

THE EFFECT OF CELLULAR ORGANIC MATTER PRODUCED BY CYANOBACTERIA MICROCYSTIS AERUGINOSA ON WATER PURIFICATION

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The aim of this paper is to investigate the influence of COM (Cellular Organic Matter) produced by *Microcystis aeruginosa* on the process of water purification by destabilisation and subsequent aggregation of the impurity particles. The research was carried out with a raw water into which COM was added. The removal efficiency of the most significant components of COM, i.e. polysaccharides and proteins, was investigated. It was found that the removal efficiency of polysaccharides and proteins was dependent on the reaction conditions (pH, type of destabilisation reagent and its dosage). The removal efficiency of COM was relatively low. It was about 46% and 41% using ferric sulphate and aluminium sulphate aggregation, respectively. In comparison to the other organic components of COM, mainly polysaccharides, the proteins are removed with a higher efficiency. The GPC analyses of the residual COM showed that the proteins of higher molecular weight were aggregated with a higher efficiency.

KEY WORDS: AOM (Algal Organic Matter), COM (Cellular Organic Matter), Destabilisation, Aggregation, Reaction Conditions, Water Purification.

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Cílem práce je opis vlivu buněčných organických látek (COM) produkovaných sinicí *Microcystis aeruginosa* na proces úpravy vody pomocí destabilizace a následné agregace znečišťujících příměsí. Výzkum byl uskutečňován se syntetickou surovou vodou, do které byly přidány COM získané laboratorní kultivací *M. aeruginosa*. V průběhu laboratorních testů byla sledována především účinnost odstranění dvou základních složek COM, tj. polysacharidů a proteinů. Bylo zjištěno, že tato účinnost závisí především na reakčních podmínkách (typu a dávce destabilizačního činidla a pH). Účinnost odstranění COM byla poměrně nízká, maximální dosažená účinnost byla 46% při destabilizaci pomocí síranu železitého a 41% při použití síranu hlinitého. Bylo zjištěno, že s vyšší účinností jsou odstraňovány proteiny, obzvláště pak proteiny s vyšší molekulovou hmotností.

KLÍČOVÁ SLOVA: organické látky produkované fytoplanktonem, buněčné organické látky, destabilizace, agregace, reakční podmínky, úprava vody.

1. Introduction

Effective removal of natural organic matter (NOM) and especially that produced by a vast development of phytoplankton (Algal Organic Matter – AOM) is one of the very significant challenges of the purification of the surface waters (Bernhardt et al., 1985, 1986, 1989; Hoyer et al., 1987; Takaara et al., 2005, 2007; Pivokonsky et al., 2006). Generally, these organic matters can be distinguished into the substances that are released by metabolic activity of micro-organisms – the extracellular organic

matter (EOM) and the substances released during the process of their decay – the intracellular organic matter (COM) (Pivokonsky et al., 2006; Lüsse et al., 1985; Hoyer et al., 1985). The COM represents a specific problem due to the occasional sudden increase in its concentration.

A great attention was given to the influence of AOM on the purification of water polluted with different impurities such as aluminosilicates, humic matter, etc. Various studies showed that the influence of EOM on the process of destabilisation and aggregation of the impurity particles could be com-

pared to that of non-ionic polymers (Bernhardt et al., 1985, 1986, 1989; Hoyer et al., 1987). At their low concentrations the efficiency of water purification increases due to the formation of inter-particle bridges between the impurity particles or adsorption to their surfaces. In contrast, at higher concentrations the AOM inhibits the process of destabilisation of the impurity particles by increasing negative charge on their surface. Furthermore, certain substances contained in AOM, primarily proteins, also inhibit the destabilisation process. It is assumed that this results from the formation of higher-valence cation complexes and hydrated ions aided by coordination bonds. The formation of these complexes results in increased consumption of destabilisation reagent and decreasing efficiency of the destabilisation process (Bernhardt et al., 1989). The extent of the inhibition effect of the AOM is dependent upon its composition which is influenced by the kind of organisms and their growth phase (Pivokonsky et al., 2006). The COM are the substances most difficult to remove from the water. The reason is that these organic compounds are released suddenly in large quantities during decay of phytoplankton. Another very important reason is different chemical composition of COM in comparison to the other NOM and EOM (Takara et al., 2005, 2007; Pivokonsky et al., 2006).

The influence of AOM on the destabilisation and aggregation efficiency is not yet adequately explained. The contribution of authors to this theme is presented in this paper. The results obtained by investigating the effect of COM pollution produced by *M. aeruginosa* on its removal efficiency by coagulation with ferric sulphate and aluminium sulphate purification are summarised in this paper.

2. Material and methods

2.1 Cyanobacteria cultivation procedure

The cyanobacteria *Microcystis aeruginosa* KUTZ. (ZAPOMELOVA 2006/2) was used in this study. Inoculums of this strain were kindly supplied by the Culture Collection of Algal Laboratory, Institute of Botany, AS CR, v. v. i., Czech Republic. The laboratory strain *M. aeruginosa* was grown in a 20 l volume of Z medium (pH = 8.5) (Strub and Schweiz, 1961) at 24 °C and regularly shaken by a shaking apparatus operating at 40 rpm. The 16h-light/8h-dark cycle was applied. The cultures were illuminated using four 40W cool-white fluorescent

lamps supplying about 6000 lux. All materials and media were sterilized by autoclaving before assembly and operation. The growth of *M. aeruginosa* was monitored by chlorophyll-a in the culture. Cyanobacterial cells were harvested on 16th day of the cultivation period in the steady-state growth (dominant number of cells was free).

