Influence of COM-peptides/proteins on the properties of flocs formed at different shear rates

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ABSTRACT

Coagulation followed by floc separation is a key process for the removal of algal organic matter (AOM) in water treatment. Besides optimizing coagulation parameters, knowledge of the properties of AOM-flocs is essential to maximizing AOM removal. However, the impact of AOM on the floc properties remains unclear. This study investigated how peptides/proteins derived from the cellular organic matter (COM) of the cyanobacterium Microcystis aeruginosa influenced the size, structure, and shape of flocs formed at different shear rates (G). Flocs formed by kaolinite, COM-peptides/proteins and a mixture of the same were studied, and the effect of intermolecular interactions between floc components on floc properties was assessed. The coagulation experiments were performed in a Taylor–Couette reactor, with aluminum (Al) or ferric sulphate (Fe) utilized as coagulants. Image analysis was performed to gauge floc size and obtain data on fractal dimension. It was found that floc properties were affected by the presence of the COM-peptides/proteins and the coagulant used. COM-peptides/proteins increased floc size and porosity and widened floc size distributions. The Fe coagulant produced larger and less compact flocs than Al coagulant. Moreover, the decrease in floc size that occurred in parallel with increase in shear rate was not smooth in progress. A rapid change for the kaolinite-coagulant suspension and two rapid changes for the suspensions containing COM were observed. These were attributed to various intermolecular interactions between floc components participating in coagulation at different G. Based on the results obtained, shear rates suitable for efficient separation of flocs containing COM were suggested.

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Introduction

The presence of algae and cyanobacteria in drinking water has become a challenge for the water industry. During the increased growth and subsequent decay of algae and cyanobacteria, the concentration of algal/cyanobacterial cells and algal organic matter (AOM) increases in the source water. AOM comprises extracellular organic matter (EOM), stemming from algal metabolic activity and cellular organic matter (COM), sometimes also termed intracellular organic matter (IOM), released into the water during cell decay. AOM affects water quality and, since it is difficult to remove, it poses...
problems for water treatment plants (Zhang et al., 2010). Several studies have shown that AOM may exert a negative impact on the coagulation of algal/cyanobacterial cells (Henderson et al., 2010) and inorganic impurities, such as clay particles (Takaara et al., 2007). However, COM-peptides/proteins have been reported to improve the coagulation of clay particles and humic substances due to mutual interactions (Pivokonsky et al., 2015; Safarikova et al., 2013). Removal of AOM and its influence on the coagulation of other particles depends on the properties of AOM, e.g. protein/non-protein ratio, molecular weight, charge density and hydrophobicity, since it is these that determine the mechanism of interaction with the coagulant and other substances (Henderson et al., 2010; Pivokonsky et al., 2012).

In addition to efficient coagulation, an important role is played by floc properties (e.g. size, shape, porosity or density) in the efficiency of the downstream separation processes (i.e. filtration, sedimentation and flotation) (Bache and Gregory, 2010; Bubakova and Pivokonsky, 2012). Floc size and diameter is usually derived by the equation below (Bouyer et al., 2004; Jarvis et al., 2005a; Parker et al., 1972):

\[ d_{av/max} = CG^{-2/3} \]  

(1)

where \( d_{av/max} \) is the average or maximal diameter of flocs in the system, \( G \) is the average/mean shear rate (velocity gradient) and \( C \) and \( \gamma \) are constants describing the strength of flocs and their tendency to break up, respectively (Jarvis et al., 2005a).

These constants, and thus also the floc size, are influenced by the composition and concentration of contaminants, type and dose of coagulant, reaction pH and the form of flow or energy dissipation, i.e. the effect of hydrodynamics. The balance that exists between forces of attraction and hydrodynamics then determines whether the flocs grow, break up or restructure (Bouyer et al., 2001; Selomulya et al., 2001; Spicer et al., 1998; Vlieghue et al., 2017). The shape and structure (represented by porosity and density) of flocs is often expressed using fractal dimension, since flocs are considered to be fractal objects which possess certain self-similarity in flocs, i.e. a repeated pattern of interconnected particles. In the equation below fractal dimension, \( D \), describes the relationship between mass, \( M \), and length, \( L \):

\[ M = L^D \]  

(2)

An important feature of self-similar fractals is that their mass and density decrease as they grow larger (Meakin, 1990).

