEC (1260 Infinity, Agilent Technologies, USA) using the Agilent Bio SEC-5100 Å and 300 Å columns connected in series and coupled with a diode array detector (DAD) operating at 280 nm (Pivokonsky et al., 2015), which is used for peptide/protein detection.

1.4. Characterization of non-proteinaceous COM

NM obtained after thermal precipitation of proteins from the COM of Chlorella vulgaris was characterized in terms of N/C (nitrogen/carbon) ratio, charge, molecular weight distribution, total carbohydrates, proteins and lipids (as fatty acids). To describe the composition of NM utilized in this study and link it to the composition of the original algal material, the total carbohydrate, protein and fatty acid content was also determined in the total COM biomass (disrupted cells of C. vulgaris) and dissolved COM biomass (extracted from cells as described in Section 1.2.). DOC and total nitrogen (TN) concentrations were determined by a TOC/TN analyzer (Torch TN, TELEDYNE TEKMAR, USA). Determination of the amount of ionisable functional groups in whole NM and in its fractions with MW < 50 kDa and >50 kDa was undertaken by potentiometric titrations performed under the nitrogen atmosphere using an Orion 960 Autotitrator (Thermo Scientific, USA). Before the experiments, the system was standardized with dilute NaOH solutions to pH 10.5. The ionic strength (I = 0.05 mol/L) was controlled with NaCl. The aliquots (500 mL) of non-proteinaceous COM containing 250 mg DOC/L were titrated to pH 2.5 using 0.05 mol/L HCl at a constant temperature of 25 ± 0.2 °C. A blank titration was performed under the same conditions. The resultant titration curve (transformed for 1 g DOC/L) was determined as the difference between titration of non-proteinaceous COM and the blank titration (Newcombe 1994). Dissociation constants and equivalence points were determined as described by Safarikova et al. (2013). Molecular weight (MW) fractionation was performed using the Amicon Ultra-15 centrifugal filters of nominal MW cut-offs of 100, 50, 30, 10, and 3 kDa (Millipore, USA) as described by Pivokonsky et al. (2014). The MW distribution was expressed as a proportional part of DOC. To determine the total carbohydrates, the MBTH (3-methyl-2-benzothiazolinone hydrizone) method was employed. In brief, 25 mg of freeze-dried algal biomass was subjected to a two-step sulfuric acid hydrolysis to convert the polymeric forms of carbohydrates into monomeric subunits. The saccharide content was then determined spectrophotometrically at 620 nm after reaction with MBTH that yields a blue-green colored solution due to complexing with the aldehyde group of carbohydrates. Glucose was used as a calibration standard, while the calibration range was 0.01–0.05 mg/mL (Wychen and Laurens, 2015). To determine protein content, sequential extraction in trichloroacetic acid and NaOH from freeze-dried samples followed by a modified Lowry method was employed (Slocombe et al., 2013). The determination of total fatty acids from the samples was performed according to the methodology previously described by Šezanka et al. (2013). Briefly, samples were extracted using a mixture of chloroform, methanol and phosphate buffer (1:2:0.8; V/V/V). Fatty acid methyl esters (FAMES) were prepared after saponification and further esterification using BF3/MeOH. The products were dissolved in hexane and analyzed by gas chromatography-mass spectrometry (GC–MS; 450-GC, 240-MS ion trap detector, Varian, Walnut Creek, CA) according to Šnajid et al. (2008). The individual fatty acids were identified and quantified using the respective chemical standards obtained from Sigma-Aldrich (Czech Republic) and Matreya, LLC (PA, USA). All the analyses were performed in triplicates.