2.2 COM preparation procedure

The COM samples were prepared by destruction of the microorganism's cells which were separated from the growth media (2 l samples) on a 0.22 µm membrane filter (Millipore, USA) on the 16th day of the cultivation time (the steady-state growth). The separated cells were mixed with ultrapure water (200 ml). The destruction of cells was performed using an ultrasonic homogenizer (HD 3200, 20 kHz, 60W), which was dipped into the beaker containing the separated cells of microorganisms. The efficiency of cells destruction was checked in an optical microscope (Optech B4T, Olympus, Japan). The residual solids (cells) were removed on a 0.22 µm membrane filter, and the filtrates concentrated ten-times in a rotary evaporator (Laborota 4000 HB/G1, Heidolph, Germany) at 30 °C. The concentrated COM was stored at -18 °C.

2.3 COM characterisation procedure

Determination of protein portion from the COM

The COM consists primarily of carbohydrates and proteins. Therefore, portions of proteins (DOC_P) and non-protein (carbohydrates) substances (DOC_{NP}) were measured during the experiments. Proteins were isolated from the COM using H₂WO₄ as a protein precipitant (Dawson et al., 1986). The protein precipitate was then separated from the dissolved organic matter by filtration through a 0.22 µm membrane filters (MF, Millipore, USA) and DOC_{NP} was analysed in the filtrate. The protein portion DOC_P is calculated as follows:

$$\text{DOC}_P = \text{DOC}_T - \text{DOC}_{NP}, \quad (1)$$

where DOC_T is the total DOC of the COM.

The protein precipitations were carried out in triplicate and errors of DOC_P were less than 5%. The methodology of determination of protein portion from the COM can be found in Dawson et al. (1986) and Pivokonsky et al. (2006).

DOC analysis

A Shimadzu TOC-V_{CPH} analyzer was used for organic carbon analysis. Dissolved organic carbon (DOC) was calculated as the difference between the total carbon and inorganic carbon measurements for samples filtered through 0.22 µm membrane filter (MF, Millipore). All measurements were conducted in triplicate and errors were less than 2%.

Molecular weight fractionation

The aqueous COM samples were dialyzed against 0.05M phosphate buffer (pH 7.0) using dialysis membrane (Amersham Bioscience Corp., MW cut off: 10 kDa). The dialyzed samples were applied to the gel permeation chromatography (GPC) for the apparent molecular weight fractionation. The DOC_T concentration of all the COM samples was 100 mg l⁻¹ to eliminate potential concentration effects on GPC. The MW fractionation was performed by HPLC system (Agilent 1100 series, Agilent Technologies) with diode array detector (DAD). The Zorbax GF-250 (9.4 mm x 250 mm, 4 µm) and GF-450 columns (9.4 mm x 250 mm, 6 µm) were used for the GPC. The separation range applied was 4,000–900,000 for globular proteins using GF-250 and GF-450 columns in series. The buffer used for MW fractionation was 0.05M phosphate buffer (pH 7.0). The flow rate of mobile phase was 2.00 ml min⁻¹ at the temperature of 23°C. The sample volume was 20 µl. The maximum absorption wavelength (λ_{\max} = 280 nm) was used for measurement of MW of the COM samples. The wavelength of 280 nm was used especially for the protein detection. Data analysis was performed using Agilent Technologies Chemstation software. The system was calibrated using the following gel filtration standards (Sigma-Aldrich Co.): cyanocobalamin (1.35 kDa), ribonuclease-a (13.7 kDa), myoglobin (17 kDa), carbonic anhydrase (29 kDa), albumin (66 kDa), alcohol dehydrogenase (150 kD), β -amylase (200 kDa), apoferritin (443 kDa), thyroglobulin (669 kDa) and immunoglobulin (900 kDa). A calibration curve was based on a linear relationship between the retention time and the actual log MW. In all cases, a good linear correlation between the retention time and log MW was observed for the calibration curves (R^2 = 0.996). BioRad gel filtration standards of bovine gamma globulin (158 kDa) and chicken ovalbumin (44 kDa) were used as control samples. Standard error

was ± 1.05 kDa for gamma globulin and ± 0.62 for chicken ovalbumin. Reproducibility of the MW fractionation of COM samples was very good, with MW deviations of less than 3% from repeated measurements.

2.4 Coagulation procedure

Water

The ultrapure water with added NaHCO₃ was used in these experiments. The COM of a concentration DOC_T = 7.0 mg l⁻¹ was prepared. Other relevant water quality parameters were: pH = 8.3, KNK_{4.5} = 1.99 mmol l⁻¹, Fe = 0.007 mg l⁻¹, Al = 0.004 mg l⁻¹.