A number of studies have investigated the effect of hydrodynamics on floc properties, many of them employing artificial model particles, such as silica, kaolinite, latex and polystyrene (Jarvis et al., 2005b; Oles, 1992; Selomulya et al., 2001; Soos et al., 2007; Spicer et al., 1998). However, the effect of hydrodynamics on floc properties in natural water has largely been overlooked (Bubakova et al., 2013; Bubakova and Pivokonsky, 2012). Moreover, floc properties that arise through the presence of AOM or algal/cyanobacterial cells have received precious little attention (Gonzalez-Torres et al., 2017; Gonzalez-Torres et al., 2014; Henderson et al., 2006; Pivokonsky et al., 2009). In this context, it has been observed that flocs formed by algal cells and/or AOM were larger and more fragile than flocs stemming from inorganic particles or NOM (Gonzalez-Torres et al., 2014; Henderson et al., 2006). Furthermore, floc size was found to be more dependent on the initial concentration of AOM than the concentrations of coagulants (Pivokonsky et al., 2009).

However, information is very limited on floc properties arising through AOM, and thorough evaluation has yet to take place on differences between such flocs and those formed by other impurities. Furthermore, studies describing mutual interactions between AOM and other impurities during coagulation (Pivokonsky et al., 2016; Safarikova et al., 2013) indicate that said interactions may also alter the properties of flocs, and thus their separation.

Therefore, the aim of this study was to examine the properties of flocs instigated by AOM, namely COM-peptides/proteins derived from cyanobacterium Microcystis aeruginosa, at different shear rates. Specific objectives were to compare the properties of COM-flocs with floc properties initiated by inorganic particles (kaolinite) and those stemming from a mixture of COM and kaolinite. Moreover, the influence of COM-kaolinite interactions on floc properties was assessed. Finally, two types of coagulant – ferric sulphate and aluminium sulphate – were utilized in this study, and their impact on floc properties was evaluated. The significance of the presented research lies in the comparison of the influence of various floc components and their intermolecular interactions on the floc properties.

1. Materials and methods

1.1. Materials

1.1.1. Kaolinite

Kaolinite particles (Sedlecky kaolin a. s., Czech Republic) of size <4 \( \mu \)m were dispersed in ultrapure water. The point of zero charge (pzc) of kaolinite was determined to be at pH 2.9 by potentiometric titration, as described by Safarikova et al. (2013). Thus, at pH > 2.9, the negative charge prevailed in the kaolinite particles.

1.1.2. COM-peptides/proteins

Peptides/proteins were acquired from the COM of cyanobacterium Microcystis aeruginosa (strain Zap. 2006/2, CCALA, Institute of Botany, CAS, Czech Rep.) cultivated at the Institute of Hydrodynamics, CAS. The culture was harvested on the 16th day of cultivation, during the stationary growth phase, and separated from the culture media via a 0.45 \( \mu \)m membrane filter (Millipore, USA). COM was extracted by ultrasonication in an ice bath on an ultrasonic homogenizer (UP400S, Hielsher Ultrasonics, Germany), followed by filtration through a 0.45 \( \mu \)m membrane filter. The peptide/protein fraction was isolated from the COM by a two-step precipitation procedure utilizing ammonium sulphate, (NH4)2SO4, at 0°C. The detailed methodology for cultivation, COM extraction and peptide/protein isolation is described by Safarikova et al. (2013). The COM-peptides/proteins were further characterized in terms of molecular weight (MW) distribution by high performance size exclusion chromatography (HPSEC) using
Agilent Bio SEC-5 columns connected in series. The HPLC system (Agilent Technologies, USA) was coupled with a diode array detector (DAD) operated at 280 nm. The methodology of HPSEC is described in detail elsewhere (Pivokonsky et al., 2012). 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.

For the sake of simplification, the COM-peptides/proteins are referred to as “COM” hereinafter. Their concentrations in the experiments were expressed as dissolved organic carbon (DOC).