1.5. Coagulation tests

Traditional jar tests were applied to study the coagulation of the non-proteinaceous COM of C. vulgaris. Model water was ultra-pure water with alkalinity adjusted to 1 mmol/L (utilizing 0.1 mol/L NaHCO3), pH 8.0 and initial DOC concentration of 5 mg/L. Two coagulants were compared: aluminum sulphate (Al2(SO4)3·18H2O; Sigma Aldrich, USA), the most common conventional coagulant, and polymeric coagulant polyaluminium chloride (PACl; Kemwater ProChemie, Czech Republic; basicity 43% ± 5%, Al content 9.0% ± 0.3%), which is utilized for its high efficiency in terms of charge neutralization and a wide range of coagulation pH values owing to the
high stability of \( \text{Al}_{13} \) polymers (Wu et al., 2007). Stock solutions of 1% coagulants were utilized in the tests. The doses of coagulants and pH values were optimized by testing with coagulant doses ranging from 1 to 15 mg/L Al (0.037–0.556 mmol/L Al; 0.2–3.0 mg Al/mg DOC) in the pH range 3.0–10.5. The target pH values were reached by adding predetermined amounts of 0.1 mol/L NaHCO\(_3\), 0.1 mol/L NaOH or 0.1 mol/L HCl before supplementation with the coagulant. The experiments were performed using a variable speed eight position paddle stirrer (LMK 8) and 60 min of settling. The supernatants were analyzed for DOC and residual Al by ICP-OES (5110 Series, Agilent Technologies, USA). Each sample was analyzed in triplicate.

2. Results and discussion

2.1. Character of non-proteinaceous COM

Non-proteinaceous COM was isolated in two steps from the cells of Chlorella vulgaris, i.e., isolation of water-soluble COM from the cells and isolation of the non-proteinaceous fraction from water-soluble COM. To provide insight into the composition of original and isolated fractions, the carbohydrate, protein and lipid content in the total COM (cells including both water-soluble and insoluble components), water-soluble COM (extracted from cells) and NM (extracted from water-soluble COM) was quantified as detailed in Table 1. It can be seen that the portion of proteins and especially lipids differed in the total and dissolved COM biomass of C. vulgaris. This is given by the fact that some proteins and most lipids are insoluble in water. According to the literature, protein content in C. vulgaris dry biomass varies with growth conditions 4%–40% (Becker, 1994; Laurens et al., 2014; Safi et al., 2014; Yeh and Chang, 2012). Some of the protein is bound to cell walls (about 20%) and the membranes of organelles, and some is soluble in cytoplasm (Becker, 1994; Safi et al., 2014). Herein, the portion of proteins in water-soluble COM of C. vulgaris determined by the modified Lowry method was about 10% of dry weight (DW), which conforms with the yield of the thermal precipitation method separating about 9% of DOC. The same amount of proteinaceous DOC (9%) was acquired with the salting out method. HPSEC-DAD at 280 nm analyses showed that some organics of apparent MW 800–6000 Da remained in the COM after thermal precipitation (data not shown), indicating the presence of low-MW peptides. The presence of nitrogenous compounds, which may contain amino acids, peptides, nucleotides and nucleic acids, was confirmed also by measuring TN. The N/C ratio was about 0.1.

In general, C. vulgaris can reach 5%–50% lipids per biomass DW depending on cultivation conditions (Becker, 1994; Yeh and Chang, 2012). During the separation of water-soluble COM, lipids apparently stayed in the solid parts of cells and were removed by filtration, as they are relatively insoluble in water. The analyses of fatty acids showed that they formed about 26% of the total COM biomass, while both water-soluble and non-proteinaceous COM contained negligible concentrations of lipids and fatty acids, composed mainly of palmitic and stearic acid. Similarly, Yeh and Chang (2012) identified palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids as major components of lipids produced by the C. vulgaris ESP-31 strain.

Carbohydrates formed 21%, 18% and 20% of the total COM, water-soluble COM and non-proteinaceous COM DW, respectively. In the literature, the carbohydrate content again varies with growth conditions and methods used and ranges 12%–60% of biomass DW (Laurens et al., 2014; Templeton et al., 2012; Yeh and Chang, 2012). Sui et al. (2012) extracted water-soluble polysaccharides of C. vulgaris forming about 13%–19% of biomass DW.

It should be noted that besides proteins, lipids and carbohydrates, algal biomass includes other substances, which forms majority in the case of water-soluble COM and NM. These substances are of low MW as indicated by MW fractionation (see Fig. 1) and comprise aldehydes,

| Table 1 – Carbohydrate, protein and lipid content in total cellular organic matter (COM), water-soluble COM and non-proteinaceous COM of Chlorella vulgaris expressed as dry weight portions. |
|----------------|----------------|----------------|----------------|
| Fraction        | Total COM      | Water-soluble COM | Non-proteinaceous COM |
| Carbohydrate    | 21%            | 18%             | 20%             |
| Protein         | 23%            | 10%             | 0%              |
| Lipid           | 26%            | 0.04%           | 0.05%           |

Fig. 1 – Molecular weight fractionation of non-proteinaceous COM (NM) of Chlorella vulgaris before coagulation (a) and after coagulation by aluminum sulphate (alum) (Al dose 10 mg/L; pH 7.3) (b) and polyaluminium chloride (PACl) (Al dose 10 mg/L; pH 7.3) (c) expressed as DOC portions.
hydrocarbons, amines, glycolic acids, nucleotides and nucleic acids, amino acids, peptides and aminosugars (Pivokonsky et al., 2016).

