Charles University in Prague

Faculty of Science

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Study program: Environmental Sciences

Adsorption of organic compounds onto activated carbon in water treatment process

Ph.D. Thesis

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Prague, 2014

Prohlášení

Prohlašuji, že jsem tuto práci zpracovala samostatně, s uvedením všech použitých informačních zdrojů a literatury. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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

In Prague 2014

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Ivana Kopecká

Financial support

The research for this thesis has been funded by the Czech Science Foundation under the projects No. P105/11/0247 and No. P105/10/P515 and the Grant Agency of the Academy of Sciences of the Czech Republic under the project No. IAA20600902.

This thesis was prepared in co-operation with the Institute of Hydrodynamics, Academy of Sciences of the Czech Republic, v. v. i.

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Acknowledgements

I would like to thank my supervisor Martin Pivokonský and supervisor-consultant Petra Hnaťuková for expert consultancy, friendly guidance and support throughout my Ph.D. study; the Institute of Hydrodynamics, ASCR, v. v. i. for providing the conditions for preparation of this work; and all colleagues and students from a group of Drinking Water Treatment for their help in the laboratories at the Institute of Hydrodynamics, ASCR, v. v. i.

LIST OF PUBLICATIONS

The thesis is based on the following 4 papers and 2 conference contributions:

Publication 1

Kopecká, I., Pivokonský, M., Pivokonská, L., Hnaťuková, P., Šafaříková, J., 2014. Adsorption of peptides produced by cyanobacterium *Microcystis aeruginosa* onto granular activated carbon. *Carbon* 69, 595-608.

Ivana Kopecká was responsible for preparation and running the experiments, laboratory and data analyses and writing the manuscript.

Publication 2

Pivokonský, M., Šafaříková, J., Pivokonská, L., Barešová, M., Kopecká, I., 2014. A comparison of the character of algal extracellular versus cellular organic matter produced by cyanobacterium, diatom and green alga. *Water Research* 51, 37-46.

Ivana Kopecká participated in the laboratory work and manuscript preparation.

Publication 3

Šafaříková, J., Barešová, M., Pivokonský, M., Kopecká, I., 2013. Influence of peptides and proteins produced by cyanobacterium *Microcystis aeruginosa* on the coagulation of turbid waters. *Separation and Purification Technology* 118, 49-57.

Ivana Kopecká participated in the laboratory work and manuscript preparation.

Publication 4

Hnaťuková, P., Kopecká, I., Pivokonský, M., 2011. Adsorption of cellular peptides of *Microcystis aeruginosa* and two herbicides onto activated carbon: Effect of surface charge and interactions. *Water Research* 45, 3359-3368.

Ivana Kopecká was responsible for running the experiments and data evaluation; she also participated in the manuscript preparation.

Publication 5

Kopecká, I., Šafaříková, J., Pivokonský, M., Hnaťuková, P., 2012. Effect of cellular peptides of *Microcystis aeruginosa* on granular activated carbon adsorption of

herbicides. In Proceedings of the Conference “6th IWA International Conference for Young Water Professionals - IWA YWPC 2012”, 10-13 July, 2012, Budapest, Hungary.

Ivana Kopecká was responsible for running the experiments, data analyses and writing the manuscript.

Publication 6

Kopecká, I., Hnaťuková, P., Pivokonský, M., 2011. Adsorpce pesticidů na granulovaném aktivním uhlí při úpravě vody: vliv organických látek produkovaných fytoplanktonem. In Proceedings of the Conference *“*Modernizácia a optimalizácia úpravní vôd – 2. ročník", Stará Lesná, Slovensko, 2011. ISBN 978-80-969974-4-2.

Ivana Kopecká was responsible for running the experiments, data analyses and writing the manuscript.

ABSTRACT

The dissertation thesis focuses on the application of activated carbon for the removal of low molecular weight algal organic matter (AOM) produced by phytoplankton during drinking water treatment, as well as on the effect of AOM on adsorption of anthropogenic micropollutants contained in raw water. The results of this study have been published in international peer-reviewed journals in 4 papers and in 2 conference contributions.

The efficiency of AOM removal was studied in laboratory equilibrium and kinetic experiments using different types of granular activated carbon and cellular peptides with molecular weight < 10 kDa produced by cyanobacterium *Microcystis aeruginosa*. It has been previously confirmed that these peptides are removed with difficulty during the conventional water treatment based on coagulation/flocculation processes and therefore, other methods need to be applied for their restriction. The effect of solution properties on peptide adsorption was assessed by the tests at different pH values and at variable ionic strengths. The negative impact of peptides on the adsorption of organic micropollutants present in raw water was simulated using competitive adsorption experiments with herbicides alachlor and terbuthylazine and fresh activated carbons and carbons preloaded with cellular peptides.

The experimental results proved that activated carbon can be an effective adsorbent for natural and anthropogenic organic pollutants in case of properly designed adsorption conditions. Adsorption efficiency depends strongly on solution pH value, which affects both the protonation/deprotonation of pollutants' functional groups and surface charge of activated carbon. The amount of adsorbed peptides markedly increases with the decrease of pH value to acidic region (from pH 8.5 to pH 5) and with the increasing portion of secondary micropores and mesopores in activated carbon structure. Moreover, adsorption is significantly affected by the solution ionic strength, which can enhance or reduce peptide removal through the influence on electrostatic interactions in adsorption system. The effect of ionic strength depends on the pH value and the type of activated carbon. Among COM peptides, those with molecular weights below 4.5 kDa were removed preferentially and had negative effect on the removal of both herbicides owing to the competitive adsorption. This was apparent mainly at acidic pH value (pH 5) and thus corresponded well to higher adsorption of peptides under such conditions.

ABSTRAKT

Disertační práce se zabývá využitím aktivního uhlí při odstraňování nízkomolekulárních organických látek produkovaných sinicemi a řasami (AOM – Algal Organic Matter) při úpravě pitné vody a také vlivem těchto látek na adsorpci antropogenních mikropolutantů, které jsou v surové vodě přítomny. Základem práce jsou výsledky publikované ve čtyřech mezinárodních recenzovaných časopisech s impakt faktorem a dvou konferenčních příspěvcích.

Účinnost odstraňování AOM byla studována pomocí rovnovážných a kinetických laboratorních testů s různými typy granulovaného aktivního uhlí a buněčnými peptidy s molekulovou hmotností < 10 kDa produkovanými sinicí *Microcystis aeruginosa*, u kterých bylo již dříve prokázáno, že jsou velmi obtížně odstranitelné konvenční úpravou pitné vody pomocí koagulace/flokulace, a je tak třeba je odstraňovat jinými procesy. Vliv vlastností roztoku na adsorpci peptidů byl posuzován prostřednictvím testů při různých hodnotách pH a iontové síly. Negativní vliv peptidů na adsorpci organických mikropolutantů přítomných v surové vodě byl simulován prostřednictvím kompetitivních adsorpčních testů s herbicidem alachlorem a terbuthylazinem a uhlím čistým i předem zatíženým buněčnými peptidy.

Výsledky provedených testů prokázaly, že aktivní uhlí je v případě vhodného nastavení podmínek adsorpce velmi dobrým adsorbentem pro odstraňování přírodních i antropogenních organických polutantů. Účinnost adsorpce velmi silně závisí na pH roztoku, které ovlivňuje jak protonizaci/deprotonizaci funkčních skupin polutantů, tak i náboj povrchu aktivního uhlí. V případě peptidů jejich naadsorbované množství výrazně roste s poklesem pH do kyselé oblasti (z pH 8,5 na pH 5) a zároveň s růstem zastoupení sekundárních mikropórů a mezopórů ve struktuře aktivního uhlí. Adsorpce je také výrazně ovlivněna iontovou silou roztoku, která může adsorpci peptidů v závislosti na pH a typu použitého aktivního uhlí posílit nebo potlačit prostřednictvím vlivu na elektrostatické síly v adsorpčním systému. Mezi peptidy byly přednostně adsorbovány ty s nižší molekulovou hmotností pod 4,5 kDa. Prokázán byl i negativní vliv nízkomolekulárních peptidů na odstranění obou herbicidů z roztoku díky jejich kompetitivní adsorpci. Tento vliv byl patrný zejména při nízkém pH (pH 5), což koresponduje s vyšší adsorpcí peptidů za těchto podmínek.

CONTENTS

LIST OF ABBREVIATIONS AND SYMBOLS

a_m	Langmuir parameter of adsorption capacity at maximum
AC	Activated carbon
ALA	Alachlor
AOM	Algal organic matter
b	Langmuir constant
BET	Brunauer-Emmett-Teller method
BJH	Barrett-Joyner-Halenda method
C_0	Initial concentration of adsorbate
C_e	Equilibrium concentration of adsorbate
C_t	Concentration of adsorbate at specific time
CC	Cell counting
COM	Cellular organic matter
CV	Coefficient of variation
d	Diameter
DAD	Diode array detector
DBPs	Disinfection by-products
DOC	Dissolved organic carbon
DOC_{NT}	Non-protein dissolved organic carbon
DOC_P	Protein dissolved organic carbon
DOC_T	Total dissolved organic carbon
DOM	Dissolved organic matter
EC	Electrical conductivity
EOM	Extracellular organic matter
ESI+	Positive electrospray ionization
FTL	Filtrasorb TL830
F400	Filtrasorb 400
GAC	Granular activated carbon
GC-ECD	Gas chromatography with electron capture detector
HPI	Hydrophilic fraction
HPIA	Hydrophilic acid
HPIB	Hydrophilic base
HPIN	Hydrophilic neutral
HPLC	High performance liquid chromatography
HPO	Hydrophobic fraction
HPOA	Hydrophobic acid
HPOB	Hydrophobic base
HPON	Hydrophobic neutral
HPSEC	High performance size exclusion chromatography
IC	Inorganic carbon
IEF	Isoelectric focusing
IS	Ionic strength
IUPAC	International Union of Pure and Applied Chemistry
K_f	Freundlich parameter of adsorption capacity
k_1, k_2	Kinetic rate constants
LC/MS/MS	Liquid chromatography with mass spectrometry
m	Adsorbent dose/mass
MC	Microcystin
MC-LR	Microcystin-LR
MC-RR	Microcystin-RR
MC-YR	Microcystin-YR

MF	Microfiltration
MW	Molecular weight
n	Freundlich constant
N_r	Number of a specific functional group
N_t	Normality of titration agent
NOM	Natural organic matter
N1240	Norit 1240
OD	Optical density
p	Adsorbate pressure
p_0	Saturated vapour pressure of adsorbate at measurement temperature
pE	Equivalence point
pI	Isoelectric point
pI_T	Total isoelectric point
pK	Dissociation constant
PAC	Powdered activated carbon
pH	Potential of hydrogen
pH_{pzc}	pH of the point of zero charge
PIC	Picabiol 12x40
PSD	Pore size distribution
Q	Surface charge
q_e	Adsorbed amount at equilibrium
q_t	Adsorbed amount at specific time
R^2	Coefficient of determination
S_{BET}	Specific surface area measured by the Brunauer-Emmett-Teller method
S_{meso}	Surface area of mesopores
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
SD	Standard deviation
SPE	Solid phase extraction
SUVA	Specific UV absorbance
T	Time
Tu	Turbidity
TBA	Terbuthylazine
TC	Total carbon
THMs	Trihalomethanes
TOC	Total organic carbon
TPI	Transphilic fraction
UF	Ultrafiltration
US EPA	United States Environmental Protection Agency
$UV_{254\ nm}$	UV absorbance at 254 nm
UV/VIS	Ultraviolet-Visible Spectroscopy
V	Solution volume
V_0	Initial volume of titration agent
V_{micro}	Volume of micropores
V_t	Volume of titration agent at specific time
V_{total}	Total pore volume

1 GENERAL INTRODUCTION

1.1 Activated carbon

Activated carbon (AC) is a carbon-based material with microcrystalline structure very similar to that of graphite. However, more detailed analyses revealed that structure of both materials slightly differs mainly with respect to the orientation of carbon layers and the interlayer spacing. The spacing is narrower in AC, ranging between 0.34 and 0.35 nm (compared with 0.335 nm in case of graphite), and the layers are also less ordered. This disorder imparts specific adsorption properties to the activated carbon and it is caused by the defects such as vacant lattice sites, and the presence of several heteroatoms, e.g. oxygen, hydrogen, nitrogen or sulphur (Bokros, 1969; Bansal and Goyal, 2005).

Activated carbon is traditionally used in many fields of gas- and liquid-phase applications as an efficient and versatile adsorbent for both organic and inorganic compounds. It is utilized in the treatment of municipal and industrial wastewater, in purification of air, chemical, food and pharmaceutical products. Adsorption onto activated carbon has also been cited by the US EPA as one of the best available environmental control technologies for the removal of colour, odour and taste, and other natural and anthropogenic organopollutants from drinking water (U.S. EPA, 1984; Ebie et al., 2001).

A variety of materials with high carbon content and low amount of inorganic impurities is used for AC production. Activated carbons applied for water purification are most often produced from heterogeneous materials such as bituminous coal, charcoal, lignite, wood, peat or coconut shells (Knappe, 2006). The economic aspect of AC production is also important, therefore cheap and easily accessible materials such as waste from agriculture and food industry (e.g. sugar-cane waste, fruit stones, rice bran) are often utilized (Mohan and Singh, 2002; Kobya a kol., 2005; Suzuki et al., 2007). The manufacture of activated carbon involves two important steps – carbonization and activation. The raw precursor material is firstly heated (carbonized) in an inert atmosphere at 700 – 1100 °C to remove volatile compounds and to produce basic char material composed of condensed polyaromatic sheets with a poorly developed pore structure (Knappe, 2006; Marsh and Rodríque-Reinoso, 2006). Then, the further development of internal pore structure takes place during thermal activation carried out at 800 – 1000 °C with carbon dioxide, steam, or a mixture of both. The processes of carbonization and thermal activation are usually

performed sequentially. In some cases, the raw material is carbonized and activated simultaneously in the presence of chemicals such as H_3PO_4, KOH, HNO_3. This process, referred as chemical activation, is carried out usually at 450 – 600 °C and it is used especially in the manufacture of some wood-based ACs that are employed in water treatment (Knappe, 2006; Marsh and Rodríque-Reinoso, 2006).

The porosity of activated carbon is heterogeneous in character due to the presence of pores of different sizes, and it is also the reason why activated carbon has such a large specific surface area (S_{BET}) available for adsorption or chemical reactions. AC structure consists of micropores (d < 2 nm), mesopores (2 ≤ d ≤ 50 nm) and macropores (d > 50 nm), where d means diameter. Micropore region is in detail subdivided to primary (d < 0,8 nm) and secondary micropores (0,8 nm < d < 2 nm) (IUPAC, 1972). Pore size distribution (PSD), i.e. a proportion of each type of pores in carbon structure, depends on the raw material and the activation technique used in the manufacture of the adsorbent (Newcombe, 2006).