1.2. Methods

1.2.1. Coagulation tests

The coagulation of COM, kaolinite and a mixture of both by either aluminum sulphate (Al2(SO4)3.18H2O; Sigma–Aldrich, USA) or ferric sulphate (Fe2(SO4)3.9H2O; Sigma–Aldrich, USA) was performed in a Taylor–Couette reactor. The following suspensions were utilized in the coagulation experiments: (1) kaolinite (10 mg/L, 35 NTU), (2) COM (5 mg/L DOC), (3) a mixture of kaolinite and COM (10 mg/L kaolinite and 5 mg/L DOC). The mentioned concentrations were chosen with respect to concentrations usually present in raw water sources. Kaolinite and COM were dosed into the ultrapure water with alkalinity adjusted to 1.5 mmol/L (75 mg/L CaCO3) using NaHCO3. The target pH values were reached by adding predetermined amounts of 0.1 mol/L NaOH or 0.1 mol/L HCl prior to supplementation with the coagulant. The doses of coagulants and pH values were optimized by coagulation tests according to Pivokonsky et al. (2012) and Safarikova et al. (2013). Table 1 summarizes the optimized coagulant doses, optimized pH values, residual coagulants, and residual impurities (residual kaolinite is expressed as turbidity and residual COM is expressed as DOC) arrived at after the coagulation tests had been conducted. With respect to low coagulant doses, sweep flocculation (also called enmeshment) is not considered a coagulation mechanism (Gregory and Duan, 2001). The global shear rates applied during the coagulation experiments comprised $G = 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 150, 160,$ and $180 \text{ sec}^{-1}$. The retention period of the suspension in the Taylor–Couette reactor was 60 min., which corresponded to its subsequent steady state. All tests were repeated five times. Image analysis was performed after each coagulation test.

1.2.2. Taylor–Couette mixing device

The Taylor–Couette reactor (TC) (IH CAS, Czech Rep.) was selected as the mixing device for the coagulation experiments due to its well-defined velocity field and the possibility for non-invasive observation of the resultant flocs. The TC comprises two concentric cylinders made from Plexiglas (Umaplex, Perspex), with an inner rotating cylinder. White and black inner cylinders were employed to give a contrasting background for the ferric and aluminum flocs, respectively. The outer cylinder was transparent and permitted photographic imaging of the ferric and aluminum flocs, respectively.

The TC was of radius $R_2$ of 73.5 mm while the outer one ($R_1$) was of radius 85.5 mm, which resulted in a gap of $d = R_2 - R_1 = 12$ mm in width and radius ratio of $\eta = R_1/R_2 = 73/85.5 = 0.8596$. The height of the cylinders was $H = 385$ mm, giving the aspect ratio of $T = H/d = 385/12 = 32.08$. The inner cylinder was driven by a variable speed drive with a torque-meter. The hydrodynamic conditions in the TC were characterized by global shear rate, $G$, calculated according to the following equation:

$$G = \sqrt{\frac{P_i}{\mu \pi}} = \sqrt{\frac{\omega M}{\nu \mu}} = \sqrt{\frac{2\pi fM}{V \mu}} \quad (3)$$

where $P_i$ represents the power dissipated in the aggregation space, $V$ is the volume of the aggregation space, $\mu$ is the dynamic viscosity of the fluid, $\omega$ is the angular velocity of inner cylinder rotation, $M$ is the torque and $f$ is the rotation frequency.

1.2.3. Analyses of floc properties

The size, number, and size distribution of the flocs formed during coagulation in the TC reactor was determined by image analysis, which involved the following three steps: (1) illuminating flocs in the TC reactor with a laser light sheet (width = $1.2 \pm 0.1$ mm) generated by a laser diode ($\lambda = 675$ nm, power capacity 20 mW); (2) recording images of flocs on a Pentax K-3II digital camera (Asahi Co., Japan) with an smc PENTAX D FA Macro 50 mm f/2.8 lens (Asahi Co., Japan); (3) processing the images with image analysis software (Sigma Scan 5.0).

<table>
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<tr>
<th>Table 1 – Coagulation conditions (optimal coagulant doses, optimal pH and temperature) and residual water parameters after coagulation (turbidity, DOC, Fe/Al).</th>
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<tr>
<td><strong>Al (Al2(SO4)3.18H2O)</strong></td>
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<td><strong>Kaolinite</strong></td>
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<td>Optimal coagulant dose * (mmol/L Al/Fe)</td>
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<td>Coagulation pH *</td>
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<td>Fe (mg/L)</td>
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<td>Al (mg/L)</td>
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</table>

* The optimal coagulant doses and optimal pH were determined on the basis of the highest removal efficiencies of all floc components (kaolinite as turbidity, COM as DOC and Al/Fe).
The digital images (23.5 × 15.6 mm) were taken in RAW format (6016 × 4000 pixels), which gave the pixel size of approximately 3.9 × 3.9 μm. Only flocs larger than 4 pixels (7.8 × 7.8 μm) were retained in the image analysis process to eliminate digital background noise. The images were then converted from RAW into BMP gray-scale format, after which thresholding was applied (Appendix A Fig. S1). Finally, Sigma Scan 5.0 software was employed to calculate the following: the projected area, \( A \); the perimeter, \( P \); the minimal and maximal dimensions, \( l_{\text{min}} \) and \( l_{\text{max}} \), respectively, of all the imaged flocs; and the total number of flocs. The equivalent floc diameter, \( d \), of each floc was calculated from the projected area, as follows:

\[
d = \sqrt{4A/\pi}
\]