MW fractionation through Amicon Ultra-15 centrifugal filters (Fig. 1a) showed that the NM of *C. vulgaris* contained a high portion (67%) of low-MW compounds (<3 kDa) and the second biggest fraction was >100 kDa (22%). Compared with other algal species (Baresova et al., 2017; Pivokonsky et al., 2014), MW distribution of the NM of *C. vulgaris* followed the same pattern as the NM of green alga *Chlamydomonas geitleri* and diatom *Fragilaria crotonensis*, while the NM of cyanobacteria *Microcystis aeruginosa* and *Merismopedia tenuissima* differed, but still contained large portions of low-MW substances (Table 2). The same MW fractions for PM are shown in Table 2 for comparison. In the case of PM, the portions of fractions under 3 kDa were small. The COM of both cyanobacteria species contained a substantial portion of high-MW proteins. In both studies indicated in Table 2 (Baresova et al., 2017; Pivokonsky et al., 2014), COM was acquired in the same way as in this study, and proteins were isolated from NM with the salting out method. Changes in MW fractions after coagulation by alum and PACl (Fig. 1b, c) are discussed in Section 2.2.

Since charge and the content of functional groups are important from the perspective of coagulation, potentiometric titration of NM was performed. The titration curve (Fig. 2) shows the number of protons which the NM is able to accept under the given pH value and which is equal to the amount of dissociated functional groups (in millimoles of H⁺ ions per 1 g of DOC). The curve provides three buffer regions with equivalence points and dissociation constants, which may be attributed to various functional groups. The dissociation constant $pK_1 = 3.5$ may be attributed to carboxyl (–COOH) functional groups. For example, saccharide alginic acid is composed of poly-D-mannuronic and L-guluronic acid with $pK_a = 3.38$ and 3.65.

### Table 2 – Comparison of molecular weight (MW) fractions of non-proteinaceous and proteinaceous COM of different algal species.

<table>
<thead>
<tr>
<th></th>
<th><em>Chlorella vulgaris</em></th>
<th><em>Chlamydomonas geitleri</em></th>
<th><em>Fragilaria crotonensis</em></th>
<th><em>Merismopedia tenuissima</em></th>
<th><em>Microcystis aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-proteinaceous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW &lt; 3 kDa</td>
<td>67%</td>
<td>66%⁴</td>
<td>63%⁴</td>
<td>41%⁵⁴</td>
<td>48%⁴</td>
</tr>
<tr>
<td>MW &gt; 100 kDa</td>
<td>22%</td>
<td>22%⁴</td>
<td>22%⁴</td>
<td>10%⁵⁴</td>
<td>35%⁴</td>
</tr>
<tr>
<td><strong>Proteinaceous</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MW &lt; 3 kDa</td>
<td>NA</td>
<td>0%⁴</td>
<td>2%⁴</td>
<td>5%⁵⁴</td>
<td>6%⁴</td>
</tr>
<tr>
<td>MW &gt; 100 kDa</td>
<td>NA</td>
<td>3%⁴</td>
<td>6%⁴</td>
<td>86%⁵⁴</td>
<td>22%⁴</td>
</tr>
</tbody>
</table>

NA – not analyzed.

⁴ Pivokonsky et al. (2014).

⁵ Baresova et al. (2017).