Adsorption of pollutants onto AC is influenced by the chemical properties of carbon surface, which contains several functional groups located mainly at the edges of graphene layers. The most abundant and important are the groups with oxygen as a heteroatom, because they directly affect the charge of carbon surface depending on pH value. The dissociation of acidic oxygen groups (e.g. carboxyl, phenol or lactone) coupled with the proton release to the solution, is responsible for a net negative charge of AC (Moreno-Castilla, 2004). On the other hand, the net positive charge is usually assigned to the basic oxygen complexes (e.g. pyrone, chromene, quinone groups), nitrogen-containing functional groups, inorganic impurities (metal oxides) on the carbon surface and/or to the existence of delocalized π-electron systems of carbon basal planes, which accept protons from the aqueous solution (Moreno-Castilla, 2004; Newcombe, 2006).

1.2 Activated carbon in drinking water treatment process

Adsorption process using carbonaceous porous media, such as activated carbon, is frequently integrated in the drinking water treatment chain for removing undesirable impurities of both natural and anthropogenic origin (Delgado et al., 2012). Due to the large specific surface and heterogeneous pore structure, AC is most often used to remove taste and odour compounds, NOM, disinfection by-products (DBPs), cyanobacterial toxins,

pharmaceuticals, pesticides, heavy metals etc. (Bjelopavlic et al., 1999; Humbert et al., 2008; Bond et al., 2011; Ho et al., 2011a; Zhang et al., 2011a; Delgado et al., 2012; Oh and Kim, 2014).

Various types of activated carbon are applied for the water purification, with granular activated carbon (GAC) and powdered activated carbon (PAC) being the most frequently used ones. GAC with particle size of 0.4 – 2.5 mm (Newcombe, 2006) is employed continuously in different types of filters/adsorbers as a final polishing step after coagulation/flocculation and before disinfection. The removal of target compounds takes place via adsorption of the soluble ones, physical filtration of the particulate ones, and if microorganism biofilm colonizes GAC particles, via biodegradation (Gibert el al., 2013). PAC formed from very fine particles with diameter 10 – 100 μm (Newcombe, 2006) is usually applied only for short periods of unexpected deterioration in raw water quality. In this case, it is dosed directly to the treated water, most often before or simultaneously with the dosing of coagulant. Activated carbon is also frequently used in combination with other treatment techniques. Dixon et al. (2011) presented a study on the use of multiple barrier approach incorporating coagulation, PAC, and ultrafiltration (UF) for the removal of extracellular and intracellular toxins (via cell removal) from naturally occurring cyanobacterial blooms of *Anabaena circinalis* and *Microcystis flos-aquae*. Campinas and Rosa (2010) also confirmed in the study on *Microcystis aeruginosa* that the combination of PAC/UF system seems to be a suitable treatment method for the control of cyanobacteria and their toxins. Moreover, a technology which combines PAC adsorption with microfiltration (MF) membrane is already being employed in some experimental plants (Matsushita et al., 2008). The extensive review of Ho et al. (2011b) provides insight into biological treatment options for optimum removal of cyanobacterial metabolites. This work quotes biological GAC filters as the attractive treatment option since they offer the advantage of two removal mechanisms, adsorption and biodegradation.

1.3 Organic pollutants in water

Surface waters employed in drinking water production may contain a variety of organic pollutants of both natural and anthropogenic origin. Natural pollutants are generally recognized as NOM (Natural Organic Matter) that are represented by humic compounds

(e.g. humic and fulvic acids) formed by the different ways of degradation and transformation of vegetable and animal materials in aquatic environment or as a consequence of elution from soils (Frimmel, 1998). Besides the humic compounds, NOM involves algal organic matter (AOM) that is produced by phytoplankton and provides a significant contribution to the heterogeneous NOM mixture mainly in the summer season (Hoyer et al., 1985; Leenheer and Croué, 2003). In this period, water treatment plants often have to deal with increased concentrations of another important group of pollutants, especially pesticides of anthropogenic origin that get into the environment mainly from agricultural production (den Hond et al., 2003; Humbert et al., 2008).

1.3.1 Algal organic matter

Algal organic matter (AOM) is a heterogeneous mixture of organic compounds such as peptides, proteins, mono-/oligo- and polysaccharides, lipids, amino sugars, uronic acids and other organic acids (Fogg, 1983; Huang et al., 2009), among which proteinaceous and polysaccharide substances dominate (Myklestad, 1995; Henderson et al., 2008a). The same trend was confirmed by Pivokonsky et al. (2006) who evaluated the production and the composition of AOM produced by the cyanobacteria *Anabaena flos-aqua* and *Microcystis aeruginosa* and the green alga *Scenedesmus quadricauda*, and divided AOM in protein and non-protein compounds (carbohydrates). Nevertheless, the composition of AOM is not constant, but it varies in dependence on the species, growth phase, geographical and seasonal life conditions (Pivokonsky et al., 2006; Henderson et al., 2008a).

According to the way of AOM release, extracellular and cellular substances can be distinguished in water environment. Extracellular organic matter (EOM) is produced during the phytoplankton living through metabolism. On the other hand, the release of large amount of cellular organic matter (COM) occurs after microorganisms' death when the cells fall prey to lysis and degradation (Henderson et al., 2008a).

In recent years, the increasing concentration of AOM is observed frequently almost in all types of surface water due to the occurrence of algae and cyanobacteria (Zamyadi et al., 2013). These microorganisms are omnipresent thanks to their viability and unassumingness for the living conditions. Moreover, the natural eutrophication of water is often strengthened and heavily disrupted as a result of human activities in agriculture and

industry, and contributes then to the expansion of algal blooms also in drinking water reservoirs (Fang et al., 2010; O'Neil et al., 2012). As a consequence, significant challenges in drinking water treatment occur (Her et al., 2004).

Several studies have confirmed that high concentration of AOM in the raw water may lead to low efficiency of coagulation and the related increasing consumption of coagulants (Bernhardt et al., 1985; Henderson et al., 2010; Takaara et al., 2010; Ma et al., 2012; Pivokonsky et al., 2012). Other serious problems are membrane fouling (Her et al., 2004; Teixeira and Sousa, 2013) and high formation of disinfection by-products in chlorination and chloramination of waters with AOM (Huang et al., 2009; Fang et al., 2010). From a consumer's perspective, the production of taste and odour compounds affecting the organoleptic properties of water (Zhang et al., 2011a), and harmful cyanobacterial toxins (Campinas and Rosa, 2006; Huang et al., 2007), is also undesirable.

The coagulation inhibitory effects are often related to COM compounds of protein character and their ability to form complexes with Al or Fe coagulants (Takaara et al., 2007; Takaara et al., 2010). Pivokonsky et al. (2012) ascertained that the removability of COM peptides/proteins produced by cyanobacterium *Microcystis aeruginosa* and mechanisms of their coagulation by Fe are strongly dependent on pH value. The highest coagulation efficiency was achieved in the pH range from 4 to 6 by the charge neutralization. At pH 6-7, which is often used for the coagulation of organic matter in practice, the coagulation of peptides and proteins was disturbed by the formation of metal-organic complexes (Bernhardt et al., 1985; Pivokonsky et al., 2012; Sano et al., 2012).

In terms of molecular weight (MW) distribution of coagulating AOM, the coagulation/flocculation processes are highly effective in removing organic molecules with high MW, whereas low-MW ones remain in the solution after the treatment (Pivokonsky et al., 2009). This was also confirmed in the study of Pivokonsky et al. (2012), where COM proteins of *M. aeruginosa* with MW > 10 kDa were completely removed under optimum reaction conditions in contrast to the peptides of MW of approximately 1, 2.8, 4.5, 5.5, 6.8, 8.5 and 10 kDa which remained in the solution. Therefore, these COM peptides represent residual DOM which is not supposed to be aggregated and separated during the chemical water treatment. This COM fraction may also include high concentrations of toxic microcystin–LR (Campinas and Rosa, 2006; Huang et al., 2007) and requires further treatment methods, for example adsorption onto GAC or PAC (Campinas and Rosa, 2006;

Bond et al., 2011). However, the specific mechanisms governing the adsorption of many organic compounds on activated carbon are still ambiguous (Moreno-Castilla, 2004) and need to be studied more, as is also the case of AOM.

1.3.2 Pesticides

Pesticides are chemicals or biological substances used to kill or control pests. Chemical pesticides are synthetic organic and inorganic compounds typically of anthropogenic production, but there are also exceptions which occur naturally in the environment, such as plant derivatives or inorganic minerals (Waxman, 1998). Pesticides are classified into several groups according to the type of target organisms that they suppress or destroy. The three most important groups are herbicides (against plants), insecticides (against insects), and fungicides (against fungi and molds). The general properties of most pesticides include persistence in the environment, the ability to accumulate in organisms and their tissues, and often difficult degradability (Krieger, 2010). Due to the ability of these compounds to pass through the soil and pollute surface water and groundwater, they also constitute a risk for water quality. This can be a serious problem mainly in agricultural areas and areas of pesticide production (Ormad et al., 2008).

The use of conventional water treatment technologies based on chemical coagulation using Al or Fe salts, flocculation and sedimentation is not effective for pesticide removal (Miltner et al., 1989; Badriyha et al., 2003). It was found that an inclusion of other technological processes, such as clarification, softening, filtration, recarbonization, and chlorination, usually does not lead to an increase in the removal efficiency over 30 % (Miltner et al., 1989). On the other hand, the preoxidation step with chlorine or sodium hypochlorite can help to improve the pesticide removal, but the fundamental problem associated with this treatment lies in the generation of by-products with proven carcinogenic power such as trihalomethanes (THMs) (Ormad et al., 2008; Huang et al., 2009). Another possibility is the application of ozone in the prior oxidation step instead of chlorine agents as it has higher oxidation power and does not generate such hazardous by-products (Sorlini and Collivignarelli, 2005). However, ozonization often presents a big disadvantage in terms of higher economic costs (Hladik et al., 2005). Other frequently applied and very effective methods are membrane technologies such as nanofiltration or reverse osmosis

(Plakas and Karabelas, 2012), and especially the adsorption on powdered or granular activated carbon (Badriyha et al., 2003; Faur et al., 2008; Humbert et al., 2008; Ormad et al., 2008) which has lower financial demands than membrane technologies. This method is very appropriate in terms of removal efficiency thanks to the heterogeneous porous structure of AC and the high internal surface area. Depending on the process conditions and the pesticide type, the maximum efficiency of optimised process can usually reach from 80 to 95 % (Badriyha et al., 2003; Ormad et al., 2008).

The occurrence of pesticides with respect to the quality of water intended for human consumption is regulated by the collective legislation of the European Union under the Drinking Water Directive No. 98/83/EC and incorporated in the national regulation of Ministry of Health of the Czech Republic under No. 252/2004. These regulations establish the maximum permissible limit of 0.5 μg L^{-1} for the total amount of individual active ingredients of pesticides, and 0.1 μg L^{-1} for a single active ingredient detected and quantified through monitoring. These limits are also valid for two herbicides, alachlor and terbuthylazine, which have been selected to represent anthropogenic water micropollutants for the purpose of this study.

Alachlor (ALA) or 2-chloro-2',6'-diethyl-N-methoxymethyl acetanilide (Figure 1) belongs to a large group of chlorinated pesticides. It is widely used as a herbicide in North America and Europe for control of annual grasses and broadleaf weeds in crops, primarily on corn, sorghum, and soybeans. Despite the fact that acetanilide herbicides have been classified as strong carcinogens, alachlor belonged between the four most widely used pre-emergence herbicides in the U.S. at the end of the last century (Waxman, 1998; Badriyha et al., 2003). Alachlor is also widely used in Asia (e.g. China and Japan) and often detected in local rivers and groundwater, which brings about serious problems as alachlor has been found to disrupt the endocrine system (Zhu et al., 2006). The application of commercial products with its content was banned in the Czech Republic in 2007. Despite alachlor should not be used in agricultural applications now, it still can be detected in water due to leakages from old stocks of preparations with its contents.

Terbuthylazine (TBA) or 6-chloro-N-(1,1-dimethylethyl)-N'-ethyl-1,3,5-triazine-2,4-diamine belongs to chlorotriazine herbicides, which are very effective inhibitors of photosynthesis and form a wide group of compounds used for pre- and post-emergence weed control. TBA is widely used in crop farming, mostly for maize protection in the early

spring (Mercadante et al., 2013). The concentration of TBA rises in the environment since it has been introduced instead of the better known atrazine, a possible human carcinogen, which was banned in several regions of the world because of frequent ground water pollution (Dezfuli et al., 2006; Mercadante et al., 2013). The physical properties of TBA indicate lower water solubility and stronger soil sorption compared to atrazine, and therefore lower water loading is assumed (WHO, 1998). In the Czech Republic, TBA is continuously monitored by the Czech Hydrometeorological Institute as it is applied for pre- and post-emergence protection of maize, potatoes, grapevine, and fruit trees.

a) b)

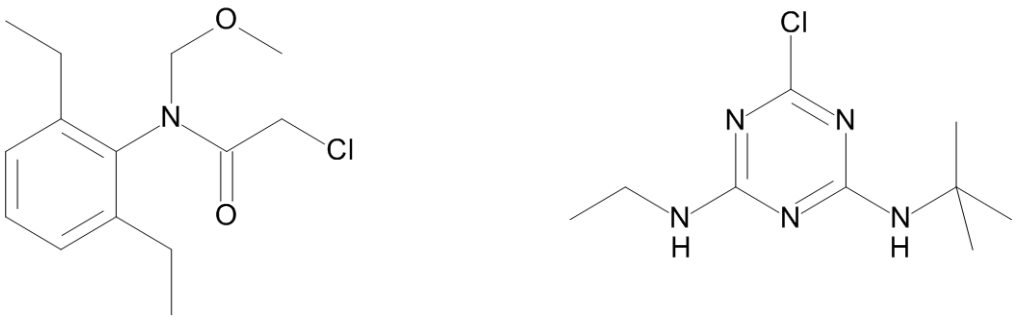

Figure 1. The molecular structures of herbicides alachlor (a) and terbuthylazine (b).

1.4 Removal of organic pollutants by activated carbon

The vast majority of recent adsorption studies dealing with the removal of NOM is focused on humic and fulvic material (Matilainen et al., 2006; Kristiana et al., 2011; Velten et al., 2011; Matsui et al., 2012; Gibert et al., 2013) or generally on dissolved organic matter (DOM) passed through a 0.45 µm filter (Schreiber et al., 2005; Gur-Reznik et al., 2008; Wei et al., 2008). Only a limited number of studies has addressed the specific adsorption of AOM (Zhang et al., 2011a; Zhang et al., 2011b) although both groups of these compounds are together classified as natural organic matter (NOM).

1.4.1 Factors affecting adsorption

It is generally accepted that adsorption of organic molecules, such as NOM, onto AC is influenced by several factors that can be divided into physical and chemical (Dastgheib et al., 2004; Moreno-Castilla, 2004).