Furthermore, the average floc diameter, \( d_{\text{avr}} \), (referred to as “floc size” hereinafter) was calculated from the \( d \) values for all the flocs present in one image (Bouyer et al., 2004; Bubakova and Pivokonsky, 2012). The structural characteristics of the flocs were derived from the two-dimensional fractal dimension, \( D_f \), determined from the image analysis data. Said fractal dimension, \( D_p \), represents the compactness or density of the flocs and is given by the following formula (Bubakova et al., 2013; Chakraborti et al., 2007):

\[
A \propto d_{eq}^{D_f}
\]

where \( A \) is the projected area, \( d_{eq} \) is the equivalent floc diameter calculated from the measured values for minimal and maximal dimensions \( l_{\text{min}} \) and \( l_{\text{max}} \), respectively, according to the following equation:

\[
d_{eq} = \sqrt{l_{\text{min}}l_{\text{max}}}
\]

This calculation of \( d_{eq} \) guarantees its independence of \( A \) (Bagheri et al., 2015). \( D_f \) lies in the interval (1, 2). Dense flocs possess a higher value for \( D_f \) than porous and lacunar flocs. Surface properties are calculated from the perimeter, \( P \), and the projected area, \( A \), as the perimeter-based fractal dimension, \( D_{pf} \), see below (Bubakova et al., 2013; Chakraborti et al., 2007; Li et al., 2006):

\[
A \propto D_{pf}^2
\]

where \( D_{pf} \) lies in the interval (1, 2). Fractal objects increase their perimeter more rapidly than their size (projected area). Hence, if \( D_{pf} \) tends towards 2, the flocs are irregular and branched, whereas they are somewhat spherical in shape if \( D_{pf} \) tends towards 1.

1.2.4. Statistical processing and reproducibility of results

Statistical analysis was performed in Statistica 7.1 software (TIBCO Software, Inc.). Testing normality for the equivalent diameter, \( d \), involved conducting a one-sample Kolmogorov–Smirnov test with a significance level of 0.95 (\( \alpha = 0.05 \)). The null hypothesis, i.e. the size distribution of the flocs possesses normal distribution, was not rejected (Appendix A Table S1), thus the arithmetic mean was chosen to characterize the dataset.

Reproducibility of results was verified for all suspension variants at the G values of 40 and 160 sec \(^{-1} \). The dataset of \( d \) and the number of flocs were tested by analysis of variance (ANOVA). The one-way ANOVA was used to test the null hypothesis that the means of parameters \( d \) and number (relevant floc components) were equal for individual measurements at both of the tested shear rates. At the significance level of 0.95 (\( \alpha = 0.05 \)), the null hypothesis was not rejected for all suspensions. There was no statistically significant difference between the five experiments of each experimental variant (\( p \)-value > \( \alpha \)-level and \( F_{\text{crit}} \) > \( F \)-value) (Appendix A Table S2).

2. Results and discussion

2.1. Number of flocs, size, and size distribution

The dependence of floc size on shear rate, \( G \), is illustrated in Fig. 1a. In agreement with previous research, it was confirmed that the size of the flocs decreased concurrently with increase in shear rate (Bubakova et al., 2013; Bubakova and Pivokonsky, 2012; Dyer and Manning, 1999; Tambo and Hozumi, 1979). The extent of such decrease was greatly dependent on the substances participating in the coagulation, i.e. the primary components of the flocs, such as kaolinite, COM and the coagulant. The floc size increased in the following order: kaolinite+Al < kaolinite+Fe < COM+Al < kaolinite+COM+Fe < COM+Fe < kaolinite+COM+Fe.

Studies by Gonzalez-Torres et al. (2017, 2014), who coagulated cells of \( M. \) aeruginosa and Chlorella vulgaris together with the associated AOM released into cultivation media, also found that floc size diminished alongside a rise in \( G \) (evaluated in the range 9.4–167 sec \(^{-1} \)). Moreover, they showed that floc size was dependent on the composition of the AOM, specifically on the distribution of protein and carbohydrate throughout the flocs.