![Fig. 2 – Titration curves of non-proteinaceous COM of *Chlorella vulgaris* (NM), and its fractions with molecular weight > 50 kDa (NM > 50 kDa) and < 50 kDa (NM < 50 kDa). Equivalence points (pEq₁–pEq₃) and dissociation constants (pK₁–pK₃) assigned to different functional groups are depicted for NM.](image)
respectively. The constant $pK_2 = 6.2$ may be assigned to the second $-\text{COOH}$ group of dicarboxylic acids. The constant $pK_3 = 9.5$ may be attributed to the collective effect of functional groups dissociating at alkaline pH values, such as $-\text{OH}$, $-\text{SH}$, $-\text{NH}_2$, and $=\text{NH}_3^+$ (Chang, 2005). It should be taken into consideration that NM is a mixture of compounds containing various functional groups whose dissociation constants depend not only on the type of group, but also on the overall net charge of the molecule. The number of titratable groups in whole NM is very similar to figures from our previous studies obtained for humic substances (Pivokonsky et al., 2015) or the COM of *Merismopedia tenuissima* (Baresova et al., 2017). The titration curves for NM fractions with MW > 50 kDa and < 50 kDa will be discussed in Section 2.2.

### 2.2. Coagulation of non-proteinaceous COM

Dose optimizations were performed for Al concentrations of 1–15 mg/L in a pH range of 3.0–10.5 with initial DOC concentration of 5 mg/L for both coagulants. The results are detailed in Figs. 3 and 4. In these figures, pH ranges 4.0–9.0 and 5.5–10.5 are indicated for alum and PACl, respectively, because no DOC and Al removal was observed outside these pH ranges. When alum was applied, coagulation started with a dose of 3 mg/L Al, and the highest DOC removal efficiencies (up to 25%) were achieved at doses of 8, 10 and 12 mg/L Al (1.6, 2 and 2.4 mg Al/mg DOC) at pH 6.6–8.0 (Fig. 3a). Residual aluminum dropped to the lowest level of 0.3 mg/L in the pH range 7.1–7.5 (Fig. 4a). In the case of PACl, coagulation started with a dose of 3 mg/L Al, and the highest DOC removal efficiencies (up to 25%) were achieved at doses of 8, 10, 12 and 15 mg/L Al (1.6, 2, 2.4 and 3 mg Al/mg DOC; Fig. 3b). DOC removal efficiencies of 19%–25% were obtained at pH range 7.5–9.0, while residual aluminum reached the lowest level of 0.5 mg/L in a very narrow pH range of 7.6–7.8 (Fig. 4b). This narrow pH range can be better observed in Fig. 5, which details the coagulation curves for the optimal doses of PACl and alum (10 mg/L Al, i.e., 2 mg Al/mg DOC).

The coagulation of non-proteinaceous COM has been poorly investigated up to now. Pivokonsky et al. (2009) coagulated COM of *M. aeruginosa* with ferric sulphate and aluminum sulphate and analyzed the PM and NM content before and after coagulation. They found that under optimal conditions ferric sulphate removed about 74% of PM and 12% of NM, while aluminum sulphate removed about 50% of PM and 22% of NM. Cui et al. (2016), who investigated the coagulation of effluent organic matter from wastewater by AlCl$_3$ and PACl, also demonstrated a higher removal of protein (75% and 69%, respectively; determined by the modified Lowry method) than saccharides (30% and 40%, respectively; determined by the colorimetric method) at Al dose of 3.2 mg Al/mg DOC.

Studies focusing on the coagulation of algal PM confirm higher DOC removal at lower coagulant doses (Pivokonsky et al., 2012, 2015) than those needed for NM removal. PM was also removed at lower pH values, at which metal coagulants hydrolyse to form positively charged hydroxopolymers (Stumm and Morgan, 1996), and was therefore prone to a charge neutralization mechanism. Specifically, Pivokonsky et al. (2012) found that peptides/proteins of *M. aeruginosa* in DOC concentrations of 1–8 mg/L were removed in the pH range 4–6 by ferric sulphate (at a dose of 7 mg/L Fe, i.e., 0.88–7 mg Fe/mg DOC) with a removal of 60%–85% depending on the initial DOC concentration. In the study by Pivokonsky et al. (2015), the same peptides/proteins of *M. aeruginosa* (DOC = 5 mg/L) were coagulated by alum (at a dose of 2 mg/L Al, i.e., 0.4 mg Al/mg DOC) in the pH range 5.2–6.7 with a removal efficiency of 75%.

---

**Fig. 3** – Optimization of coagulant dose and pH value – DOC removal efficiencies for aluminum sulphate (a) and polyaluminium chloride (b). At least three samples were analyzed in each pH interval for each coagulant dose. Initial DOC = 5 mg/L.
Therefore, NM required four to five times more alum than PM. In both studies mentioned, Fe/Al residuals dropped below 0.1 mg/L.