In terms of a physical interaction, it is important to have a good compatibility of the adsorbent PSD relative to MW distribution of the NOM mixture. This factor can subsequently determine which compound will be preferentially adsorbed and removed from the treated water. Several studies demonstrated that organic molecules are more likely to be adsorbed in pores of approximately the same size as the adsorbate, as there are more contact points and more favourable adsorption energy (Newcombe et al., 1997; Ebie et al., 2001; Kilduff and Karanfil, 2002). Moreover, low-MW organic matter is adsorbed to a greater extent than the larger molecules due to the size exclusion effects (Matilainen et al., 2002; Cheng et al., 2005). It was also ascertained that entirely microporous AC have the lowest efficiency for adsorption of humic NOM with nominal MW between 500 and 30 000 Da presented in river water and other drinking water sources (Newcombe et al., 1997; Bjelopavlic et al., 1999). All these NOM fractions are adsorbed in the secondary micropores (0.8 nm < d < 2 nm) and mesopores and only and only NOM of very low MW can enter primary micropores (d < 0.8 nm). The increase in the volume of carbon larger micropores and mesopores indicates the more open pore structure of AC and may enhance the NOM uptake in many cases (Pelekani and Snoeyink, 1999; Pelekani and Snoeyink 2000; Li et al., 2003a; Dastgheib et al., 2004).

From the chemical interaction perspective, the important factors are the character and MW distribution of NOM, surface charge and functional group composition of the AC and NOM, as well as the solution chemistry (e.g. pH value, ionic strength) (Dastgheib et al., 2004; Moreno-Castilla, 2004). It is generally acknowledged that the adsorbent PSD plays a major role in the adsorption of NOM onto AC, particularly when the adsorption is governed by non-specific interactions (dispersive van der Waals interactions, hydrophobic interactions, hydrogen bonds), which are always attractive. But in the case of adsorption of organic molecules, which can be present in the solution in ionic form and therefore exhibit a charge, electrostatic interactions are also very prospective (Newcombe and Drikas, 1997; Bjelopavlic et al., 1999; Moreno-Castilla, 2004). These interactions can be either attractive or repulsive (Bjelopavlic et al., 1999) and are determined by the solution pH, which controls the de-

/protonation of surface functional groups identified in both AC structure and NOM (Boehm, 1966; Newcombe and Drikas, 1997; Mangun et al., 2001). Another key factor that controls the adsorption of NOM onto AC is solution ionic strength. It often influences not only the adsorbent-adsorbate electrostatic interactions but also the interactions between NOM molecules through the shielding effect produced by the added salt (Moreno-Castilla, 2004; Campinas and Rosa, 2006).

1.4.2 Competitive adsorption between NOM and micropollutants

Until now, the simultaneous adsorption of natural (e.g. NOM) and anthropogenic organopollutants (e.g. pesticides, herbicides) has been studied only for NOM of humic character, not for AOM. Hence, the negative effect of NOM and subsequent suppression of pesticide removal by AC has been proved only for humic and/or fulvic acids due to their competitive adsorption (Newcombe, 2006; Schideman et al., 2007; Humbert et al., 2008). Many studies revealed that NOM can restrict the adsorption ability of AC for micropollutants as it is usually present in raw water in much higher concentrations (mg L^{-1}) than problematic target species such as pesticides (µg L^{-1}) (Bjelopavlic et al., 1999). This process is known as carbon fouling and it can have deleterious effect on AC adsorption as it changes the surface properties of carbon filters. Consequently, both the working life of the AC absorbers and adsorption capacity can be reduced (Humbert et al., 2008). Capacity reductions of one or two orders of magnitude and large reductions in the rate of adsorption are no exception to the rule (Pelekani and Snoeyink, 1999).

The competition between NOM and micropollutants is influenced by many factors. The most important is usually the relationship between pore size distribution of activated carbon and molecular weight of the adsorbates. Adsorption of micropollutants is located in micropores of AC due to their very low molecular weight (maximum of tens or a few hundreds of Da), therefore low-MW NOM is generally believed to provide the greatest competition (Newcombe et al., 1997; Humbert et al., 2008). On the other hand, some micropores available for micropollutant adsorption may not be able to admit larger NOM (Ebie et al., 2001) and size exclusion effect occurs (Newcombe, 2006).

NOM fractions which are not sufficiently removed by coagulation process and penetrates the water treatment chain may affect adsorption capacity of activated carbon filters

predominantly by two different mechanisms. NOM either directly competes with micropollutants for the same adsorption sites (direct competition) or can block their access to the micropores by adsorption near pore enterings (pore blockage) which usually leads to narrowing of the pores or their complete closing and consequent reduction of the AC surface area available for adsorption (Pelekani and Snoeyink, 1999; Ebie et al., 2001).

The mechanism of pore blockage is usually induced by NOM with higher molecular weight and in case of AC with pores large enough to admit the micropollutant but too small to admit the NOM (Pelekani and Snoeyink, 1999). Furthermore, this competition mechanism proves much more negatively for adsorbents having a very narrow PSD and containing predominantly or exclusively pores of one size, in particular micropores. In this case, NOM blocks the access to the pores as is described above. Broadening of the PSD through the presence of secondary micropores and mesopores in AC structure seems to be possible solution. It provides more surface area available for NOM adsorption (Dastgheib et al., 2004; Cheng et al., 2005) and thus reduces the negative impact on micropollutant removal (Pelekani and Snoeyink, 1999).

The second competitive mechanism, the direct site competition, most likely occurs between compounds of similar molecular weight and structure (Pelekani and Snoeyink, 1999). Thus, this competition is expected mainly for small NOM molecules, which can occupy similarly small-sized pores with high energy as micropollutants and compete with them for the same adsorption sites (Li et al., 2003a, 2003b; Schideman et al., 2007).

1.4.3 Adsorption of AOM

Although the inclusion of adsorption onto AC in the water treatment plants can also assist the coagulation/flocculation based treatment of algae (Henderson et al., 2008b), the studies focused primarily on the adsorption of associated AOM are still limited (Table 1) and systematic evaluation of adsorption effectiveness is necessary. Moreover, the understanding of AOM adsorption on AC is of a major significance as many of drinking water treatment plants face up to the constantly increasing concentrations of AOM due to the eutrophication of surface waters (O'Neil et al., 2012; Paerl and Paul, 2012). Another reason for studying the adsorption of AOM is the different characteristics of the two groups of NOM (humic substances vs. AOM) such as origin, chemical composition, MW distribution, content of

functional groups, surface charge or hydrophobicity (Ebie et al., 2001; Leenheer and Croué, 2003; Chang, 2005). Thus, an evaluation of AOM adsorption by AC may help to reveal their treatment particularities and to contribute then to its better removal and practical use of AC adsorbers in drinking water facilities.

Table 1 Adsorption studies dealing with algal organic matter removal

Study	Microorganism	Adsorbate	Treatment process/experiment	Activated carbon
Matsushita et al. (2008)	*Anabaena planktonica*, *Anabaena smithii*, *Anabaena spp*.	cyanobacteria cells, intra- and extracellular geosmin	laboratory-scale ceramic MF experiments and pilot-scale AC adsorption/coagulation/ ceramic MF experiments	N-PAC (d_{50} = 7.6 µm) and S-PAC (0.65 µm) (Futurama Chemical Industries Co., Ltd., Japan)
Campinas and Rosa (2010)	*Microcystis aeruginosa*	EOM (extracellular matter excreted during growth) and AOM (total extracellular and intracellular matter released during cell lysis)	laboratory-scale experiments with hollow-fibre cellulose acetate UF membrane/PAC	PAC Norit SA-UF (Cabot Norit Americas Inc., USA)
Zhang et al. (2011a)	*Microcystis aeruginosa*	dimethyl trisulfide (98 %) and *β*-cyclocitral (90 %) (Sigma Aldrich) in natural water to simulate products of *M. aeruginosa*	laboratory-scale batch and kinetic experiments	GAC unspecified (Calgon Carbon Corp., USA)
Zhang et al. (2011b)	*Microcystis aeruginosa*	cyanobacteria cells, AOM, MC-LR, MC-RR, MC-YR	laboratory-scale experiments with a hollow-fibre polyvinyl chloride UF membrane and AC	coconut shell PAC unspecified (China)

Matsushita et al. (2008) studied the removal of cyanobacteria *Anabaena planktonica*, *Anabaena smithii* and *Anabaena spp.*, as well as the associated musty odour compound geosmin, from cyanobacterium-containing solutions. Both laboratory-scale experiments with MF membrane and pilot-scale experiments using a hybrid system of PAC adsorption, coagulation, and ceramic MF were performed. The MF membrane alone could remove only the intracellular geosmin contained in cyanobacterial cells and not extracellular geosmin because the pores of the membrane were much larger than the geosmin molecules. The addition of commercially available PAC (median particle size 7.6 µm) improved the geosmin removal but only to a limited extent. On the other hand, operation of the hybrid system with

micro-ground PAC (median particle size 0.65 µm) completely removed both intracellular and extracellular geosmin and proved that this type of system can simultaneously and effectively remove both the cyanobacteria and the geosmin from water.

The study of Campinas and Rosa (2010) focused on the PAC contribution to the control of membrane fouling caused by autochthonous NOM derived from *Microcystis aeruginosa* as AOM/EOM and allochthonous NOM (humic and tannic acids) in a PAC/UF hybrid system. The system combined a hydrophilic hollow-fibre cellulose acetate membrane and a commercial highly mesoporous PAC SA-UF Norit with low average particle diameter (6 µm). PAC was not able to promote or affect the reversible membrane fouling, but it controlled the irreversible one. The addition of PAC to UF also contributed to the enhancement of the overall removal of AOM (mixture of intra- and extracellular matter), although it was apparently ineffective for the highly hydrophilic EOM compounds.

Zhang et al. (2011a) investigated equilibria, kinetics and thermodynamics of adsorption of dimethyl trisulfide and *β*-cyclocitral, two algal odorants often produced by the cyanobacterium *M. aeruginosa*, onto GAC. Authors also focused on the presence of NOM during the adsorption and its possible effect on odorant removal. It was determined that NOM fraction with a low nominal molecular weight < 1000 Da showed the greatest inhibiting effect as it had a potential to access the same pores as odorants and could compete for the same active sites.

The study of Zhang et al. (2011b) evaluated the effect of PAC addition on immersed UF for the laboratory treatment of algal-rich waters. Even if a UF membrane alone could achieve an absolute removal of *M. aeruginosa* cells, it was less effective for the removal of AOM released into water. The UF membrane rejected a part of the high-MW AOM through a sieving mechanism, but the removal of organic matter below 1 kDa had to be enhanced by PAC adsorption. It was also inferred that apart from adsorption function, PAC may also decrease the content of AOM in water through the influence on the algal metabolism. The addition of PAC decreases the light intensity in the PAC/UF system, and therefore this may lead to lower production of proteins and carbohydrates.

Many other studies focus on the AC adsorption of cyanobacterial toxins, especially on the most commonly occurring microcystins (MCs) (Pendleton et al., 2001; Campinas and Rosa, 2006; Huang et al., 2007; Ho et al., 2011b). Even though these pollutants are not targeted in

this study, the results evaluating their adsorption could be useful for further research on AOM.

Based on the information mentioned in previous chapters, especially the lack of adsorption studies dealing with the adsorption of AOM onto activated carbon or its competitive effect on adsorption of micropollutants, and the fundamental differences between properties of humic NOM and NOM of algal/cyanobacterial origin (i.e. AOM), **the main objective of the thesis was to evaluate the application of activated carbon for the removal of AOM under different conditions during drinking water treatment.** This objective and its solutions are addressed mainly in the Publication 1.

The component objectives that contribute to the achievement of the main objective of the study were as follows:

- to characterise AOM produced by cyanobacterium *Microcystis aeruginosa* with respect to its composition and MW distribution (Publication 2);
- to identify and then to separate the low-MW fraction of cellular organic matter (COM) produced by *M. aeruginosa* (namely peptides with MW < 10 kDa), which is hardly removable by coagulation/flocculation but it can be adsorbed onto AC due to its specific properties (Publication 1, Publication 3);
- to characterise COM peptides < 10 kDa with respect to the properties affecting the adsorption (e.g. MW distribution, surface charge, content of functional groups) (Publication 1, Publication 4);
- to quantify the adsorption capacity of selected adsorbents for peptide removal under different solution conditions (pH and ionic strength) (Publication 1);
- to identify AC characteristic affecting adsorption of AOM (e.g. PSD, surface charge) (Publication 1, Publication 4, Publication 5);
- to determine which MW fraction of the peptides is preferably removed by adsorption onto AC (Publication 1, Publication 4, Publication 5);
- to evaluate the adsorption of organic micropollutants onto AC (herbicides alachlor and terbuthylazine) and the competitive adsorption effect of COM products on their removal (Publication 4, Publication 5, Publication 6);

- to identify and then describe the mechanisms and interactions acting in the adsorption of natural and anthropogenic organic pollutants onto AC (Publication 1, Publication 4).

The objectives specified above were achieved during several series of laboratory experiments. The obtained results have then been published in international peer-reviewed journals in 4 papers and in 2 conference contributions, which are consecutively presented in the next sections of the thesis.

CARBON 69 (2014) 595–608

Available at www.sciencedirect.com

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

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ARTICLE INFO

Article history:
Received 2 August 2013
Accepted 23 December 2013
Available online 3 January 2014

ABSTRACT

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

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

1. Introduction

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

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0008-6223/$ - see front matter
http://dx.doi.org/10.1016/j.carbon.2013.12.072

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

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

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

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

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

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

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

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

2. Experimental

2.1. Granular activated carbon

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

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

2.1.1. Surface area and pore size distribution analysis

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

2.1.2. Surface charge determination

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

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

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

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

2.2. Cultivation of Microcystis aeruginosa and preparation of COM

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

2.3. Isolation of COM peptides

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

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

2.4. Characterisation of COM peptides

2.4.1. DOC analysis

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

2.4.2. Molecular weight fractionation

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

2.4.3. Determination of isoelectric points and charge

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

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

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

2.5. Analysis of microcystins

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

2.6. Equilibrium adsorption experiments and data modeling

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

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

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

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

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

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

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

and

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

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

2.7. Kinetic adsorption experiments and data modeling

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

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

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

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

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

and

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

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

3. Results and discussion

3.1. GAC characterisation

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

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

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

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

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

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

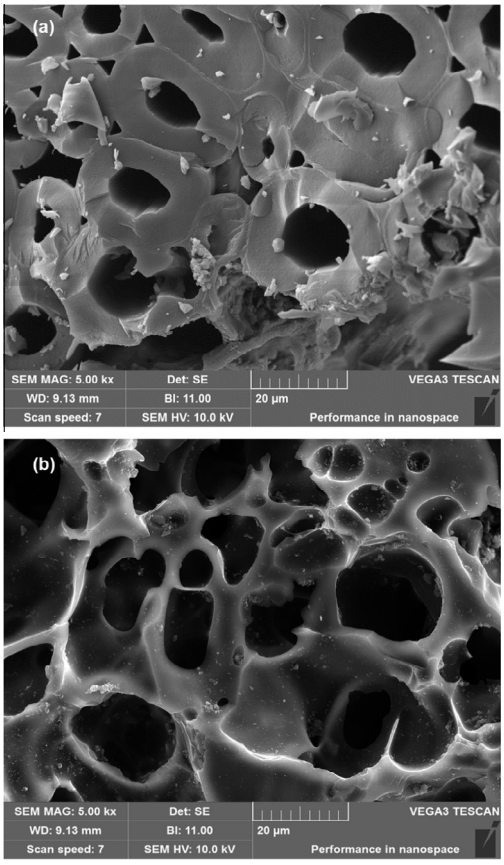

Fig. 1 – SEM micrographs of external surface structure of vegetal-based GAC PIC (a) and coal-based GAC FTL (b) scanned by Vega 3 (Tescan, Czech Republic).