Jar tests

The COM removal during water purification was investigated by jar tests. The LMK 8 (Institute of Hydrodynamics, AS CR, v. v. i., Czech Republic) variable speed eight station flocculator with a paddle type stirrer housing standard 2 l beakers fitted with a variable speed drive and equipped with an infinite speed controller and revolution counter was used for jar tests. The tested volume of water in the beakers was 1.5 l. Ferric sulphate and aluminium sulphate were applied as destabilising reagents (coagulants). The procedure consisted of a 1 minute of homogenization agitation (\bar{G} = 200 s⁻¹), 15 minutes of aggregation agitation (\bar{G} = 70 s⁻¹) and 60 minutes of settling.

The effect of coagulation was ascertained by:

- (i) direct comparison of water quality indicators: content of cation of destabilising reagent (concentration of Fe / c_{Fe} / and Al / c_{Al} /), dissolved organic carbon DOC, pH value and alkalinity (ANC_{4.5}). Methodology of chemical analyses is in details described in Polasek and Mutl, 1995.
- (ii) determination of the degree of aggregation α , calculated according to the relationship

$$\alpha_A = \frac{C_0 - C_F}{C_0}, \quad (2)$$

where C_0 is the concentration of indicator monitored (Al, Fe, DOC) at the point of testing and C_F is the concentration of the same indicator determined in the centrate of the sample C_0 after its treatment by centrifugation under defined conditions (3000 rpm, T = 20 min) (Hereit et al., 1980; Polasek and Mutl, 1995).

(iii) determination of the test of aggregation, which enables the aggregates formed to be ascribed to one of the four basic size-categories, namely: nonaggregated particles (*NA*), primary aggregates (*PR*), micro-aggregates (*MI*) and macro-aggregates (*MA*). The technologically significant categories of particles are determined according to the following relationships:

$$MA = \frac{C_0 - C_5}{C_0}, MI = \frac{C_5 - C_{60}}{C_0}, PR = \frac{C_{60} - C_{60F}}{C_0},$$

$$NA = \frac{C_{60F}}{C_0}, \quad (3-6)$$

and

$$MA + MI + PR + NA = 1, \quad (7)$$

where C_0 , C_5 and C_{60} – the concentrations of the monitored determinant measured in the samples taken at the beginning of sedimentation, after 5, and 60 minutes of sedimentation and C_{60F} – a concentration of the monitored determinant measured in concentrate of the C_{60} sample after its treatment by centrifugation under defined conditions (3000 rpm, $T = 20$ min) (Hereit et al., 1980; Polasek and Mutl, 1995).

3. Results and discussion

The results of COM characterisation show that the COM of *M. aeruginosa* is composed of protein and non-protein organic matters. The protein portion determined as DOC_P was measured to be about 59.9% of DOC_T in the COM and the non-protein organic is the balance of 40.1%. The proteins characterized by 21, 85, 234, 359, 470 kDa and more than 900 kDa were identified in the *M. aeruginosa* COM (Fig. 1). The protein concentration in the raw water was $DOC_P = 4.12 \text{ mg l}^{-1}$ and non-protein concentration was $DOC_{NP} = 2.86 \text{ mg l}^{-1}$. The character of the COM produced by cyanobacteria *M. aeruginosa* and other cyanobacteria and green algae are discussed in sufficient details in literature (Pivokonsky et al., 2006).

The dependence of removal of the COM produced by *M. aeruginosa* on dosage of destabilisation reagent is shown in Figs. 2 and 3. It is evident that residual concentration of total organic matter (DOC_T) drops up to a dosage of $0.160 \text{ mmol l}^{-1}$ (90 mg l^{-1}) with ferric sulphate and $0.105 \text{ mmol l}^{-1}$ (70 mg l^{-1}) with aluminium sulphate aggregation. Thereafter residual concentrations of DOC_T remain virtually unchanged. The aggregation efficiency of

organic matter is relatively low and reaches its highest value of about $\alpha DOC_T = 0.45$ with ferric sulphate and $\alpha DOC_T = 0.41$ with aluminium sulphate (Figs. 4 and 5). Maximum aggregation efficiency is attained in the range of high dosage of destabilisation reagent when a considerable Fe and Al concentrations remain non-aggregated (Figs. 2 and 3).

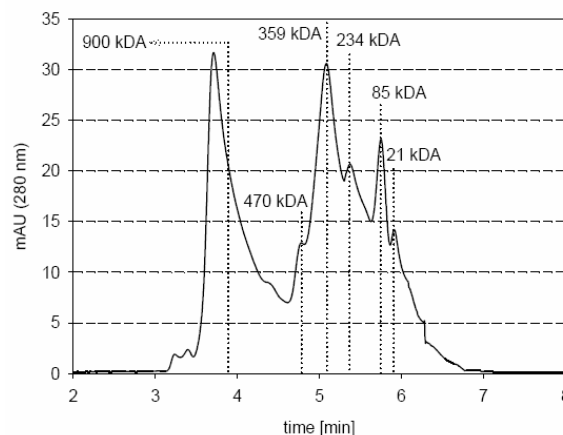


Fig. 1. GPC profile for the COM in raw water.
Obr. 1. GPC profil COM v surové vodě.