Two distinct trends are apparent in Fig. 1a. Firstly, the presence of COM in the flocs led to a substantial rise in floc size. It is not surprising that the peptide/protein fraction of COM, comprising large biopolymers with molecular weights of up to 1077 kDa (as determined by Safarikova et al. (2013)), created larger flocs than kaolinite particles. Similar observations were made by Henderson et al. (2006), who ascertained that cells + AOM produced substantially larger flocs than those formed either by natural organic matter (NOM, i.e. organic matter primarily humic in character) or kaolin. Likewise, Vandamme et al. (2014) who coagulated Chlorella vulgaris cells with and without AOM, reported that AOM caused a rise in floc size when alum and chitosan were applied as coagulants. These findings indicate that AOM polymers behave similarly to synthetic polymeric flocculants that increase floc size (Fabrizi et al., 2010).

Secondly, the results in Fig. 1a show that ferric sulphate (Fe) produced flocs almost twice as large as those created by aluminium sulphate (Al), regardless of the type of impurity. Similar results were achieved by Gonzalez-Torres et al. (2014) who investigated flocs formed by cells+AOM of \( M. \) aeruginosa and ferric chloride or aluminium sulphate. Furthermore, approximately twice the proportion of large flocs called macroaggregates (i.e. flocs larger than 1000 μm) was observed for the Fe coagulant than the Al coagulant by Pivokonsky et al. (2009) for coagulation of COM of \( M. \) aeruginosa at \( G = 70 \) sec \(^{-1} \).
for 15 min. Additionally, Jarvis et al. (2012, 2005b) reported that their Fe-coagulant produced significantly larger NOM-flocs than Al.

Several explanations exist for such difference in floc size for ferric and aluminum coagulants. Fe tends to produce larger hydrolysis products than Al, as the Fe-O distance in Fe hydrolysis products is longer (2.00 Å) than the Al-O distance in Al hydrolysis products (1.89 Å) (Persson, 2010). In addition, higher coagulant doses of Fe than Al were applied to coagulate kaolinite particles, as shown in Table 1, thus producing a larger amount of Fe precipitates than Al. However, Fe doses were lower than those for Al for coagulation of COM and kaolinite + COM. Nevertheless, in the presence of COM, the size of proteins, which is known to differ with a change in pH value due to protein folding and unfolding, plays a crucial role in the resultant size of flocs. Many proteins unfold at pH values of less than 6 or greater than 9 (Creighton, 1993), which is accompanied by increase in their size. This may be the reason why flocs formed at pH values for coagulation by Fe are larger than those formed at pH values for coagulation by Al (see Table 1).

The most substantial differences in floc size were observed at the lowest shear rates (up to $G = 50 \text{ sec}^{-1}$ for Al-flocs and up to $G = 70 \text{ sec}^{-1}$ for Fe-flocs), potentially related to forces of attraction, i.e. interactions acting between primary components (further discussed in Section 2.3.), prevailing over hydrodynamic ones at the mentioned shear rates. As the shear rate increased, the forces (attraction vs. hydrodynamic) gradually attained balance. Finally, though, the effect of hydrodynamics prevailed over the forces of attraction (Coufort et al., 2005); therefore, the variances in floc size between the individual floc types became less pronounced at the highest shear rates.

Fig. 1 shows data on size distribution for three selected shear rates: 40, 80, and 140 sec$^{-1}$. With increase in the value G, size distributions became narrower, indicating that the floc suspensions were more homogeneous through increase in collision frequency (Bouyer et al., 2004; Bubakova et al., 2013), and the fact that hydrodynamic forces did not allow the flocs to grow any larger. The size distributions of COM+Al and kaolinite+COM+Al and of COM+Fe and kaolinite+COM+Fe were more similar to each other than the size distributions of COM+Al and COM+Fe and of kaolinite+COM+Al and kaolinite+COM+Fe; this suggested that the type of coagulant utilized had a greater impact on the size distribution of flocs formed by COM than the presence of kaolinite in the suspensions. The effects of coagulant type on floc size are discussed above.