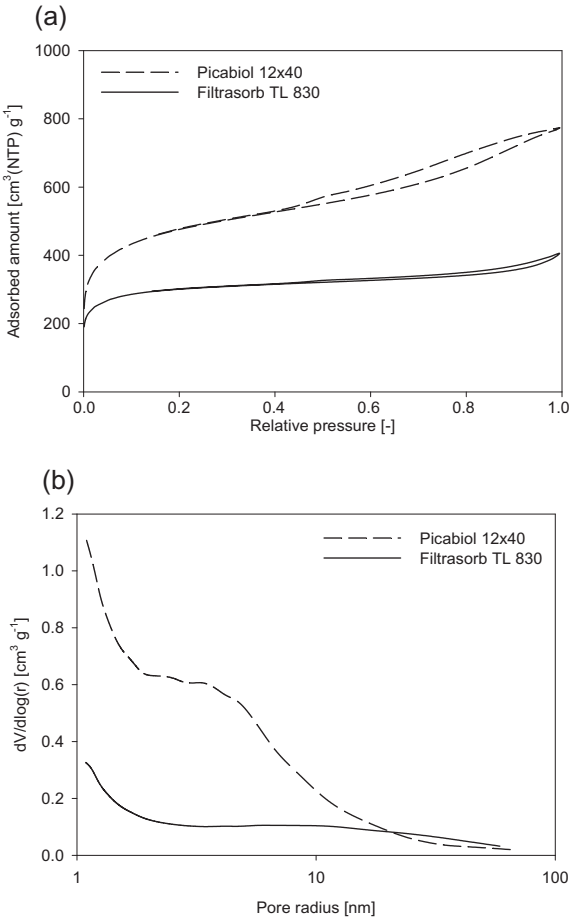

Fig. 2 – Nitrogen adsorption–desorption isotherms (77 K) (a) and pore-size distributions of GAC PIC and FTL evaluated from adsorption branches of sorption isotherms (b).

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

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

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

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

3.2. *Characterisation of COM peptides*

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

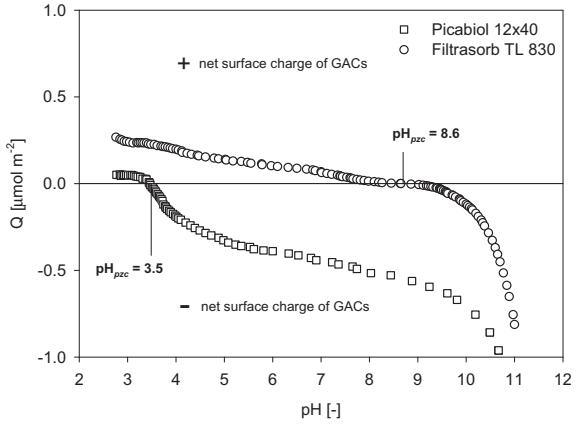

Fig. 3 – Dependence of the net surface charge (Q) of GAC PIC and FTL on experimental pH and estimated values of pH$_{pzc}$ in a 0.01 M NaCl background electrolyte.

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

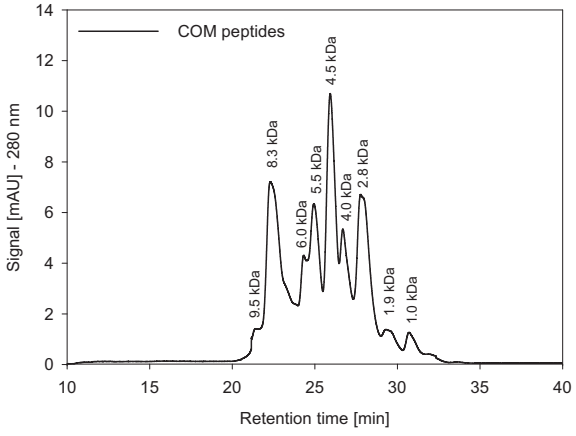

Fig. 4 – Molecular weight distribution of peptides isolated from COM produced by cyanobacterium *Microcystis aeruginosa*.

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

3.3. *Equilibrium adsorption of COM peptides*

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

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

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

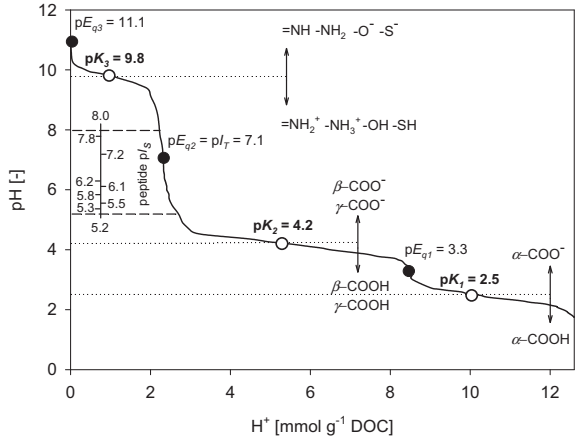

Fig. 5 – pH-titration curve of COM peptides with dissociation constants (pK_1, pK_2, pK_3) and equivalence points (pEq_1, pEq_2, pEq_3) related to the effect of different functional groups. pI_T represents the total isoelectric point of the peptide mixture. The isoelectric points (p*I*s) of single peptides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

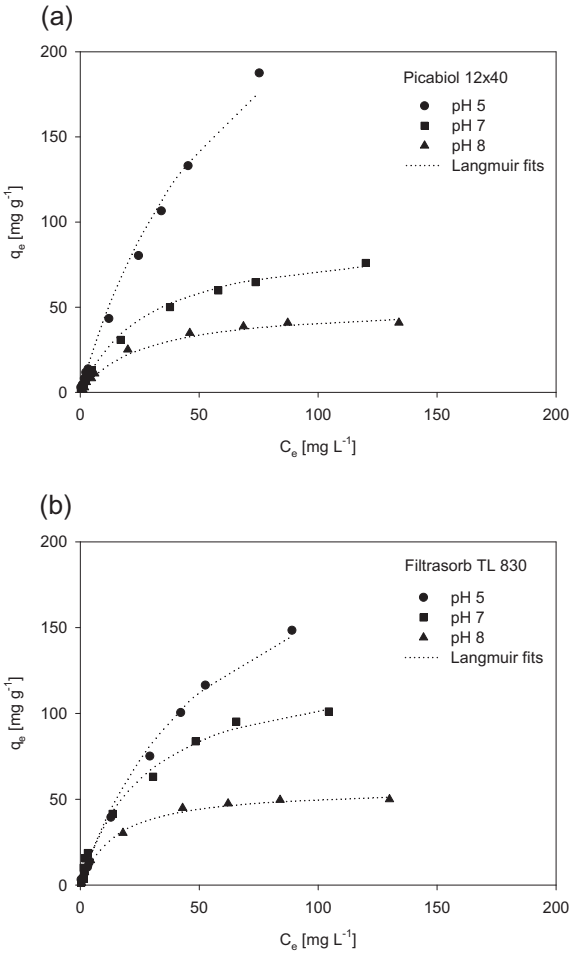

Fig. 6 – Adsorption isotherms of COM peptides at pH 5, 7 and 8 on PIC (a) and FTL (b). C_e and q_e represent equilibrium solution concentration and uptake of COM peptides, respectively. Langmuir fits were calculated from measured data according to Eq. (3).

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

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

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

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

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

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

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

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

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

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

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

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

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

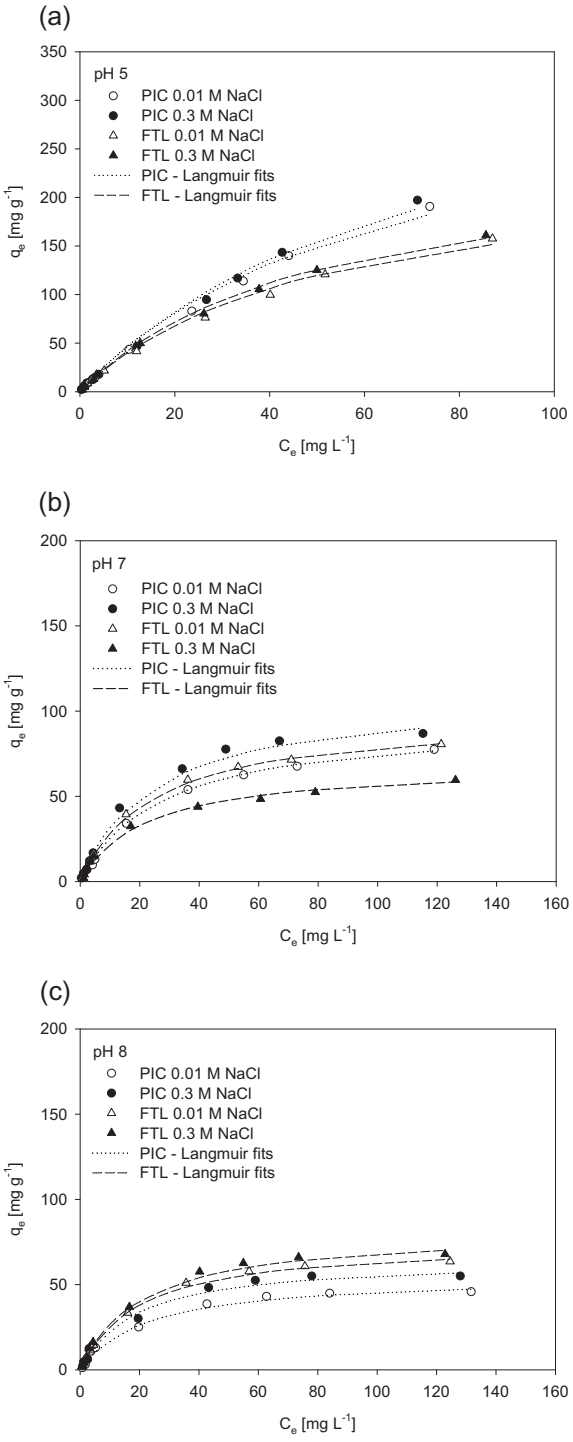

Fig. 7 – Adsorption isotherms of COM peptides at low (0.01 M NaCl) and high (0.3 M NaCl) ionic strengths on PIC and FTL at pH 5 (a), pH 7 (b) and pH 8 (c). C_e and q_e represent equilibrium solution concentration and uptake of COM peptides, respectively. Langmuir fits were calculated from measured data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

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

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

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

3.5. Adsorption kinetics of COM peptides

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

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

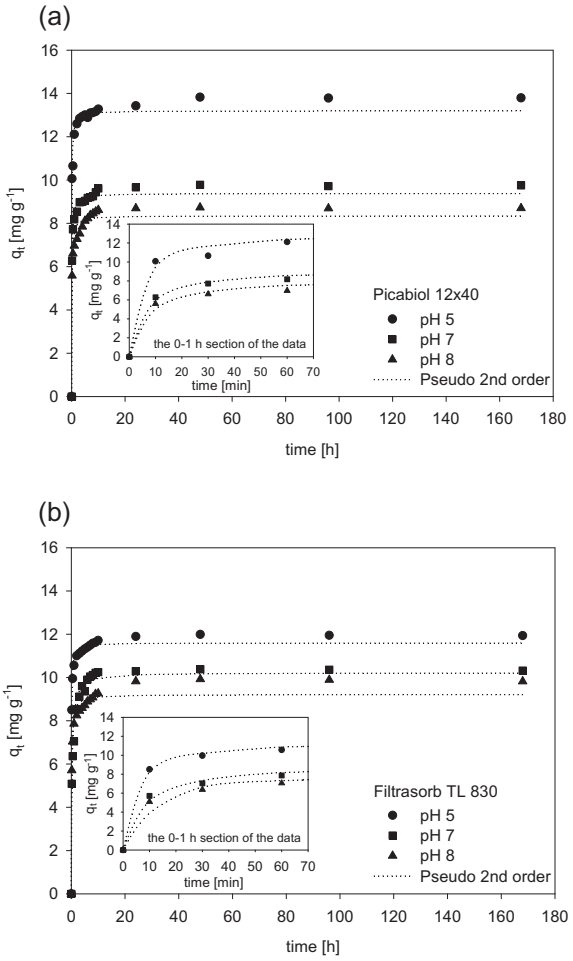

Fig. 8 – Adsorption kinetics of COM peptides on PIC (a) and FTL (b) at pH 5, 7 and 8. The value q_t represents the uptake of COM peptides at a specific time (t). Pseudo 2nd order kinetic fits were calculated from measured data according to Eq. (7).