In contrast to the curves of residual concentration of organic matter (DOC_T), the concentration curves of residual Fe and Al have a quite distinct optimum ($c_{Fe} = 0.12 \text{ mg l}^{-1}$, $c_{Al} = 0.16 \text{ mg l}^{-1}$) as shown in Figs. 2 and 3. The residual Fe reaches its maximum $c_{Fe} = 1.23 \text{ mg l}^{-1}$ at a dosage around $0.018 \text{ mmol l}^{-1}$ (10 mg l^{-1}) $Fe_2(SO_4)_3 \cdot 9H_2O$ and $pH = 7.56$. With a further increase in dosage, the residual Fe gradually decreases up to its minimum $c_{Fe} = 0.12 \text{ mg l}^{-1}$ at a dosage of $0.125 \text{ mmol l}^{-1}$ (70 mg l^{-1}) and $pH = 6.33$. A further increase in ferric sulphate dosage results in another sharp increase in residual Fe up to $c_{Fe} = 1.91 \text{ mg l}^{-1}$ which is reached at a dosage of $0.178 \text{ mmol l}^{-1}$ (100 mg l^{-1}) and $pH = 5.43$ and thereafter increases gradually until alkalinity of the water was fully utilised (Fig. 2) at a dosage of about $0.231 \text{ mmol l}^{-1}$ (130 mg l^{-1}).

The residual Al reaches its maximum $c_{Al} = 1.29 \text{ mg l}^{-1}$ at a dosage around $0.030 \text{ mmol l}^{-1}$ (20 mg l^{-1}) $Al_2(SO_4)_3 \cdot 18H_2O$ and $pH = 7.58$ (Fig. 3). After reaching this maximum, residual Al gradually decreases to its lowest value $c_{Al} = 0.16 \text{ mg l}^{-1}$ which is attained at a dosage of $0.075 \text{ mmol l}^{-1}$ (50 mg l^{-1}) and $pH = 6.67$. With a further increase in dosage, the residual Al gradually increases until alkalinity of the water ($ANC_{4.5}$) is fully utilised (Fig. 3) at a dosage of about $0.210 \text{ mmol l}^{-1}$ (140 mg l^{-1}).

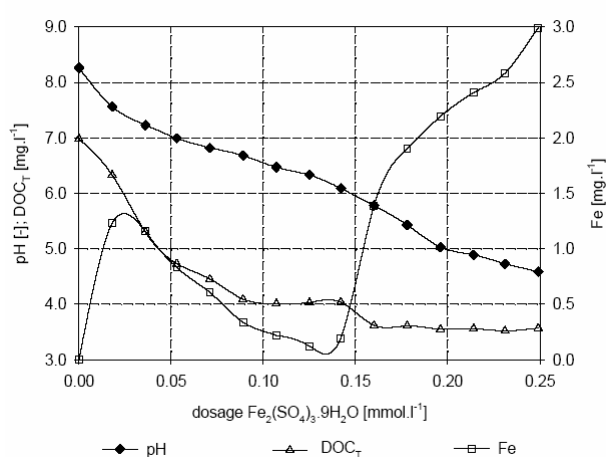


Fig. 2. Dependence of residual Fe and DOC_T on ferric sulphate dosage.

Obr. 2. Závislost zbytkového železa a DOC_T na dávce síranu železitého.

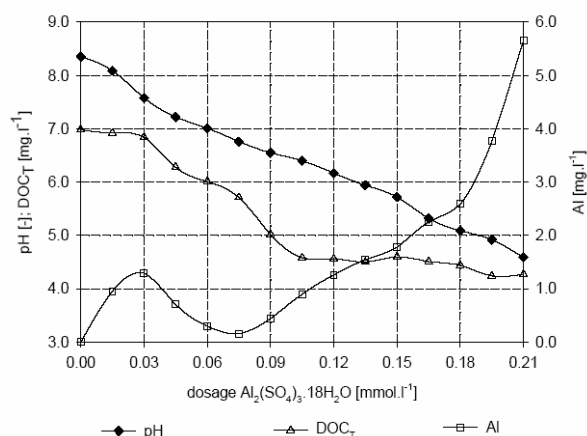


Fig. 3. Dependence of residual Al and DOC_T on aluminium sulphate dosage.

Obr. 3. Závislost zbytkového hliníku a DOC_T na dávce síranu železitého.

The efficiency of Fe-aggregation is about $\alpha_{\text{Fe}} = 0.90$ in a dosage range between 0.071 and $0.178 \text{ mmol l}^{-1}$ (40 and 100 mg l^{-1}). The maximum aggregation efficiency $\alpha_{\text{Fe}} = 0.92$ is attained at a dosage of $0.160 \text{ mmol l}^{-1}$ (90 mg l^{-1}) (Fig. 4). Similarly, the efficiency of Al-aggregation is about $\alpha_{\text{Al}} = 0.90$ also in a dosage range between 0.060 and $0.150 \text{ mmol l}^{-1}$ (40 and 100 mg l^{-1}). However, the maximum $\alpha_{\text{Al}} = 0.92$ is attained at a dosage of $0.060 \text{ mmol l}^{-1}$ (40 mg l^{-1}) (Fig. 5). Comparison of the residual Fe and Al ions shows that the lowest Fe concentration is attained at a dosage of $0.125 \text{ mmol l}^{-1}$ (70 mg l^{-1}) whereas the lowest Al concentration is attained at a dosage of $0.075 \text{ mmol l}^{-1}$ (50 mg l^{-1}). In contrast to that the lowest residual $\text{DOC}_T = 3.6$

mg l^{-1} is attained at a dosage of $0.178 \text{ mmol l}^{-1}$ (100 mg l^{-1}) of ferric sulphate whilst in the case of aluminium sulphate the lowest $\text{DOC}_T = 4.2 \text{ mg l}^{-1}$ is attained at a dosage of $0.195 \text{ mmol l}^{-1}$ (130 mg l^{-1}).