### 2.2. Fractal dimension of flocs

The dependence of fractal dimensions, $D_2$ and $D_{pf}$, used to describe floc structure and shape, on shear rates is shown in Fig. 3. As expected, $D_2$ rose and $D_{pf}$ dropped concurrently with rise in G. This indicated that the flocs became more compact, i.e. their density increased while porosity decreased (Fig. 3a), as well as more regular in shape, i.e. closer to a sphere/ellipsoid (Fig. 3b), with increase in G. Bubakova and Pivokonsky (2012) and Bubakova et al. (2013) reported similar results for natural water (turbidity 2.9 NTU for both and DOC 4.5 and 3.8 mg/L, respectively) coagulated by ferric sulphate at $G = 28–307$ and 21.2–347.9 sec$^{-1}$, respectively. Li et al. (2006) who coagulated kaolin by aluminium sulphate or cationic polyacrylamide at $G = 11.3–342 \text{ sec}^{-1}$, observed an analogous trend. They utilized a three-dimensional fractal dimension, $D_F$, (possessing values between 1 and 3) to describe the floc structure. Therein, $D_F$ increased in parallel with rise in G, meaning that the floc structure was more compact. Furthermore, Wang et al. (2017) concluded that flocs became less porous and more compact with increase in G, when they coagulated wastewater using composited polyferric chloride–polydimethylallylammonium chloride (PFC-PDM) and non-composited polyferric chloride (PFC) under various coagulation mechanisms at $G = 24.8–547.9 \text{ sec}^{-1}$.
Herein, the compactness of flocs (Fig. 3a) decreased in the following order: kaolinite+Al > COM+Al > kaolinite+Fe > COM+Fe > kaolinite+COM+Fe > kaolinite+COM+Al. The higher value for $D_2$ for kaolinite+Al and COM+Al than the kaolinite+Fe and COM+Fe flocs probably stemmed from the shorter Al-O distance (1.89 Å) than Fe-O distance (2.00 Å) (Fabrizi et al., 2010). The most porous flocs were those formed by all three primary components – kaolinite, COM, and the coagulant. The variations in structure (compactness) between individual floc types could also be attributed to the distinct levels of pH at which coagulation took place (Table 1), this determining the formation of different Al/Fe hydrolysis products (Duan and Gregory, 2003). Moreover, in the case of the COM-flocs, the given pH value influenced protein folding (and thus floc structure), as mentioned in Section 2.1. This would also explain the inconsistency in the order of floc types according to their increase in $D_2$ and decrease in size. While the structure is influenced by the form of the primary component, which is pH-dependent (Gregory, 1998), size is determined additionally by the concentration of said component (or volume of mass) (Pivokonsky et al., 2009). Thus, the largest flocs are not necessarily the most porous.

The situation became more complicated for $D_{pf}$ (Fig. 3b). At $G = 70–120$ sec$^{-1}$, the order of $D_{pf}$ for the various floc types was inversely proportional to $D_2$, meaning that the more compact the flocs, the more regular their shape. At $G < 70$ sec$^{-1}$, the curves for $D_{pf}$ crossed over each other. The shape of the flocs containing COM was obviously more affected by $G$ than the kaolinite–flocs. This phenomenon is discussed in the following Section 2.3. At $G > 120$ sec$^{-1}$, the differences between the fractal dimensions of flocs formed by different substances diminished, and the curves for kaolinite+Fe, COM+Fe, kaolinite+COM+Fe and kaolinite+COM+Al resembled each other. This was probably due to corrosion of the floc surfaces at high $G$, resulting in all of them obtaining a similar shape.

Although fractal dimensions have been used to describe floc structure in many studies (Bubakova et al., 2013; Chakraborti et al., 2007; Vlieghe et al., 2017; Wang et al., 2017, 2009), they varied in experimental conditions, i.e. type and concentration of coagulant and impurity, $G$ value, method for gauging fractal dimension, and so on. Therefore, it is not possible to compare the absolute values derived for fractal dimension, either with each other or with the results given herein.

### 2.3. Floc components and their interaction mechanisms

Papers in the literature on the coagulation of kaolinite particles or/and AOM show that the interaction mechanisms between impurities and coagulants are primarily governed by their predominant net charge and/or the presence and amount of charged functional groups (Henderson et al., 2010; Pivokonsky et al., 2012; Safarikova et al., 2013). It should be noted that the charge of kaolinite, AOM, and Al/Fe coagulants is highly dependent on pH value, and that consistent pH optimization is a prerequisite for efficient floc formation and separation. The predominant net charges of the primary components coagulated in this study are detailed in Table 2, as derived from the titration curves of the individual primary components (Appendix A Fig. S2).