3.6. Molecular weight distribution of COM peptides after adsorption

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

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

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

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

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

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

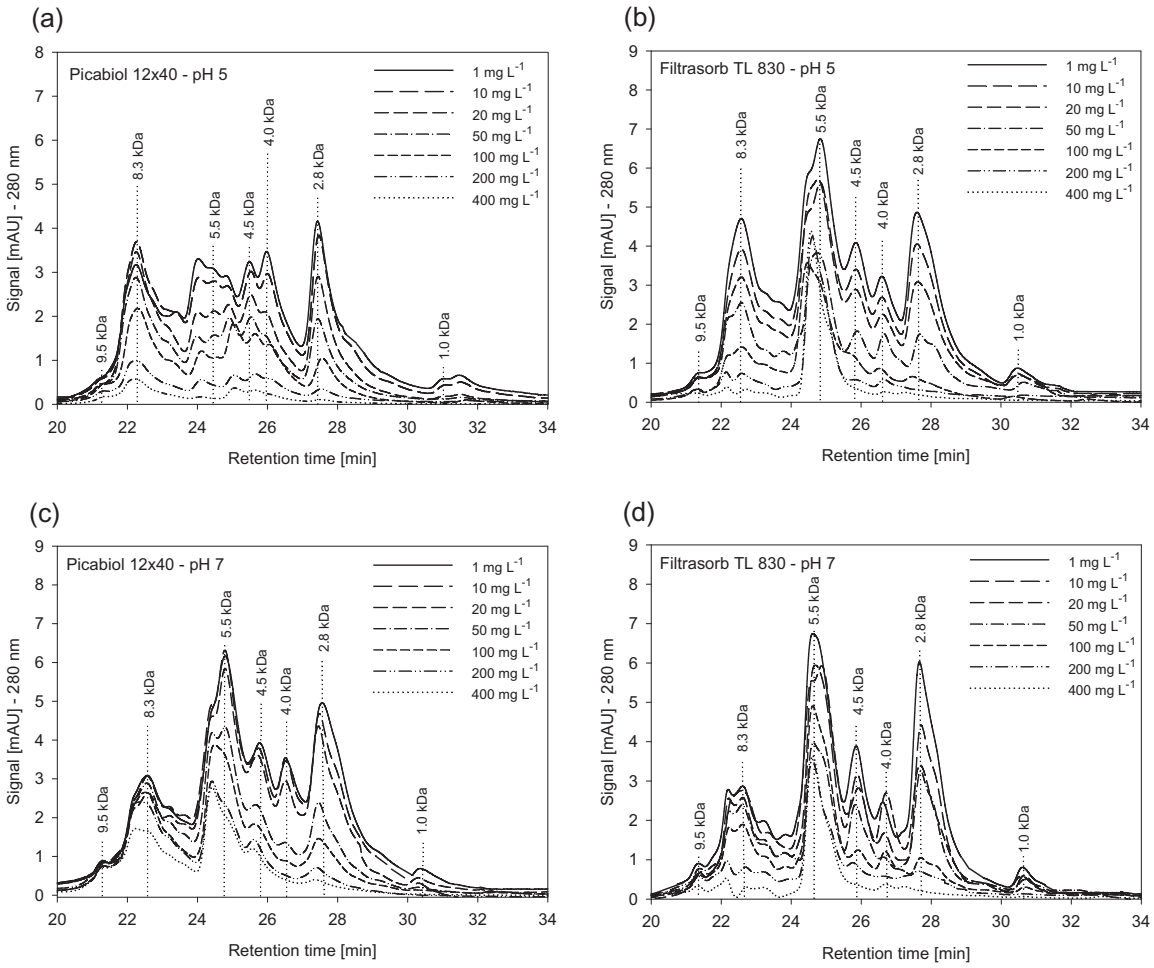

Fig. 9 – Molecular weight distributions of COM peptides remaining in the solution after adsorption on PIC (a), (c) and FTL (b), (d) at pH 5 and pH 7.

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

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

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

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

4. Conclusions

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

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

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

Acknowledgements

The research project has been funded by the Czech Sciences Foundation under the Project No. P105/11/0247. The authors acknowledge the financial assistance on this project.

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

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ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form
12 December 2013
Accepted 15 December 2013
Available online 24 December 2013

Keywords:
Algal organic matter
Extracellular organic matter
Cellular organic matter
Peptide/protein content
Hydrophobicity
Molecular weight fractionation

ABSTRACT

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

1. Introduction

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

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0043-1354/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.watres.2013.12.022

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

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

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

2. Material and methods

2.1. Algal species

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

2.2. Culture conditions

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

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

2.3. Growth monitoring

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

2.4. AOM characterization

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

2.4.1. SUVA analyses

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

2.4.2. Preparation of EOM samples

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

2.4.3. Preparation of COM samples

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

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

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

2.4.5. Determination of hydrophilic and hydrophobic fractions

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

2.4.6. Molecular weight fractionation

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

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

3. Results and discussion

3.1. Algal growth

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

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

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

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

3.2. *Peptide/protein and non-peptide content*

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

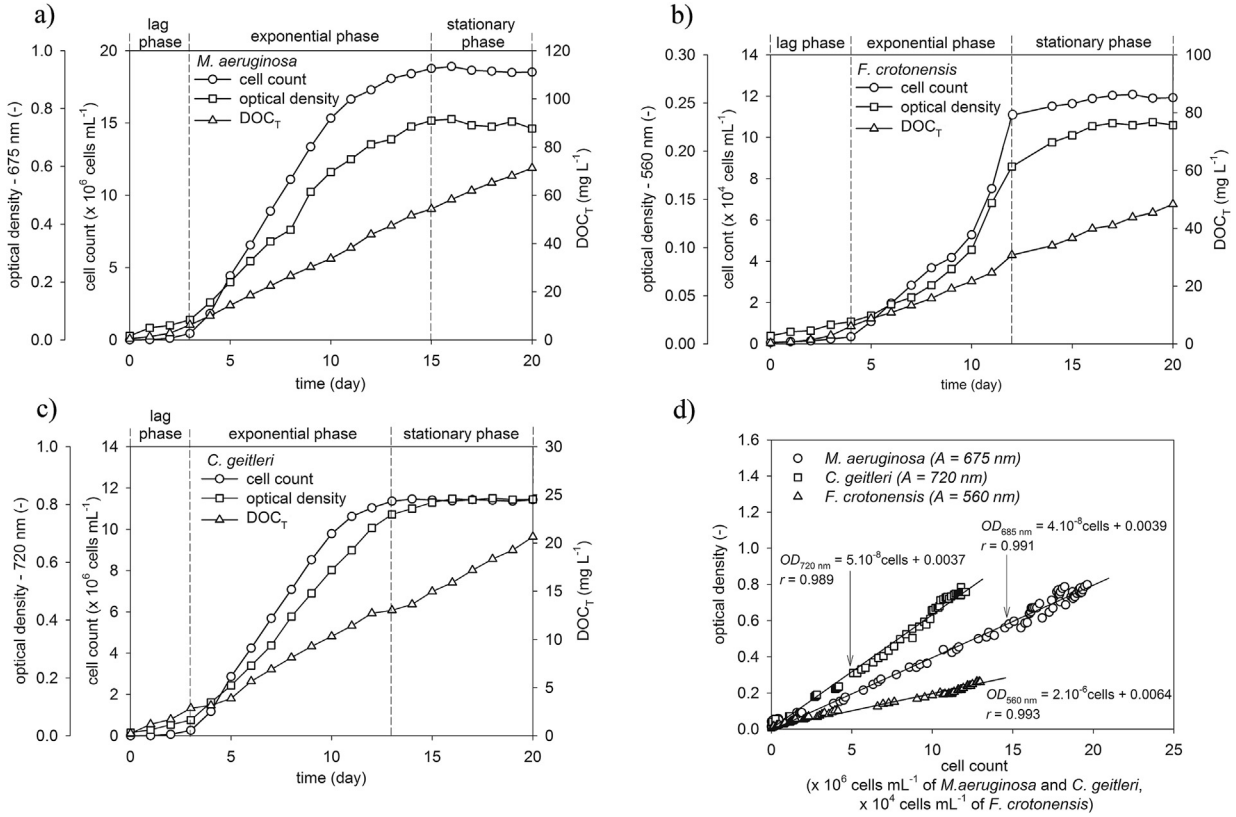

Fig. 1 – Growth curves of *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c) with growth phases marked, and linear correlations between optical densities and cell counts (d).

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

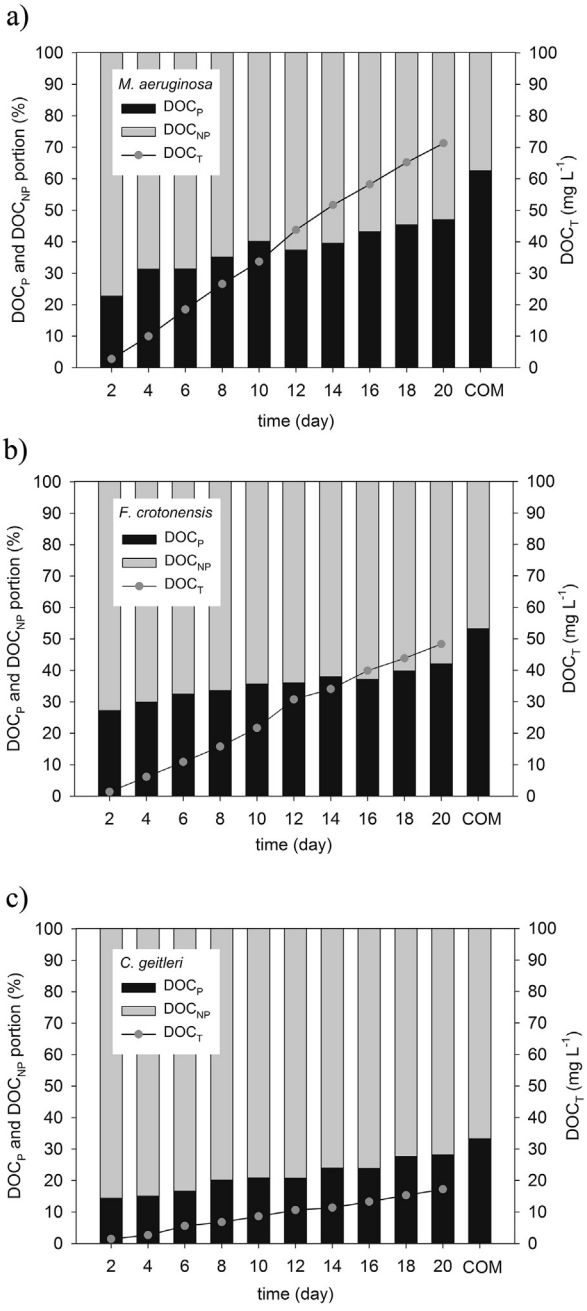

Fig. 2 – Portions of peptide/protein (DOC_P) and non-peptide (DOC_{NP}) organic matter from the total amount of organics released into cultivation media (DOC_T) as a function of cultivation time, and from COM of *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c).

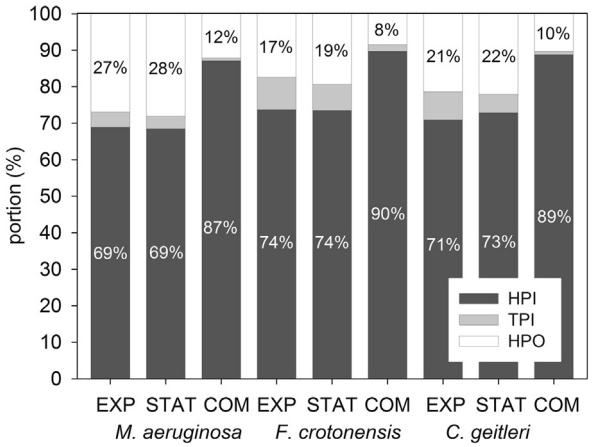

Fig. 3 – Hydrophilic (HPI), transphilic (TPI) and hydrophobic (HPO) fractions of EOM at exponential (EXP) and stationary (STAT) growth phases and of COM.

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

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

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

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

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

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

3.3. Hydrophilic and hydrophobic fractions and the SUVA values

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

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

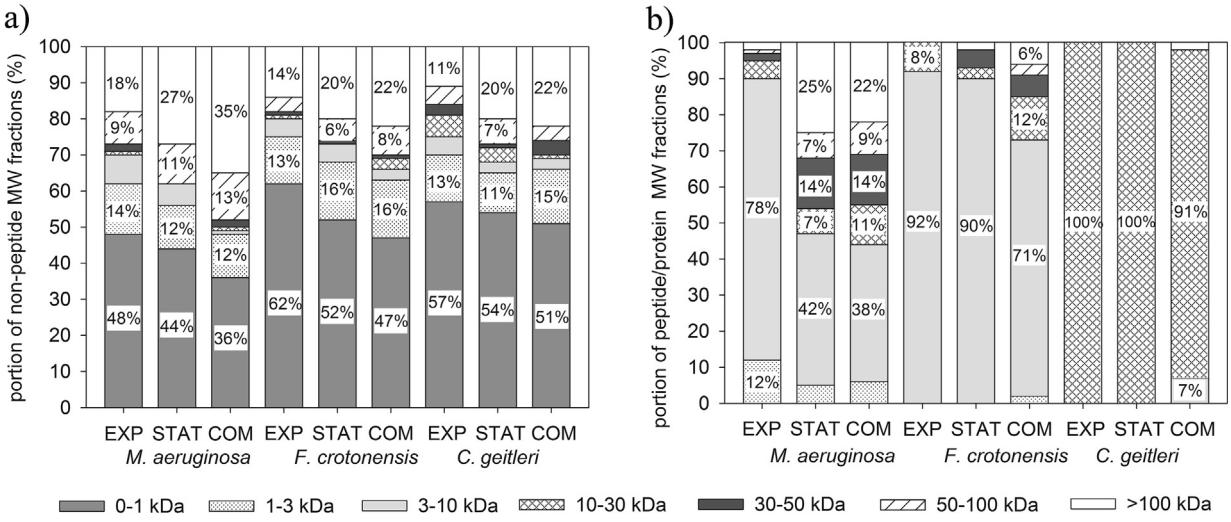

Fig. 4 – Molecular weight (MW) fractionation of non-peptide (a) and peptide/protein (b) portions of EOM at exponential (EXP) and stationary (STAT) growth phases, and of COM determined by centrifugation-driven filtration.

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

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

3.4. *Molecular weight fractionation*

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

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

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

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

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

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

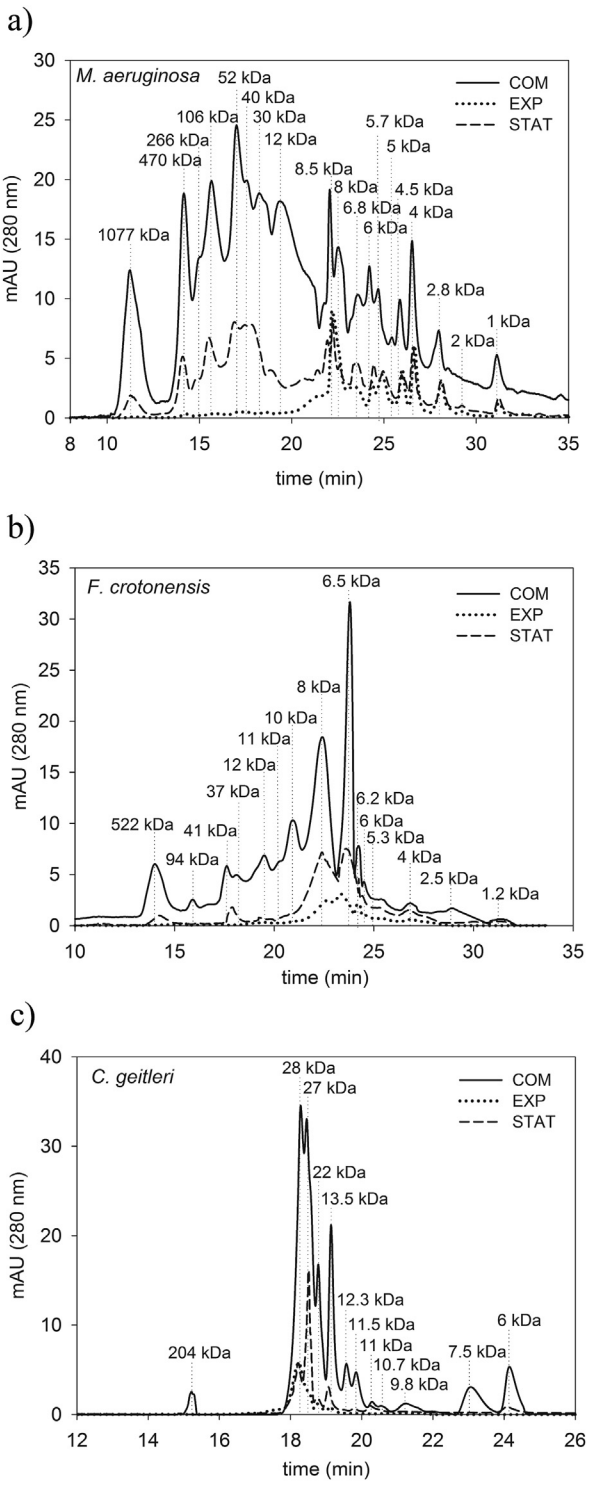

Fig. 5 – Molecular weight (MW) distributions of peptide/protein portions of EOM at exponential (EXP) and stationary (STAT) growth phases, and of COM determined by HPSEC for *M. aeruginosa* (a), *F. crotonensis* (b) and *C. geitleri* (c).