It is evident from the results obtained that the efficiency of COM aggregation is dependent on pH values. It was found that the dissolved organic substances are the most efficiently separated in a pH ranging between 5.0 and 6.5 and sometimes even at $\text{pH} < 5.0$ (Edzwald et al., 1982; Edwards and Amiratharajah, 1985; Polasek and Mutl, 1995, 2005; Gregor et al., 1997; Pivokonska and Pivokonsky, 2007). The pH value influences also the prevailing type of Fe-hydroxopolymer which is mainly characterized by the magnitude of surface charge. The charge magnitude determines efficiency of a hydroxopolymer as destabilisation reagent (Polasek and Mutl, 1995, 2005).

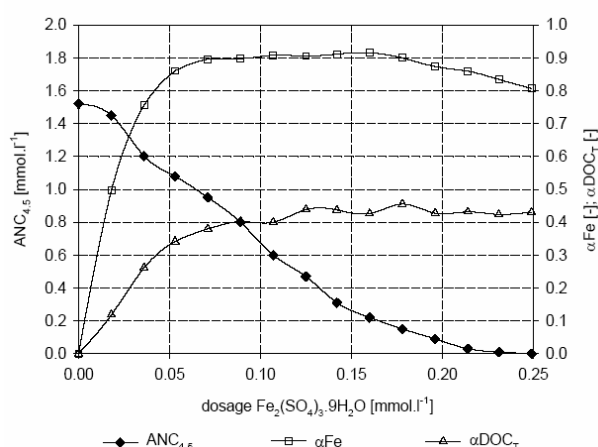


Fig. 4. Dependence of α_{Fe} and α_{DOC_T} on ferric sulphate dosage.

Obr. 4. Závislost α_{Fe} a α_{DOC_T} na dávce síranu železitého.

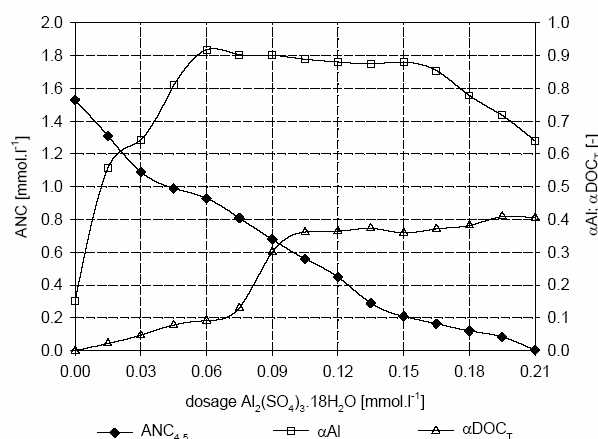


Fig. 5. Dependence of α_{Al} and α_{DOC_T} on aluminium sulphate dosage.

Obr. 5. Závislost α_{Al} a α_{DOC_T} na dávce síranu hlinitého.

Figs. 6 and 7 show comparison of the changes in residual protein (DOC_P) and non-protein (DOC_{NP}) organic matters with ferric and aluminium sulphate dosages. In the case of ferric sulphate (Fig. 6) DOC_P gradually decreases with dosage to around 1.5 mg l^{-1} at a dosage of $0.089 \text{ mmol l}^{-1}$ (50 mg l^{-1}) and with a further increase in dosage it decreases very slowly to a concentration of 1.07 mg l^{-1} which is attained at a dosage around $0.160 \text{ mmol l}^{-1}$ (90 mg l^{-1}) and thereafter it remains unchanged. In contrast, DOC_{NP} is reduced by 0.4 mg l^{-1} at a dosage of $0.018 \text{ mmol l}^{-1}$ (10 mg l^{-1})

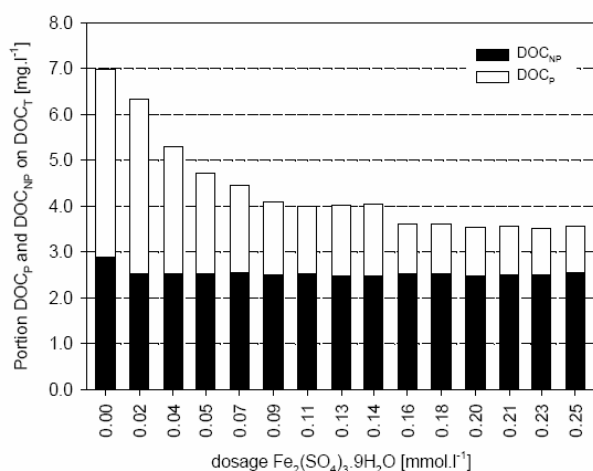


Fig. 6. Dependence of DOC_T , DOC_{NP} and DOC_P on ferric sulphate dosage.