Herein, relatively high residual aluminum concentrations, i.e., about 0.3 and 0.5 mg/L for alum and PACl, respectively, were achieved after coagulation under optimal conditions and 60 min of sedimentation (Fig. 4). These Al concentrations do not comply with the regulated limit of 0.2 mg/L of the EC drinking water directive (Directive 98/83/EC). High Al residuals stem from the high coagulant doses utilized to coagulate NM compared to the coagulation of COM peptides/proteins mentioned previously or the coagulation of AOM (Bařešová et al., 2017; Henderson et al., 2010). For instance, Bařešová et al. (2017) found that the ferric sulphate dose required to remove the COM of cyanobacterium *Merismopedia tenuissima* (removal rate of 43%–53%) was 1 mg Fe/mg DOC. Furthermore, Henderson et al. (2010) achieved the highest removals (71%, 55 and 46%, respectively) of AOM produced by *Chlorella vulgaris*, *Microcystis aeruginosa* and diatom *Asterionella formosa* at alum doses of 0.8, 1.2 and 1.5 mg Al/mg DOC, respectively. The high Al residuals achieved in this study are further discussed in Section 2.3.

MW fractionation of residual organic matter after coagulation provides insights into the coagulation pathways. In the

![Fig. 4 – Optimization of coagulant dose and pH value – Al residual concentrations for aluminum sulphate (a) and polyaluminium chloride (b). At least three samples were analyzed in each pH interval for each coagulant dose. Initial DOC = 5 mg/L.](image)

![Fig. 5 – Residual DOC and aluminum concentrations as a function of pH value for the optimized doses (10 mg/L Al) of aluminum sulphate (a) and polyaluminium chloride (b). Initial DOC = 5 mg/L.](image)
case of alum, fractions with MW > 50 kDa were fully removed by coagulation, whereas fractions with MW 3–50 kDa were removed partly and substances with MW < 3 kDa remained in the treated water (Fig. 1b). In the case of PACl, compounds larger than 10 kDa were fully removed by coagulation, while fraction with MW 3–10 kDa was removed partly and fraction under 3 kDa remained in the treated water (Fig. 1c). A number of previous studies ascertained that low-MW organics are reluctant to coagulate (Gregor et al., 1996; Liu et al., 2018; Pivokonsky et al., 2012, 2015). In the study by Liu et al. (2018), compounds of MW > 100 kDa isolated from the COM of M. aeruginosa were coagulated with a removal of 70% by AlCl3 and 80% by PACl, while the fractions with MWs 30–100 kDa, 5–30 kDa and < 5 kDa exhibited a removal of 20%–40%. PACl showed higher DOC removal for the >100 kDa and < 5 kDa fractions than AlCl3. This agrees with our results, where PACl was more efficient in removing 3–10, 10–30 and 30–50 kDa fractions than alum (Fig. 1b, c). For algal COM peptides/proteins, Pivokonsky et al. (2012, 2015) found that peptides of MW < 10 kDa were coagulated by neither ferric nor aluminum sulphate. Herein, NM contained a large portion of low-MW compounds <3 kDa (about 67%), which is most likely the reason for its low removal. Based on the data shown in Table 2, it can be assumed that the NM of the mentioned algal species will be also coagulated with low efficiencies due to the high content of low-MW compounds. The low-MW fraction includes intermediate products of algal metabolism, such as aldehydes, hydrocarbons, amines, glycolic acids, nucleotides and nucleic acids, amino acids, peptides, aminosugars and mono- and oligosaccharides (Pivokonsky et al., 2016). The portion of carbohydrates after coagulation substantially decreased from 20% of DW before coagulation to 0.5%–2% of DW after coagulation by both coagulants, indicating that carbohydrates (saccharides) were prone to coagulation. The N/C ratio after coagulation was very similar to before coagulation (0.11), suggesting that a part of nitrogen-containing compounds, e.g. high-MW aminosugars, were removed by coagulation.

As the fraction with MW > 50 kDa was fully removed by both coagulants utilized in this study, charge characterization by potentiometric titration was performed for fractions with MW > 50 kDa and MW < 50 kDa to determine whether these fractions differ in terms of charge. The results (Fig. 2) showed that fraction above 50 kDa carried substantially less ionisable groups than fraction under 50 kDa, the titration curve of which is comparable to the titration curve of whole NM. The results of titration curves and determination of saccharide content imply that the high-MW fraction of NM, which undergoes coagulation, consists of neutral or slightly acidic polysaccharides.