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

4. Conclusions

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

Acknowledgements

The research project has been funded by the Grant Agency of AS CR under the project No. IAA20600902. The authors acknowledge the financial assistance on this project.

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Separation and Purification Technology 118 (2013) 49–57

Contents lists available at SciVerse ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

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

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ARTICLE INFO

Article history:
Received 17 May 2013
Received in revised form 28 June 2013
Accepted 29 June 2013
Available online 6 July 2013

Keywords:
Cellular organic matter (COM)
Coagulation
Microcystis aeruginosa
Peptides/proteins
Turbidity removal

ABSTRACT

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

1. Introduction

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

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

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http://dx.doi.org/10.1016/j.seppur.2013.06.049

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

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

2. Experimental

2.1. Cultivation of M. aeruginosa

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

2.2. COM peptide/protein preparation

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

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

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

2.3. COM peptide/protein characterization

2.3.1. DOC analysis

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

2.3.2. Molecular weight fractionation

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

2.3.3. Charge determination

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

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

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

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

2.4. Kaolin characterization

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

2.4.1. Kaolin charge determination

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

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

2.5. Coagulation tests

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

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

3. Results and discussion

3.1. COM peptide/protein characterization

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

3.2. Charge characteristics of coagulated constituents

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

3.2.1. COM peptides/proteins

Peptide/protein isoelectric points (p*I*) were ascertained to be approximately 4.8, 5.1, 5.3, 5.5, 5.6, 5.8, 6.1, 6.3, 6.5, 6.6, 7.0, 7.4, 7.8, 7.9 and 8.1 (Fig. 1a). The number of peptide/protein p*I*s identified by IEF corresponded to the number of peptides separated by HPSEC. It is widely recognized that peptides/proteins provide a wide range of charged sites on their surfaces due to their various functional groups, some of which are able to release or accept a proton depending on the pH of the solution (—OH, —COOH, —SH, $—NH_3^+$, $=NH_2^+$, etc.) [19]. The total number of functional groups which can dissociate within a COM peptide/protein mixture was quantified by potentiometric titrations [18,24]. The titration curve (Fig. 1a) shows the number of protons, which the peptide/protein mixture is able to accept under a given pH (during titration from pH 12 to the given pH) and which is equal to the amount of dissociated functional groups (in millimoles of H^+ ions per 1 g of DOC). The curve shows the total isoelectric point (pI_T) of the peptide/protein mixture, which lies in the pH region where isoelectric points of single peptides/proteins were identified. Moreover, the curve provides several equivalence points (pEq_1, pEq_2 and pEq_3) and dissociation constants (pK_1, pK_2 and pK_3), which can be attributed to different COM peptide/protein titratable functional groups also depicted in Fig. 1a. However, it should be taken into consideration that the titration was performed with a mixture of compounds containing a number of various functional groups, whose dissociation constants depend not only on the type of group, but also on the overall net charge of the molecule [24]. It is likely that $pK_3 = 9.94$ could be assigned to several functional groups having their dissociation constants in alkaline pH, such as —SH, —OH, $=NH_2^+$ and $—NH_3^+$ (on both side chains, i.e. ε-NH_3^+, and terminal parts of molecules, i.e. α-NH_3^+). On the other hand, dissociation constant $pK_2 = 4.13$ can be attributed exclusively to —COOH groups on COM peptide/protein side chains (β-COOH of aspartic acid with $pK_a = 3.86$ and γ-COOH of glutamic acid with $pK_a = 4.25$). Finally, $pK_1 = 2.26$ coincides with the dissociation constants of —COOH groups of the terminal amino acids in COM peptide/protein molecules (α-COOH) [19]. The amount of a specific functional group in the COM peptide/protein mixture can be determined from the number of H^+ ions added between the two equivalence points that define the beginning and the end of dissociation of this group [19]. Numbers of H^+ ions added to reach equivalence points and points of dissociation constants are depicted in Fig. 1a above the *x*-axis. According to Eq. (2), it can be assumed that the number of titratable groups in the COM peptide/protein mixture is:

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

for β-COOH and γ-COOH

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

for α-COOH

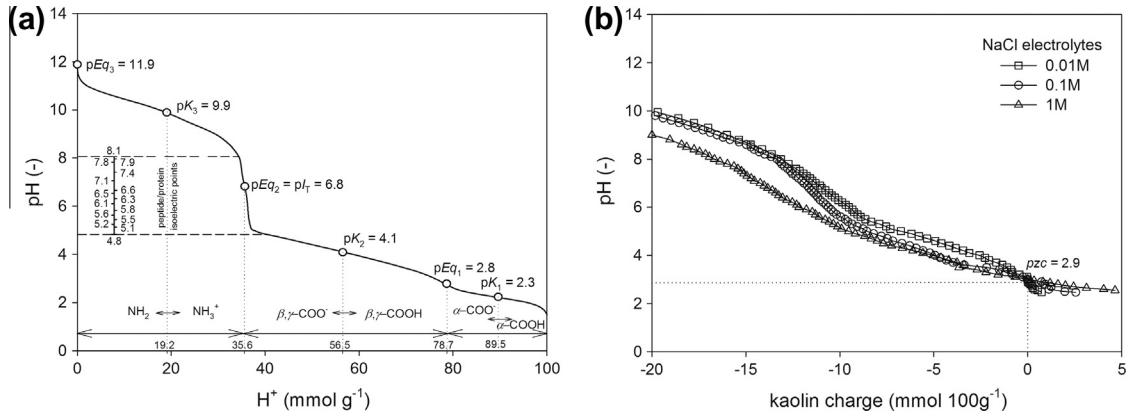

Fig. 1. (a) pH – titration curve for the COM peptide/protein mixture with equivalence points (pEq_1, pEq_2 and pEq_3), dissociation constants (pK_1, pK_2 and pK_3) and total isoelectric point (pI_T). Isoelectric points of single peptides/proteins contained in the mixture are depicted on the left. (b)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

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

for groups with dissociation constants in alkaline pH.

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

3.2.2. Kaolin

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

3.2.3. Al/Fe coagulants

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

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

3.3. Coagulation tests with kaolin

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

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

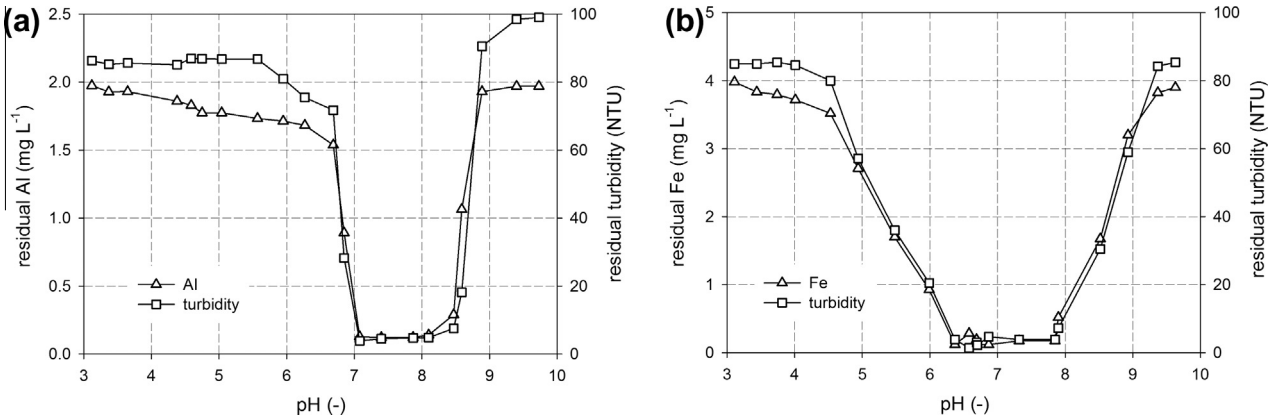

Fig. 2. Coagulation tests with kaolin and either Al (a) or Fe (b) – dependence of residual Al/Fe and turbidity on a pH value.

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

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

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

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

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

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

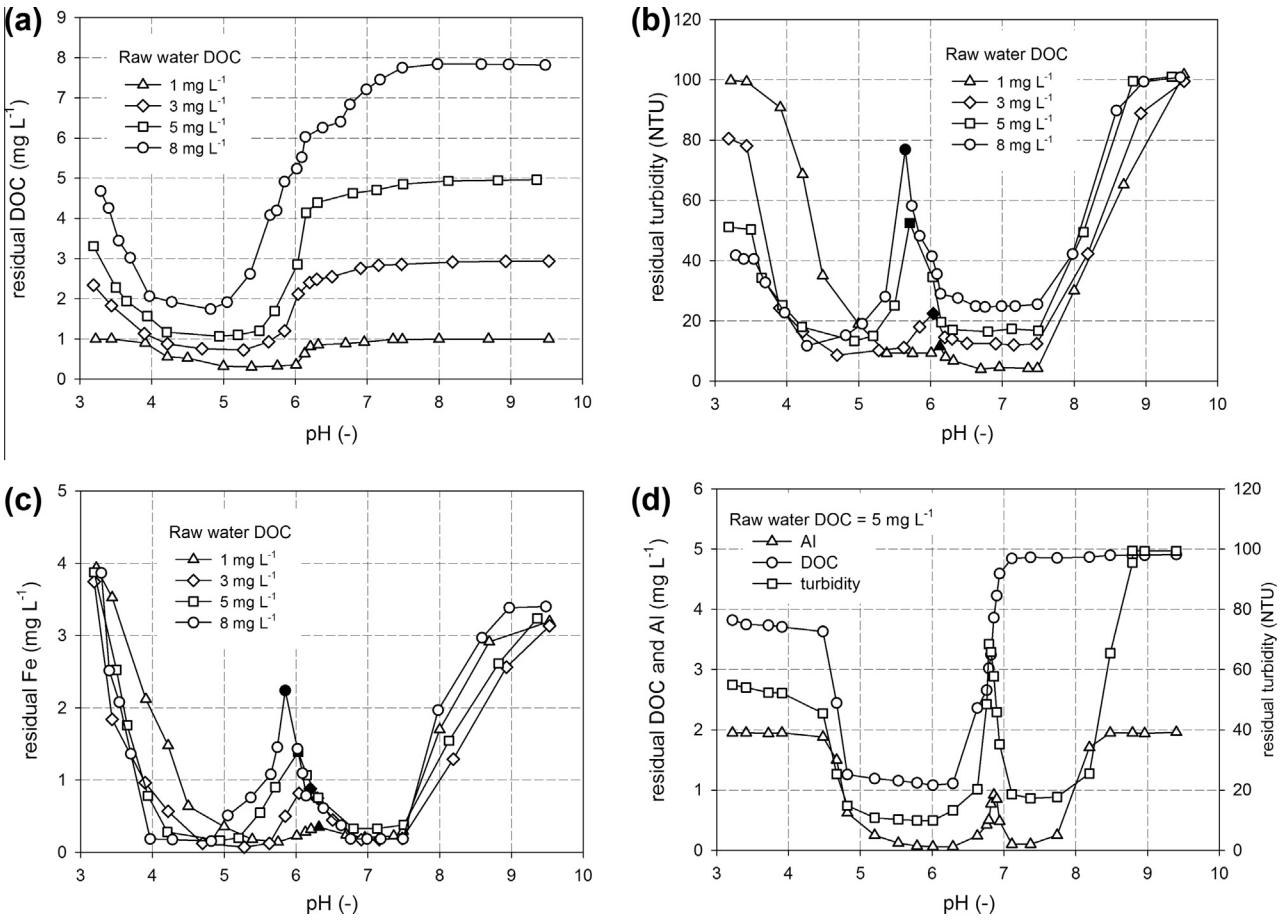

Fig. 3. Coagulation tests with Fe, COM peptides/proteins (initial DOC of 1, 3, 5 and 8 mg L^{-1}) and kaolin – dependence of residual DOC (a), turbidity (b) and Fe (c) on a pH value. (d) Coagulation tests with Al, COM peptides/proteins (initial DOC of 5 mg L^{-1}) and kaolin – dependence of residual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

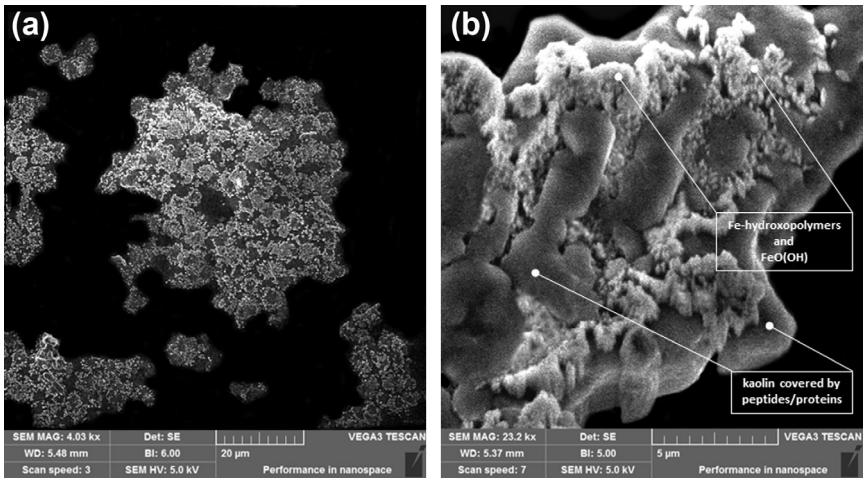

Fig. 4. SEM micrographs of aggregates formed through the interaction between Al/Fe, COM peptides/proteins and kaolin particles – complete (a) and detail (b) picture.