Obr. 6. Závislost DOC_T , DOC_{NP} a DOC_P na dávce síranu železitého.

In the case of aluminium sulphate (Fig. 7) DOC_P gradually decreases up to a dosage of $0.075 \text{ mmol l}^{-1}$ (50 mg l^{-1}) and then it drops to a value of 2.06 mg l^{-1} at a dosage of $0.105 \text{ mmol l}^{-1}$ (70 mg l^{-1}), ($\text{pH} = 6.76$ to 6.17 and $\text{ANC}_{4.5} = 0.81$ to 0.45 mmol l^{-1}). With further increase in dosage DOC_P remains unchanged. This sudden drop in the residual DOC_P concentrations indicates the optimum reaction conditions at which this drop is most likely the result of adsorption of these compounds onto the hydrolysis products of destabilisation reagents (Polasek and Mutl, 1995). Similarly, in the case of ferric sulphate, Fig. 6 shows such a sudden improvement in the residual DOC_P concentrations also between dosages 0.120 and $0.135 \text{ mmol l}^{-1}$ (80 and 90 mg l^{-1}), ($\text{pH} = 6.00 - 5.80$ and $\text{ANC}_{4.5} = 0.32 - 0.22 \text{ mmol l}^{-1}$).

The changes in non-protein organic matter (polysaccharides) concentration with dosage of the destabilisation reagents are also interesting. Its concentration drops to $\text{DOC}_{NP} = 2.54 \text{ mg l}^{-1}$ at a dosage

of $0.018 \text{ mmol l}^{-1}$ (10 mg l^{-1}) of ferric sulphate and with increasing dosage it remains unchanged. In contrast, in the case of aluminium sulphate the residual DOC_{NP} decreases very slowly with dosage to its lowest residual concentration $\text{DOC}_{NP} = 2.24 \text{ mg l}^{-1}$ at a dosage of $0.231 \text{ mmol l}^{-1}$ (130 mg l^{-1}). It is evident from comparison of the removal of DOC_P and DOC_{NP} that the reduction in COM (DOC_T) is mainly the result of the removal of protein organic matter (DOC_P). Low aggregation efficiency of the non-protein organic substances is likely the result of both the electric-neutrality of polysaccharide molecules and the fact that they contain a large quantity of OH^- ions owing to which a compact hydration layer is formed around them (Rinaudo, 2001). The low aggregation efficiency of neutral hydrophilic organic matter (polysaccharides) was confirmed also by other researchers (Croue et al., 1993; Kim and Yu, 2004; Pivokonska and Pivokonsky, 2007).

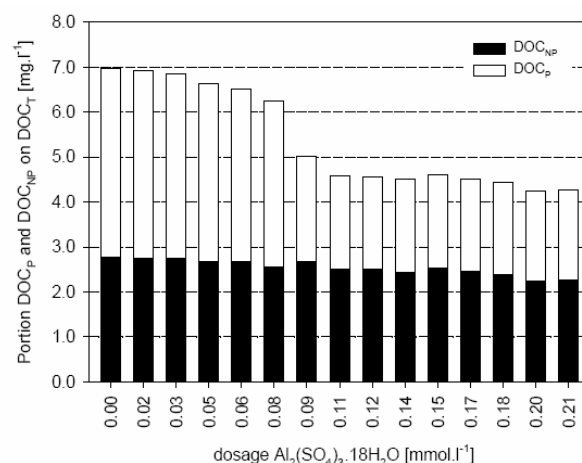


Fig. 7. Dependence of DOC_T , DOC_{NP} and DOC_P on aluminium sulphate dosage.

Obr. 7. Závislost DOC_T , DOC_{NP} a DOC_P na dávce síranu hlinitého.

The residual COM concentrations attained with both destabilisation reagents were subjected to fractionation of protein MW. The concentration of proteins identified at a dosage at which the maximum aggregation efficiency is attained, i.e. $D = 0.178 \text{ mmol l}^{-1}$ (100 mg l^{-1}) $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ ($\text{Fe} = 1.90 \text{ mg l}^{-1}$, $\text{DOC}_T = 3.61 \text{ mg l}^{-1}$, $\text{DOC}_P = 1.07 \text{ mg l}^{-1}$) and $D = 0.195 \text{ mmol l}^{-1}$ (130 mg l^{-1}) $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ ($\text{Al} = 3.77 \text{ mg l}^{-1}$, $\text{DOC}_T = 4.22 \text{ mg l}^{-1}$, $\text{DOC}_P = 2.06 \text{ mg l}^{-1}$) are compared in Tab. 1.

Table 1. Efficiency of protein aggregation.
 Tabulka 1. Účinnost agregace proteinů.