Kaolinite was effectively removed by the Fe and Al coagulants in the pH range 6.4–8.0 and 7.0–8.5, respectively, owing to the adsorption of positively charged Fe/Al hydroxide precipitates on the negatively charged surfaces of kaolin particles, thereby gradually neutralizing the charge of the kaolinite and leading to efficient coagulation (Safarikova et al.,...
In contrast, the COM-peptides/proteins of M. aeruginosa were coagulated by the Fe and Al coagulants in the pH ranges 4.0–6.0 and 5.0–6.5, respectively; this indicated that electrostatic interactions between the negatively charged acidic functional groups (–COO−) of COM and positively charged Fe or Al hydroxopolymers were important to their coagulation (Pivokonsky et al., 2012, 2015). When kaolinite and COM were removed together by the Fe or Al coagulant, the process was also influenced by electrostatic interactions of attraction between the negatively charged kaolinite surface and peptide/protein –NH3⁺ groups. The kaolinite–COM clusters were then linked by positively charged Fe or Al hydroxopolymers at the same pH values (4.0–6.0 and 5.0–6.5, respectively) as when kaolinite was absent (Safarikova et al., 2013). It is of note that Van der Waals forces, hydrogen bonding and hydrophobic interactions also participated in the given coagulation processes (Shin et al., 2008). Polar functional groups, involved in hydrogen bonding, are contained within COM and kaolinite as well as Fe/Al hydrolysis products. Additionally, the nonpolar side chains of COM and hydrophobic silica surface planes of kaolinite may take part in hydrophobic interactions. The binding energies of the aforementioned interactions decrease in the following order: electrostatic interactions (binding energy ~20–30 kJ/mol) > hydrogen bonding (~15–17 kJ/mol) > hydrophobic interactions (~1.2–6 kJ/mol) (Israelachvili, 2011). Fig. 4 details the activity of these interactions, illustrating the dependence of floc size on the shear rate in log–log plots (in accordance with Eq. (1)). It can be inferred that the measured data did not follow a single dependence with a single γ constant. This phenomenon is also reported in data from the literature (Bouyer et al., 2004; Bubakova et al., 2013; Li et al., 2006; Wang et al., 2017; Wang et al., 2009) on floc properties. However, none of the referenced studies, except that by Bubakova et al. (2013), even observed, pursued or commented on this aspect. The results of all said authors, after applying Eq. (1), are depicted in Fig. 5. In the present study, one change in the γ constant, at approximately G~120 sec⁻¹, was noted for all the types of floc (Fig. 4). This region can also be seen in works by others, as depicted in Fig. 5a–f. Furthermore, the flocs containing COM showed an additional change in γ, between values for G of 40–50 and 60–70 sec⁻¹, with reference to Al and Fe, respectively (Fig. 4c–f).

The changes in floc size can be explained by the activity of intermolecular interactions. As stated previously, electrostatic interactions, which are the strongest and most dominant ones in coagulation, are supported by other interactions with lower binding energies, such as Van der Waals forces, hydrogen bonds, and hydrophobic interactions. As the shear

### Table 2 – Net charges of primary components prevailing at the pH value used for coagulation.

<table>
<thead>
<tr>
<th>Coagulated primary components</th>
<th>Kaolinite+COM+Fe</th>
<th>COM+Fe</th>
<th>Kaolinite+COM+Al</th>
<th>COM+Al</th>
<th>Kaolinite+Fe</th>
<th>Kaolinite+Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation pH</td>
<td>4.8</td>
<td>5.8</td>
<td>6</td>
<td>6.8</td>
<td>7.2</td>
<td>7.5</td>
</tr>
<tr>
<td>Kaolinite</td>
<td>⊜</td>
<td>☓</td>
<td>⊜</td>
<td>N/A</td>
<td>☜</td>
<td>☜</td>
</tr>
<tr>
<td>COM –COOH</td>
<td>☜</td>
<td>N/A</td>
<td>☜</td>
<td>N/A</td>
<td>☜</td>
<td>☜</td>
</tr>
<tr>
<td>COM –NH₂</td>
<td>N/A</td>
<td>☜</td>
<td>☜</td>
<td>N/A</td>
<td>☜</td>
<td>☜</td>
</tr>
<tr>
<td>Al</td>
<td>☜</td>
<td>☜</td>
<td>N/A</td>
<td>☜</td>
<td>☜</td>
<td>N/A</td>
</tr>
<tr>
<td>Fe</td>
<td>☜</td>
<td>☜</td>
<td>N/A</td>
<td>☜</td>
<td>☜</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A = Not Available.