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

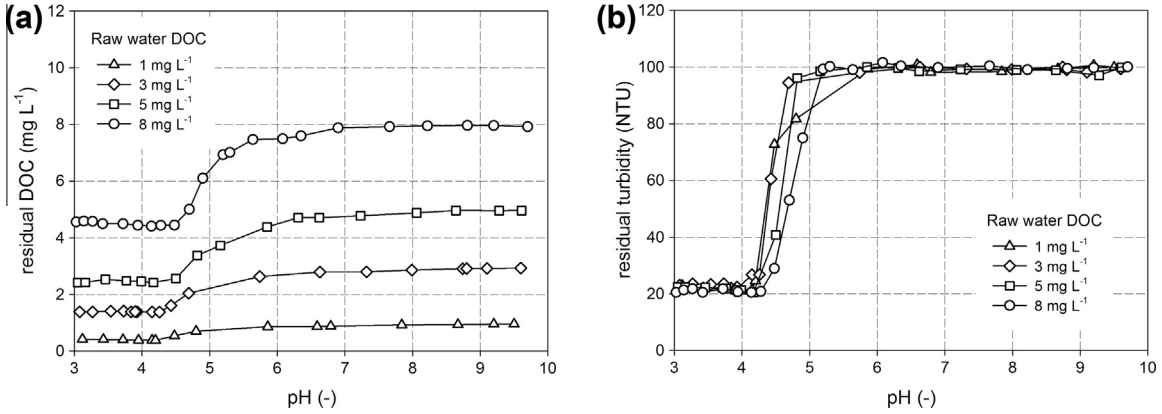

Fig. 5. Coagulation tests with COM peptides/proteins (initial DOC of 1, 3, 5 and 8 mg L^{-1}) and kaolin – dependence of residual DOC (a) and turbidity (b) on a pH value.

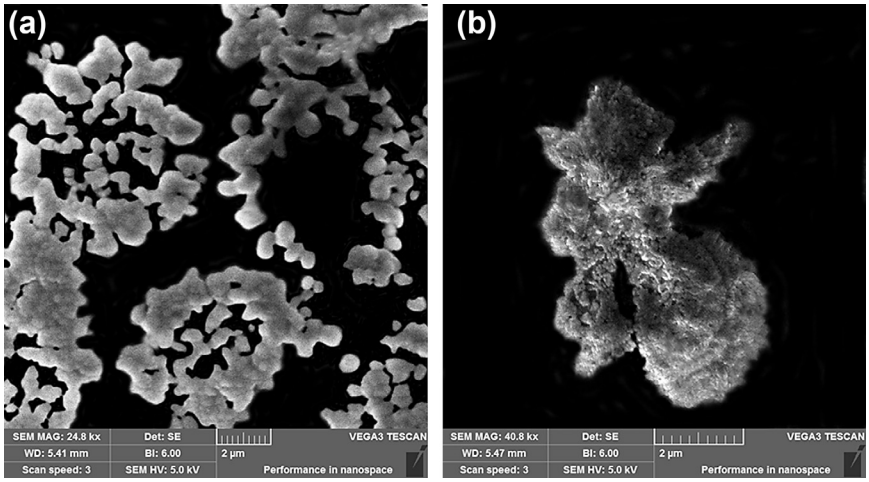

Fig. 6. SEM micrographs of aggregates formed through the interaction between COM peptides/proteins and kaolin particles (without Al/Fe) – complete (a) and detail (b) picture.

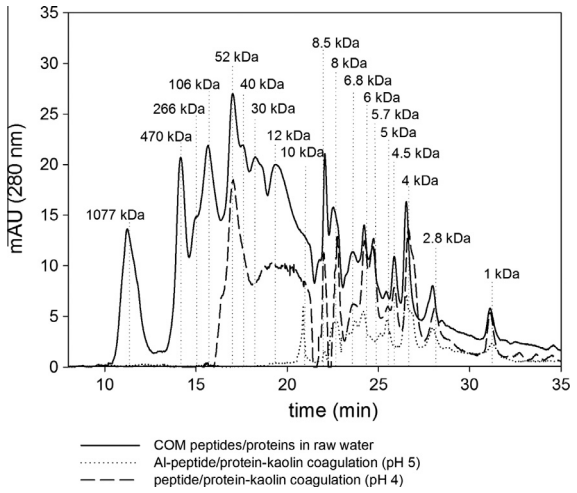

Fig. 7. Molecular weight distributions of COM peptides and proteins in raw water and residual peptides and proteins after coagulation test with: (1) Al, COM peptide/protein (initial DOC = 5 mg L^{-1}) and kaolin (the tests with Fe as a coagulant provided almost the same results; thus, only one of the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

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

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

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

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

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

3.6. Residual COM peptides/proteins

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

4. Conclusion

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

Acknowledgments

The research project has been funded by the Czech Science Foundation under the Project No. P105/11/0247. The authors acknowledge the financial assistance on this project.

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WATER RESEARCH 45 (2011) 3359–3368

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Adsorption of cellular peptides of *Microcystis aeruginosa* and two herbicides onto activated carbon: Effect of surface charge and interactions

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ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form
25 March 2011
Accepted 26 March 2011
Available online 6 April 2011

Keywords:
Cellular organic matter
Granular activated carbon
Molecular weight distribution
Surface charge
Cyanobacterial peptides

ABSTRACT

In this research, the adsorption of two herbicides, alachlor (ALA) and terbuthylazine (TBA), on granular activated carbon (GAC) in the presence of well-characterized peptide fraction of cellular organic matter (COM) produced by cyanobacterium *Microcystis aeruginosa* was studied. Two commercially available GACs were characterized using nitrogen gas adsorption and surface charge titrations. The COM peptides of molecular weight (MW) < 10 kDa were isolated and characterized using MW fractionation technique and high-performance size exclusion chromatography (HPSEC). The effect of surface charge on the adsorption of COM peptides was studied by means of equilibrium adsorption experiments at pH 5 and pH 8.5. Electrostatic interactions and hydrogen bonding proved to be important mechanisms of COM peptides adsorption. The adsorption of ALA and TBA on granular activated carbon preloaded with COM peptides was influenced by solution pH. The reduction in adsorption was significantly greater at pH 5 compared to pH 8.5, which corresponded to the increased adsorption of COM peptides at pH 5. The majority of the competition between COM peptides and both herbicides was attributed to low molecular weight COM peptides with MW of 700, 900, 1300 and 1700 Da.

1. Introduction

The use of pesticides represents a risk for water quality in agricultural areas. Many water treatment plants deal with elevated concentrations of pesticides in raw water, which have to be reduced below the established limit of the total amount of pesticides of 0.5 μg L^{-1} and 0.1 μg L^{-1} for any single pesticide in the European Union. Alachlor or 2-chloro-2,6-diethyl-N-methoxymethyl acetanilide has been one of the most commonly employed pre-emergence herbicide for crops. Terbuthylazine or 2-(tert-butylamino)-4-chloro-6-(ethylamino)-s-triazine belongs to triazine group of selective herbicides and it has been recently extensively used as a substitute for more toxic atrazine.

The removal of pesticides by conventional water treatment processes such as coagulation/flocculation/sedimentation or filtration is not effective (Miltner et al., 1989; Ormad et al., 2008). Meanwhile, the application of granular activated carbon (GAC) adsorbers is a very efficient technique for the removal of the pesticides from the water. Investigations on the application of GAC adsorbers for treatment of river water reported an alachlor removal efficiency of 75% (Badriyha et al., 2003). In the case of terbuthylazine, the lower efficiencies of about 60% were reported (Ormad et al., 2008). Triazines, owing to the presence of –NH– groups, are more hydrophilic compounds and their adsorption onto activated carbon can be obstructed (McCreary and Snoeyink, 1980).

Although the adsorption capacity of GAC adsorbers is sufficient under ideal conditions, in real water treatment systems the presence of natural organic matter (NOM), exemplified by humic substances, proteins and polysaccharides,

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doi:10.1016/j.watres.2011.03.051

could significantly reduce the efficiency of activated carbon process (Kilduff and Karanfil, 2002; Li et al., 2003a). NOM adsorbs on GAC and changes the surface properties to such extent that the trace organic compounds uptake and rate of adsorption is greatly reduced (Newcombe and Drikas, 1997). In fixed-bed GAC columns, the mass transfer zone of NOM components move down more rapidly than that of trace organic compounds due to slower adsorption kinetics of NOM. Consequently, the NOM preloads the carbon ahead of the trace organic pollutant. This results in reduced adsorption of trace organic compounds, due to direct competition for adsorption sites or pore blockage by NOM (Newcombe et al., 2002b; Li et al., 2003b). Direct site competition only happens in pores that are accessible to both NOM and the trace organic compound. Therefore, NOM components of low molecular weight (MW) compete in micropores, where the adsorption of trace organic compounds occurs. NOM molecules of higher MW adsorb in mesopores and do not compete for the same adsorption sites. In case there is not enough surface area available in mesopores, larger NOM molecules tend to cause pore blockage (Pelekani and Snoeyink, 1999).

Several factors influence the impact of preloading on activated carbon performance. One of the most important factors which determine the competitive adsorption is the pore size distribution of activated carbon relative to the molecular weight of adsorbates (trace organic compounds and NOM) (Quinlivan et al., 2005). This factor plays a crucial role particularly when the adsorption of organic molecules is governed by non-specific dispersive interactions (van der Waals interactions, hydrophobic interactions, hydrogen bonds) (Moreno-Castilla, 2004; Newcombe, 2006). Other important factors are chemical composition of NOM, surface functional group composition and surface charge of adsorbent (Newcombe and Drikas, 1997; Kilduff and Karanfil, 2002). Solution pH controls the (de)protonation of surface functional groups, which determines the surface charge of activated carbon pores and consequently the electrostatic interactions between the adsorbent and NOM molecules (Bjelopavlic et al., 1999). Ionic strength is the other key factor that controls the electrostatic interactions. Thus, these interactions, either attractive or repulsive, can be reduced by increasing the ionic strength of the solution. This is due to a shielding effect of the surface charge produced by the added salt. At certain conditions, the increase in ionic strength can improve adsorption by reduction of the intra- and intermolecular repulsions (Moreno-Castilla, 2004; Campinas and Rosa, 2006).

Until now, most attention has been given to the influence of NOM presented by humic and fulvic acids on the adsorption of trace organic pollutants (Newcombe et al., 2002b; Li et al., 2003b; Matsui et al., 2003; Quinlivan et al., 2005). A major problem for surface water purification is seasonal development of large amounts of phytoplankton, which is accompanied by a considerably increased concentration of algal organic matter (AOM) (Pivokonsky et al., 2006; Campinas and Rosa, 2010; Henderson et al., 2010). However, a review of literature revealed that no study has addressed the effect of AOM preloading on the adsorption of trace organic compounds.

The AOM is the result of metabolic activities of cyanobacteria and algae that produce extracellular organic matter (EOM) and, during decay, cellular organic matter (COM) (Takaara et al., 2007; Fang et al., 2010). The AOM is known to comprise proteins, peptides, polysaccharides, oligosaccharides, lipids, nucleic acids, amino acids and other organic acids (Hoyer et al., 1985; Leenheer and Croue, 2003) of which proteins and polysaccharides comprise the majority (Myklestad, 1995; Henderson et al., 2010). Consequently, the AOM composition can be characterized as protein and non-protein organic matter (Pivokonsky et al., 2006). In addition, under certain conditions of growth, cyanobacteria produce toxins as secondary metabolites which pose a significant health risk. The hepatotoxic and tumor-promoter microcystins are among the most commonly occurring cyanotoxins in surface water reservoirs used for water supply (Campinas and Rosa, 2006). The chemical purification of water by means of coagulation/flocculation is very sensitive to the outbreak of these substances which can cause severe problems (Bernhardt et al., 1985; Takaara et al., 2007). A previous study showed that COM from the decay of cyanobacterium *M. aeruginosa* were relatively difficult to remove by coagulation/flocculation processes. It was found that COM proteins of a higher molecular weight were removed more efficiently than COM proteins of lower molecular weight (Pivokonsky et al., 2009). Consequently, the residual fraction of COM proteins can affect adsorption of trace organic compounds such as pesticides in granular activated carbon adsorbers. In addition, water treatment plants usually have to deal with increased pesticides concentrations in the summer period, which also brings increased AOM concentrations during algal blooms in eutrophicated water.

In the present study, the competitive effect of the COM protein fraction on the adsorption of two herbicides (alachlor and terbuthylazine) was investigated at equilibrium. The protein fraction was studied in order to investigate the effect of carbon surface charge together with the charge of COM peptides on electrostatic interactions taking part in adsorption and consequent competitive effect on the adsorption of herbicides. Two chosen granular activated carbons were commercially available, designed for pesticides removal in the water treatment process. The equilibrium adsorption of both herbicides on carbons preloaded with the COM peptides of MW <10 kDa was studied. Carbons and the COM were extensively characterized in order to describe the effect of COM peptides preloading. The objectives of the study were to:

(i) elucidate the adsorption mechanism of COM peptides,
(ii) determine the influence of pH value on the competitive adsorption of herbicides and COM peptides,
(iii) identify the most likely competitive mechanism and components in this system.

2. Materials and methods

2.1. Adsorbents

The adsorbents were two commercial granular activated carbons, Filtrasorb 400 (Chemviron Carbon, USA) and Norit 1240 (Norit Americas Inc., USA). Both adsorbents were

bituminous coal-based carbons activated by steam. The sieve fraction between 0.8 and 1 mm was employed. As in Sotelo's et al. (2002) study, a full-scale diameter of GAC was used instead of a fine graded carbon, because only equilibrium adsorption experiments were performed, not kinetic experiments. Prior to experiments, the activated carbons were treated by extensive washing in ultrapure water, dried at 110 °C for 24 h and stored in a desiccator.

2.1.1. GAC characterization

Textural properties (specific surface area, micropore volume, total pore volume and mesopore surface area) of activated carbons were evaluated from 77 K nitrogen physical adsorption isotherms obtained with the volumetric instrument ASAP2020 (Micromeritics, USA). Before analysis, the samples were dried at 105 °C and 0.1 Pa for 24 h. The specific surface area (S_{BET}) was evaluated by BET method (Brunauer et al., 1938), micropore volume, (V_{micro}) and mesopore surface area (S_{meso}) by the t-plot method with Lecloux–Pirard master isotherm (Lecloux and Pirard, 1979) and pore size distribution by the advanced BJH method (Barret et al., 1951).

2.1.2. Surface charge determination

Surface charge determinations were undertaken by surface titrations using an Orion 960 Autotitrator (Thermo Scientific, USA). The carbon was titrated with 0.1 M HCl to pH 3, after the addition of 0.1 M NaOH. The titration was conducted at two electrolyte concentrations, 0.01 M and 0.3 M NaCl. A blank titration was also performed. Relative surface charge was determined from the difference between the surface titration curves and the blank curves. Relative surface charge was then plotted against pH. The pH at which the curves of two electrolyte concentrations crossed was the pH at which the absolute charge on the surface was zero (pH_{pzc}). This method was described in detail in the literature (Bjelopavlic et al., 1999).