Protein	Raw water	Purified water $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ ($D = 100 \text{ mg l}^{-1}$)		Purified water $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ ($D = 130 \text{ mg l}^{-1}$)	
MW [kDa]	DOC_{PI} [mg l^{-1}]	DOC_{PI} [mg l^{-1}]	$\alpha\text{DOC}_{\text{PI}}$ [-]	DOC_{PI} [mg l^{-1}]	$\alpha\text{DOC}_{\text{PI}}$ [-]
21	0.41	0.27	0.34	0.18	0.56
85	0.70	0	1	0.57	0.19
234	0.66	0.21	0.68	0.53	0.20
359	0.95	0.59	0.38	0.78	0.18
470	0.41	0	1	0	1
> 900	0.99	0	1	0	1
Suma	4.12	1.07	0.74	2.06	0.50

The area of the peaks of each identified protein was recalculated from the DOC_P concentration to a DOC_{PI} (DOC concentration of protein identified using molecular weight fractionation) for the determination value of the aggregation efficiency $\alpha\text{DOC}_{\text{PI}}$ (aggregation efficiency of DOC concentration of protein identified using molecular weight fractionation). It is evident from this comparison that no proteins of MW of 470 kDa and higher than 900 kDa are found in the purified water using both destabilisation reagents. Higher efficiency in the removal of high molecular organic matter, which is proven also by other authors (*Chin et al., 1994; Chow et al., 1999*), is most likely associated with their structure and the presence of dissociated functional groups on their surface.

In the case of ferric sulphate all proteins of MW = 85 kDa are removed. On the contrary, a higher efficiency in the aggregation of proteins of the lowest MW = 21 kDa is attained with aluminium sulphate. It is also evident from these results, as already discussed above, that a higher total efficiency in the aggregation of proteins is attained with ferric sulphate.

Monitoring of the size-distribution of aggregates formed by the test of aggregation (*Hereit et al., 1980; Polasek and Mutl, 1995*) offers one of the best method for the interpretation of the results of jar tests. Figs. 8 and 9 show the development of aggregates formed by both destabilisation reagents under the same hydrodynamic conditions. The residual portion of non-aggregated particles NA corresponds to the course of residual Fe and Al concentrations. As it is evident from Fig. 8 the lowest $NA = 0.10$ portion was attained with ferric sulphate dosages between 0.071 and 0.142 mmol l^{-1} (40 and 80 mg l^{-1}). The highest portion of macro-aggregates $MA = 0.58$ is attained at a dosage of 0.018 mmol l^{-1}

(10 mg l^{-1}) and thereafter it decreases with increasing dosage. As the portion of macro-aggregates decreases the portion of non-aggregated particles gradually increases with increasing dosages. The development of Al-formed aggregates is illustrated in Fig. 9. The lowest portion of $NA = 0.16$ is attained with aluminium sulphate dosage between 0.075 and 0.150 mmol l^{-1} (50 and 100 mg l^{-1}). The highest portion of macro-aggregates MA was formed at a dosage of 0.075 mmol l^{-1} (50 mg l^{-1}).

Most probably the relatively high portion of macro-aggregates formed during jar test is caused by the fact that under certain circumstances the COM substances may behave like non-ionic and anionic polyelectrolytes, which by means of the resultant adhesion enable formation of large aggregates (*Bernhardt et al., 1986, 1989; Hoyer et al., 1987*).

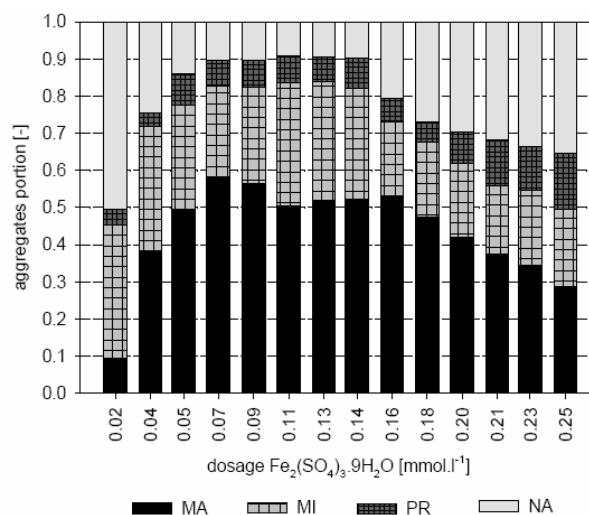


Fig. 8. Dependence of size distribution of formed aggregates on ferric sulphate dosage.

Obr. 8. Závislost velikostní distribuce tvořených agregátů na dávce síranu železitého.

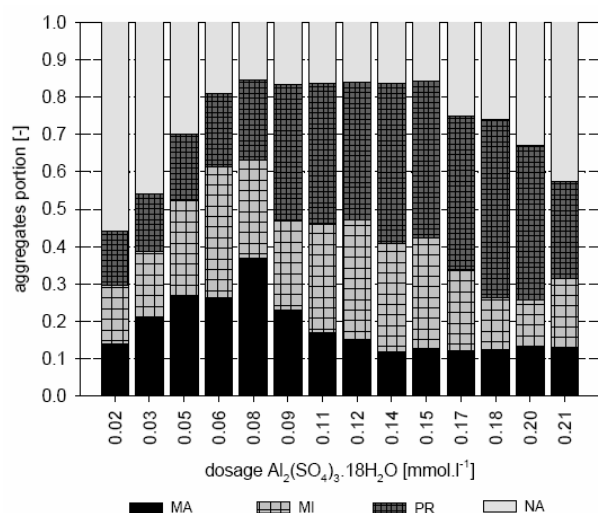


Fig. 9. Dependence of size distribution of formed aggregates on aluminium sulphate dosage.