2.3. Comparison between alum and PACl

Table 3 summarizes and compares the performance of alum and PACl in the coagulation of NM. While the same maximum DOC removal under similar coagulant doses was achieved, the two coagulants differed in pH values effective for coagulation, the lowest Al residual concentrations reached and removal efficiencies for different MW fractions. As shown by many studies (Van Benschoten and Edzwald, 1990; Wang et al., 2004; Yan et al., 2007), hydrolysis products of Al may be divided into three groups: (1) $A_{13}$ – reactive monomeric species and small polymers; (2) $A_{6}$ – medium polymer species, correlated well to tridecamer $Al_{33}OH_{34}(H_{2}O)_{12}$; (3) $A_{c}$ – colloidal or solid species of Al(OH)$_3$ precipitates. Regarding the mechanisms of coagulation, $A_{6}$ species are mainly involved in charge neutralization at slightly acidic pH values, while $A_{c}$ species are responsible for removals through adsorption and electrostatic patch effect (Yan et al., 2007). At pH values of the highest coagulation efficiency in the current study (6.6–8.0 for alum and 7.5–9.0 for PACl), the portion of $A_{c}$ is maximal for both alum and PACl, while the portion of $A_{6}$ decreases (Wang et al., 2004). These observations indicate that amorphous precipitates ($A_{c}$) are playing a major role in interactions between coagulants and NM and adsorption via both electrostatic and non-electrostatic forces, such as hydrogen bonding and van der Waals forces, are more important than electrostatic neutralization by Al hydroxopolymers for the coagulation of NM. This is supported by the fact that high-MW fraction, which was amenable to coagulation, bears very low amount of ionisable functional groups (Fig. 2). $A_{13}$ species of PACl are stable across a wide pH range and up to higher pH values than in the case of alum (Van Benschoten and Edzwald, 1990; Wang et al., 2014; Wu et al., 2007). This is probably the reason why the coagulation of NM by PACl took place at higher pH values than by alum. At pH above 7.5, the hydrolysis of PACl was favored (Wang et al., 2014) and the formation of amorphous hydroxide precipitates ($A_{c}$) from $A_{13}$ species enabled coagulation.

Difference in the lowest Al residual concentrations achieved for alum and PACl (see Table 3) also stem from different Al species distribution for alum and PACl. According to Wang et al. (2014) and Van Benschoten and Edzwald (1990), at pH values of the lowest Al residuals in this study, 5%-10% of dosed Al may be present in the form of $A_{6}$. High residual Al concentrations may thus be expected, and the portion of $A_{6}$ for PACl is higher than for alum. This is in accordance with our results and explains higher residual Al concentrations for PACl than for alum achieved in our experiments. Moreover, different Al species distribution for alum and PACl is probably the reason for different efficiencies in removing various MW fractions by alum and PACl, as shown in Section 2.2 (Fig. 1b, c). Highly stable $A_{6}$ (mostly $A_{13}$) species of PACl are able to interact with lower-MW

<table>
<thead>
<tr>
<th>Table 3 – Comparison of polyaluminium chloride.</th>
<th>aluminum sulphate</th>
<th>sulphate and polyaluminium chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al doses investigated (mg Al/mg DOC)</td>
<td>0.2–3</td>
<td></td>
</tr>
<tr>
<td>Coagulation starts at Al dose (mg Al/mg DOC)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Optimal Al doses (mg Al/mg DOC)</td>
<td>1.6; 2; 2.4</td>
<td></td>
</tr>
<tr>
<td>Maximum DOC removal (%)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>pH of DOC removal</td>
<td>6.6–8.0</td>
<td></td>
</tr>
<tr>
<td>Lowest Al residuals (mg/L)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>pH of lowest Al residuals</td>
<td>7.1–7.5</td>
<td></td>
</tr>
<tr>
<td>Coagulation efficiency (%)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>pH of highest coagulation efficiency (mg/L)</td>
<td>7.5–9.0</td>
<td></td>
</tr>
</tbody>
</table>
fractions through charge neutralization (Yan et al., 2007). Therefore, PACI was more efficient in removing 3–10, 10–30 and 30–50 kDa fractions than alum.