The contents of Table 2 were derived from the titration curves of the individual primary components (see Appendix A Fig. S2).
rate increases, any weaker interactions are not strong enough to participate in floc formation, and floc size immediately drops away. The changes in floc size (also in $D_{pf}$ value, as mentioned in the previous section) observed for the COM-flocs (at $G$ ~40–50 and 60–70 sec$^{-1}$ for Al and Fe, respectively) suggest that the presence of COM in the flocs leads to extra interactions, causing the formation of larger, irregularly shaped flocs at lower shear rates. Similar trends were reported by Li et al. (2006) and Williams et al. (1992) (Fig. 5g, h), who coagulated inorganic particles with polyacrylamide, which resemble peptides/proteins in certain properties (a ionic polymer with a nonpolar chain and $-$NH$_3^+$ and $-COO^-$ functional groups). Herein, the difference between $G$ ranges for the change in floc size for the Al and Fe coagulants may stem from the various pH values applied for coagulation by Al and Fe. As stated in Section 2.1, proteins...
When a metal salt (Al/Fe) was used as a coagulant, only a single change in $\gamma_1$ was seen (a–f). When polyacrylamide (PAM) was applied, two alterations in $\gamma$ value occurred (g). The same trend was observed for COM-peptides/proteins, which have a similar structure to PAM (nonpolar parts and $-\text{COO}^-$, $-\text{NH}_3^+$ functional groups).
tend to unfold at the pH set for coagulation by Fe (Table 1). During the process of protein unfolding, the functional groups that are buried in non-ionized forms inside the folded proteins are able to ionize (Creighton, 1993), thus enabling more electrostatic interactions to participate in floc formation. These additional electrostatic interactions could result in the greater resistance of Fe-flocs to hydrodynamics (higher G values), compared to Al-flocs.

### 2.4. Implications of floc properties for separation

For efficient coagulation, it is necessary to consider conducting a thorough analysis of source water composition and selecting a suitable coagulant with the appropriate coagulation conditions, such as pH, shear rate and mixing time. Consistent optimization of these parameters will lead to flocs being produced with properties suitable for later separation processes, with respect to maximum efficiency and low energy costs. Table 3 summarizes the recommended separation methods for the flocs formed in this study, in accordance with Edzwald (2010) and Bubakova and Pivokonsky (2012).

Bubakova and Pivokonsky (2012) reported that flocs below 100 μm in size (optimally around 60 μm) are suitable for single-stage separation by direct filtration. Larger flocs do not penetrate deeply into the filter bed and shorten filtration runs, thus sedimentation has to be applied as the first stage of separation, followed by filtration. Edzwald (2010) recommended sedimentation for flocs at the size of hundreds of μm. Moreover, the same author (Edzwald, 2010) reported that flotation was suitable for separating out flocs at sizes between 25 and 50 μm. However, this range in size pertains to turbidity-based flocs produced through coagulation by metal salts. Algal and AOM-flocs were reported to float well, and removal efficiencies of algae/AOM via flotation were higher than or comparable to sedimentation (Henderson et al., 2010). Nevertheless, Henderson et al. (2010) did not report on the sizes of algal/AOM-flocs suitable for flotation, thus the recommendation given for flocs containing COM is based solely on Edzwald (2010).

In summary, COM+Fe- and kaolinite+COM+Fe-flocs do not comply with the size suitable for direct filtration or flotation-filtration (Fig. 1a) but can be removed by sedimentation-filtration. The other types of flocs in this study (kaolinite+Fe-flocs and Al-flocs) can be separated in dependence on their size by all the separation processes listed, as given by the respective shear rate (Table 3).

### 3. Conclusions

The following conclusions can be drawn from this research. Increase in shear rate results in a decrease in floc size depending on the substances forming the flocs. Growth in floc size (alongside a drop in the number of flocs) happens in the following order: kaolinite+Al < kaolinite+Fe < COM+Al < kaolinite+COM+Al < COM+Fe < kaolinite+COM+Fe; this indicates that COM boosts floc size, and the ferric coagulant produces larger flocs than the aluminum equivalent. However, floc size changes in dependence on shear rate are not smooth in progress. Observation was of a rapid change for the kaolinite-coagulant suspension and two rapid changes for the COM-coagulant and kaolinite–COM-coagulant suspensions. These can be attributed to the engagement of different interactions between COM, kaolinite, and the coagulants at various shear rates. Further, it was found that floc suspensions are more homogeneous, and variances between the sizes of the flocs formed by different substances lessen in line with increase in shear rate. In the case of the COM-flocs, the given type of coagulant exerts more of an impact on their size distribution than the presence of the kaolinite within them. Moreover, flocs become more compact and regular in shape, and the differences in these properties of flocs formed by different substances diminish alongside rise in shear rate. Floc structure also depends on the pH that determines the form of the primary components. Since floc properties determine the suitability of flocs for subsequent separation, based on the applied shear rate and floc components, separation processes for efficient flocs removal were suggested. Direct filtration is convenient for all types of flocs (except those containing COM and Fe) produced at higher shear rates. For COM+Fe- and kaolinite+COM+Fe-flocs, and other flocs produced at low shear rates, sedimentation is required.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.11.025.

REFERENCES