Obr. 9. Závislost velikostní distribuce tvořených agregátů na dávce síranu hlinitého.

4. Conclusion

1. The removal of COM produced by cyanobacteria *M. aeruginosa* is influenced by its composition (content of protein and non-protein organic matter), type and dosage of destabilisation reagent and reaction pH.
2. Proteins of the relative MW of 21, 85, 395, 470 kDa and higher than 900 kDa are a part of COM of cyanobacteria *M. aeruginosa*. The portion of proteins measured as DOC_p represents about 59.9% DOC_T , the total content of organic matter. The removal efficiency of COM is relatively low. It is about 46% and 41% in the case of ferric and aluminium sulphate aggregation, respectively.
3. In comparison to other organic matter, mainly polysaccharides, the proteins are removed with higher efficiency. The GPC analyses of the residual COM showed that proteins of a higher MW are aggregated with a higher efficiency.
4. The test of aggregation proved the formation of considerable quantity of the macro-aggregates, mainly during Al-aggregation. Despite this, relatively large residual concentration of Fe and Al remains in the purified water.

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EFEKT CELULÁRNÍCH ORGANICKÝCH LÁTEK PRODUKOVANÝCH SINICÍ *MICROCYSTIS* *AERUGINOSA* NA ÚPRAVU VODY

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Mezi současné významné problémy úpravy povrchové vody patří problematika odstraňování organických látek přírodního původu (NOM), z nichž obzvláště obtížně odstranitelné jsou látky produkované při masovém rozvoji fytoplanktonu, tzv. algae organic matter (AOM). Tyto organické sloučeniny lze obecně rozdělit na látky, které se uvolňují vlivem metabolické činnosti mikroorganismů – extracellular organic matter (EOM) a dále pak na látky uvolňované vlivem jejich masivního odumírání – cellular organic matter (COM). Především pak COM představují pro chemickou technologii úpravy vody, vzhledem k náhlému zvýšení jejich koncentrací a specifickému složení (zvýšený podíl polysacharidů a některých proteinů), značný problém.

Doposud byla větší pozornost věnována vlivu AOM na průběh úpravy surové vody s obsahem rozdílných znečišťujících příměsí (hlinitokřemičitany, huminové látky atd.). Z publikovaných prací vyplývá, že působení

EOM na proces destabilizace a agregace znečišťujících příměsí se projevuje obdobnými rysy jaké mají neionogenní polymery. Při nízkých koncentracích dochází k zvyšování účinnosti úpravy vody tvorbou mezičásticových můstků nebo adhezí částic na jejich povrchu. Naopak při vyšších koncentracích tyto látky brání procesu destabilizace a agregace znečišťujících příměsí nárůstem negativního náboje na jejich povrchu, a tak i vzájemnému přiblížení částic. Dále se předpokládá, že některé látky obsažené v AOM, především proteiny, inhibují destabilizační proces tvorbou komplexů s vícemocnými kationty a hydratovanými ionty pomocí koordinačních vazeb. Tvorba těchto komplexů vede k nárůstu spotřeby destabilizačních činidel a ke snižování účinnosti destabilizace.

Příspěvek se zabývá problematikou vlivu COM produkovaných sinicí *Microcystis aeruginosa* na proces chemické úpravy vody destabilizací a následnou agregací znečišťujících příměsí. V průběhu výzkumu v laboratorních podmínkách byla sledována účinnost odstranění nejvýznamnějších složek COM, tj. polysacharidů a proteinů v závislosti na složení, množství a reakčních podmínkách (pH, typ a dávka destabilizačního činidla). COM v surové a upravené vodě byly stanovovány jako DOC, dále byl stanovován podíl polysacharidů a proteinů na celkové koncentraci DOC a detailně bylo složení COM popsáno pomocí relativních molekulových hmotností jejich jednotlivých složek.

Z dosažených výsledků je patrné, že odstranitelnost COM je závislá na jejich složení ale i koncentraci v surové vodě, dále pak také na typu a dávce destabilizačního činidla. Výsledky ukazují, že především podílové frakce COM nižších molekulových hmotností jsou konvenční úpravou vody velmi obtížně odstranitelné a tvoří zásadní podíl zbytkové koncentrace DOC. Účinnost odstranění COM dosahovala při použití síranu železitého hodnot $\alpha_{\text{DOC}} = 0,46$ a při použití síranu hlinitého dokonce jen cca $\alpha_{\text{DOC}} = 0,41$. Dále bylo zjištěno, že proteiny ve srovnání s ostatními organickými látkami (polysacharidy) jsou odstraňovány s vyšší účinností. Z HPSEC analýzy zbytkových koncentrací proteinů při jednotlivých dávkách destabilizačních činidel je patrné, že účinně jsou odstraňovány především proteiny s vyšší molekulovou hmotností. Naopak účinnost odstranění polysacharidů je při použití obou destabilizačních činidel velmi nízká $\alpha_{\text{DOCNP}} = 0,14\text{--}0,22$.