The different coagulation behaviors of PACI and traditional coagulant AlCl₃ was also observed by Liu et al. (2018) in the coagulation of the COM of M. aeruginosa together with kaolin. They ascertained that PACI removed 68% of COM at pH 7.8, while AlCl₃ removed 61% of COM at pH 6.9. PACI also demonstrated a slight advantage in removing fractions with MWs > 100 kDa and < 5 kDa and the hydrophilic fractions. AlCl₃ was slightly more effective in removing pigment-like organics, organics absorbing at 254 nm and transphilic fractions. Nevertheless, it is notable that neither the influence of kaolin particles nor the pH value was evaluated. The resultant pH value was based only on dose optimization experiments.

It can be concluded that the coagulation mechanisms for NM differ from those for whole AOM as well as proteinaceous AOM fractions and also for natural organic matter (NOM), which is usually represented by humic matter. Whole AOM and proteinaceous AOM were removed at acidic pH values through charge neutralization (Pivokonsky et al., 2015; Baresova et al., 2017). Similarly, as far as NOM is concerned, the aluminum-based coagulants, such as alum or aluminum chloride, are more efficient when positively-charged Al hydrolypolymers are generated under slightly acidic pH values, because NOM is mainly composed of organic compounds with negatively-charged functional groups (Sillanpää et al., 2018; Yan et al., 2008). Pre-hydrolysed metal-ion coagulants, such as PACI, also perform well at slightly acidic pH values (from pH 5) for NOM removal but may also be efficient in an alkaline pH up to 8.5–9.0 (Gao et al., 2006; Yan et al., 2008) owing to the stability of Al₁₃ species in a wide pH range.

3. Conclusions

The study investigated coagulation of non-proteinaceous AOM to which only limited attention has been paid so far, compared to algal cells and proteinaceous AOM. The following conclusions have been drawn from this research: (1) Non-proteinaceous AOM of Chlorella vulgaris was removed by aluminum-based coagulants (alum, PACI) only by 25% under optimal conditions. (2) High-MW saccharide-like organics bearing low surface charge were removed, while low-MW ones (<3 kDa) with higher surface charge formed most of residual organics after coagulation. (3) The low removal of non-proteinaceous AOM was given by the high content of low-MW compounds and may also be expected for the non-proteinaceous AOM of other algal species. (4) The prevailing coagulation mechanism of high-MW saccharide-like AOM was adsorption onto aluminum hydroxide precipitates (Al₅ species) at around neutral pH values in the case of alum (pH 6.6–7.5) and alkaline pH values in the case of PACI (pH 7.5–9.0). The lowest Al residuals were achieved at narrower pH ranges close to the minimum solubility of aluminum hydroxide. (5) Compared with previous studies dealing with the coagulation of proteinaceous AOM, NM requires higher coagulant doses and exhibits lower removal efficiencies than proteinaceous matter (25% vs. 60–85%). Additionally, proteinaceous and non-proteinaceous matter is coagulated under different pH values (acidic vs. neutral/alkaline) due to the employment of different coagulation mechanisms.

Even though PACI is widely accepted to exhibit a better coagulation efficiency in a wider pH range than traditional hydrolysing coagulants (AlCl₃ or alum), alum appears to be more suitable for non-proteinaceous AOM removal because it exhibits lower Al residuals at a wider pH range at the same DOC removal compared to PACI. The results also indicate that the various AOM components are removed by different coagulation pathways and that their removal strategy should be carefully evaluated. Based on these observations, AOM coagulation might be approached as a two-stage process: coagulation at acidic pH values for removing charged proteinaceous AOM fraction through charge neutralization and coagulation at neutral/alkaline pH values for removing neutral non-proteinaceous AOM through adsorption. This two-stage process requires further investigation as well as evaluation of the benefit of the expected increased AOM removal compared to the expense of implementing two-stage coagulation. It is significant that low-MW AOM compounds of both proteinaceous and non-proteinaceous character remaining in water after coagulation require other removal processes, such as membrane filtration, adsorption onto activated carbon or oxidative degradation. Further work is needed to determine suitable conditions for these subsequent treatments and to evaluate their impact on water quality.

Acknowledgments

This work was supported by the Czech Science Foundation (No. GA18-14445S) and by the institutional support of the Czech Academy of Sciences (RVO: 67985874).

REFERENCES