2.2. Adsorbates

Alachlor (ALA) of 99,2% purity and terbuthylazine (TBA) of 98,8% purity were supplied by Sigma–Aldrich (USA). These herbicides present two common surface water contaminants. The structural formulas of ALA and TBA are shown in Fig. 1. ALA contains benzene ring, while TBA consists of triazine heterocycle and two amino groups. They have similar molecular weight (ALA 269.8 Da, TBA 229.7 Da) and both are relatively hydrophobic (aqueous solubility at 20 °C: ALA 148 mg L^{-1}, TBA 8.5 mg L^{-1}) (Badriyha et al., 2003; Bruzzoniti et al., 2006). The stock solutions of concentration 10 mg L^{-1} were prepared by adding a weighed amount of herbicides to ultrapure water without the assistance of organic solvent. Undissolved herbicide particles were removed by filtering the solution through a 0.22 μm membrane filter (Millipore, USA). Gas chromatography with electron capture detector (Agilent Technologies 6890N, USA) and a DB-XLB capillary column (30 m × 0.32 mm i.d. × 0.17 μm film thickness) (Agilent Technologies, USA) was used for the analysis of ALA and TBA. Target herbicides were extracted from water phase by SPE columns with C18 sorbent (Agilent SAMPLI Q C18, Agilent Technologies, USA) and eluted by acetone prior to analysis.

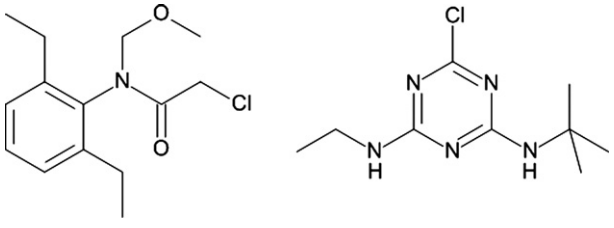

Fig. 1 – Molecular structures of alachlor (left) and terbuthylazine (right).

2.3. M. aeruginosa cultivation

The cyanobacterium *M. aeruginosa*, Kutz. (Zapomelova, 2006/2) was used in this study. Inoculum of this strain was obtained through the kind generosity of the Department of Culture Collection of Algal Laboratory, Institute of Botany, AS CR, Czech Republic. The strain of *M. aeruginosa* was cultured according to the methodology described in the literature (Pivokonsky et al., 2006, 2009). The culture of *M. aeruginosa* was harvested on the 16th day of cultivation during steady-state growth.

2.4. COM preparation

The COM samples were prepared by disruption of the microorganisms' cells, which were separated from the growth medium by a 0.22 μm membrane filter (Millipore, USA). The separated cells were stirred with ultrapure water (200 mL) and disrupted using an ultrasonic homogenizer (HD 3200, 20 kHz, 60W) for 20 min. The efficiency of cells destruction was confirmed by an optical microscope (Optech B4T, UK). The residual solids were removed by a 0.22 μm membrane filter, and the filtrates were concentrated tenfold in a rotary evaporator (Laborota 4000 HB/G1, Germany) at 30 °C. The concentrated COM was stored at −18 °C.

2.5. COM characterization

2.5.1. DOC analysis

Dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC-V_{CPH} analyzer (Shimadzu Corporation, Japan). All samples were first filtered through a 0.22 μm membrane filter (Millipore, USA). All measurements were conducted in triplicate and errors were less than 2%.

2.5.2. Determination and isolation of COM protein portion

The COM was characterized in terms of the amount of protein (DOC_P) and non-protein (carbohydrates) (DOC_{NP}) organic matter. Proteins were isolated from the COM using $(NH_4)_2SO_4$ as a protein precipitant. The protein precipitate was then separated from the dissolved organic matter by filtration through a 0.22 μm membrane filter (Millipore, USA), and DOC_{NP} was analyzed in the filtrate. The protein portion DOC_P was calculated as follows:

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

where DOC_P is the amount of protein DOC, DOC_T the total DOC of the COM, and DOC_{NP} the amount of non-protein (carbohydrate) DOC. The protein precipitations were carried out in

triplicate and errors in measurement of DOC_P were less than 5%. The protein precipitate was then dissolved in ultrapure water for the purpose of adsorption experiments.

2.5.3. *Molecular weight fractionation*

COM protein fraction was characterized in terms of molecular weight (MW) distribution. Centrifugation (4000 rpm, $T = 40$ min) was used to drive the COM protein fraction through Amicon Ultra-15 centrifugal filters of 100, 50, 30, 10 and 3 kDa NMWL (Millipore, USA). The MW distribution was expressed as DOC portion of each MW fraction. Each MW distribution was repeated in triplicate. The low MW fraction of COM peptides <10 kDa was collected as an experimental material for adsorption tests.

The MW fractionation by high-performance size exclusion chromatography (HPSEC) was performed using Agilent Bio SEC-5 100Å and 300Å (7.8 × 300 mm, 5 μm). The separation range applied was 100–1 250 000 Da using Bio SEC-5 100Å and Bio SEC-5 300Å columns in series. The HPLC system (Agilent 1100 series, Agilent Technologies, USA) was coupled with a diode array detector (DAD) operated at 280 nm. Prior to HPSEC, the maximum absorption wavelength ($\lambda_{max} = 280$ nm) of COM peptides <10 kDa was detected in UV–VIS absorbance spectra using a UV–VIS 8452A spectrophotometer (Agilent Technologies, USA). Therefore, the wavelength 280 nm was used especially for the COM peptides detection. The HPSEC mobile phase used for the MW fractionation was 0.05 M phosphate buffer (pH 7.0). The flow rate was 1 mL min^{-1} at the temperature of 23 °C and the sample injection volume was 60 μL. Data analysis was performed using Agilent Technologies Chemstation software (Agilent Technologies, USA). The system was calibrated using the following SEC standards (Sigma–Aldrich, USA): d-biotin (224 Da), cyanocobalamin (1.35 kDa), aprotinin (6.5 kDa), cytochrome c (12.4 kDa), carbonic anhydrase (29 kDa), albumin (66 kDa), alcohol dehydrogenase (150 kD), apoferritin (443 kDa) and thyroglobulin (670 kDa). A semi-log calibration curve was used to calculate the MW ($R^2 = 0.96$). BioRad gel filtration standards of chicken ovalbumin (44 kDa) and bovine gamma globulin (158 kDa) were used as control samples. Standard error was ±0.58 kDa for chicken ovalbumin and ±0.89 kDa for gamma globulin. Reproducibility of the MW fractionation of COM peptide samples was very good, with MW deviations of less than 3% from repeated measurements.

2.5.4. *Determination of protein isoelectric point*

The isoeletric point (p*I*) of isolated COM peptides <10 kDa was determined by isoelectric focusing (IEF) and was carried out with a Multiphor II electrophoresis system (Pharmacia, Sweden). The IEF gel (7.5%) was prepared using ampholines of p*I* 2.5–5.0 and 3.5–10.0 (Pharmacia, Sweden). A standard calibration curve with broad-p*I* protein calibration kit, p*I* 3.0–10.0 (Pharmacia, Sweden), was used to determine the isoelectric points. Gels were stained with silver staining kit (Bio-Rad Silver Stain, USA) and activity-stained with 0.005 M guaiacol.

2.6. *Equilibrium adsorption experiments*

Batch adsorption tests with ALA and TBA were conducted using fresh GAC and GAC preloaded with COM peptides of MW <10 kDa to determine the effect of COM peptides preloading on adsorption capacity of target herbicides on GAC. Synthetic water was prepared by herbicide stock solution and ultrapure water with alkalinity adjusted to 1.45 mmol L^{-1} by 0.125 M $NaHCO_3$. Different doses of GAC were agitated in 2 L borosilicate jars. In the case of the experiment with fresh GAC, the flasks were agitated on a magnetic stirrer for 7 days to reach adsorption equilibrium. Blanks were included to determine the initial target herbicide concentration. During COM peptides preloading experiments, different doses of fresh GAC were preloaded with COM peptides and agitated for 7 days. Initial DOC concentration of experimental solution was 6 mg L^{-1}. Furthermore, 50 mg L^{-1} of sodium azide was added to both solutions with fresh and preloaded GAC to eliminate biological activity and decomposition and to provide similar ionic strength of 12.8 mM. The experiments with preloaded GAC were undertaken at acidic and alkaline pH (pH 5.0 and 8.5) adjusted by 0.1 M NaOH and HCl. DOC samples were taken after preloading to determine the surface concentrations of COM peptides. Herbicide stock solution was then spiked into the experimental solution to obtain the initial concentration of 100 μg L^{-1} and the jars were agitated for the next 7 days to reach adsorption equilibrium. After equilibrium was reached, the GAC particles were separated by filtering the water samples through a 0.22 μm membrane filter (Millipore, USA) and the liquid-phase target herbicide concentration and DOC were measured. Afterward, the rest of the filtrates were concentrated using vacuum rotary evaporator (Laborota 4002 control, Heidolph, Germany) in order to obtain DOC concentration 100 mg L^{-1} for HPSEC determination.

The Freundlich model was used to describe the adsorption equilibrium:

$$q = K_f C_e^{1/n} \quad (2)$$

where q (μg mg^{-1}) and C_e (μg L^{-1}) represent equilibrium surface and solution concentrations, respectively, and K_f and $1/n$ are Freundlich parameters.

3. Results and discussion

3.1. *GAC characterization*

Table 1 provides information on the textural properties of the two carbons investigated. Both carbons had relatively similar surface properties, as expected for the same starting material (coal) and the steam activation method. Nevertheless, the microporosity (V_{micro}/V_{total}) of the carbon F400 was higher

Table 1 – Textural properties of granular activated carbons.

	N1240	F400
S_{BET} (m^2 g^{-1})	1110	1025
S_{meso} (m^2 g^{-1})	536	416
V_{total} (cm^3 g^{-1})	0.70	0.59
V_{micro} (cm^3 g^{-1})	0.29	0.30
V_{micro}/V_{total} (%)	41	50

than that of the N1240. It was 50% for the F400 and 41% for the N1240. Consequently, in terms of mesopores, the carbon N1240 had a higher mesopore surface, S_{meso} 536 $m^2\ g^{-1}$, compared to S_{meso} of F400, which was 416 $m^2\ g^{-1}$. This characteristic is important with regard to the pore blockage phenomenon. It was previously reported that large NOM molecules tend to cause pore blockage when there is not enough surface area available in mesopores (Pelekani and Snoeyink, 1999; Li et al., 2003a).

Net surface charge was determined by potentiometric titration, the results are shown in Fig. 2. The two investigated carbons displayed both positive and negative charge, depending on the pH. The carbon F400 had a pH_{pzc} of about 6.7, while N1240 was more acidic carbon with pH_{pzc} of about 5.5. Therefore, both carbons had a positive net charge at applied acidic conditions of pH 5 and negative net charge at basic pH 8.5 during adsorption experiments in this study. Negative surface charge of activated carbon is attributed to surface acidic oxygen groups, e.g. carboxyl, phenol, lactone and lactol (Newcombe, 2006). Whereas, positive surface charge is usually assigned to surface oxygen complexes of basic character like pyrones or chromenes, or to the existence of electron-rich regions within the graphene layers acting as Lewis basic centers, which accept protons from the aqueous solution and form H_30^+-π complexes (Moreno-Castilla, 2004). However, it is known that the titration method of surface charge determination measures net surface charge only; it is not possible from these results to determine the actual concentration of positive and negative sites.

3.2. COM characterization

The protein portion, determined as DOC_P, was measured at approximately 60.2% of DOC_T in the COM, and the non-protein portion organic matter made up the balance of 39.8%. The COM proteins were characterized in terms of MW distribution expressed as DOC portion of ultrafiltration fractions. Percentage distribution of the MW fractions of <3; 3-10; 10-30; 30-50; 50-100 and >100 kDa was 19.4; 1.0; 5.8; 9.9; 18.6 and 45.6%, respectively. The COM peptides fraction of MW <10 kDa, which was consequently used in equilibrium adsorption experiments, comprised 20.4 ± 3.7% of the whole COM protein portion. This fraction was chosen to represent the residual peptide fraction, which is not supposed to be aggregated and separated during chemical water treatment as was previously reported (Pivokonsky et al., 2009). Another study of Ebie et al. (2001) reported that the residual NOM after alum coagulation of a surface water displayed a range of molecular weights of approximately 1–5 kDa. Fig. 3 shows HPSEC chromatogram with apparent molecular weights of the isolated COM peptides <10 kDa. Peptides of MW of approximately 700, 900, 1300, 1700, 1900, 2300, 2700, 5300 and 6300 Da were identified as main components of this COM protein fraction. These peptides may include hepatotoxic microcystins. Since HPSEC is a size exclusion method, the molecular weight values should not be considered to be absolute values. The isoeletric points of isolated COM peptides <10 kDa were determined by isoelectric focusing (IEF). The measured values of peptides p*I* were 5.25; 5.45; 5.80; 6.10; 6.25; 7.15; 7.85; 7.95 and 8.05. The number of peptides p*I* identified by IEF corresponded to the number of peptides separated by HPSEC.

3.3. Adsorption of COM peptides

3.3.1. DOC removal

Adsorption experiments were conducted at two different GACs at pH 5 and pH 8.5. DOC concentrations were measured after a 7 day preloading. Initial DOC concentration of COM peptides was 6 mg L^{-1}. Fig. 4 shows the dissolved organic carbon removed as a function of carbon dose for the COM peptides <10 kDa. Adsorption isotherms were not used as the way of display for COM peptides adsorption because the experimental conditions resulted in a very narrow range of DOC solution and surface concentrations for the isotherms. DOC removal was approximately twice as high at pH 5 than at pH 8.5. Moreover, at pH 5, higher adsorption of COM peptides was seen on carbon N1240 compared to F400. It can be attributed to higher available pore volume of carbon N1240, see Table 1. The pore size distribution as well as the character and the MW distribution of the NOM has been found to be a major influence on the adsorption by several authors (Ebie et al., 2001; Kilduff and Karanfil, 2002; Li et al., 2003a). Some

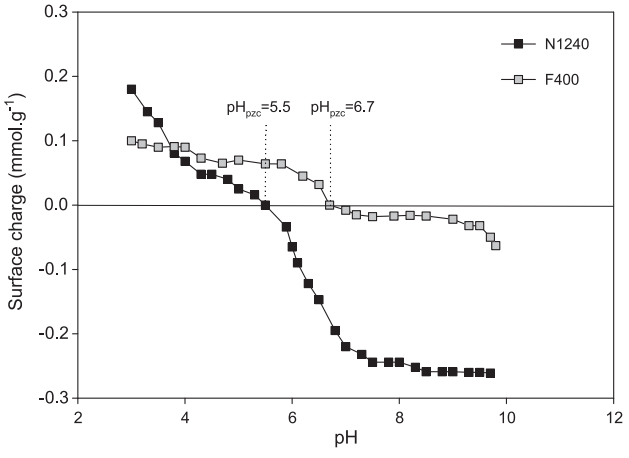

Fig. 2 – Surface charge versus pH of activated carbons (pHpzc = pH of point of zero charge).

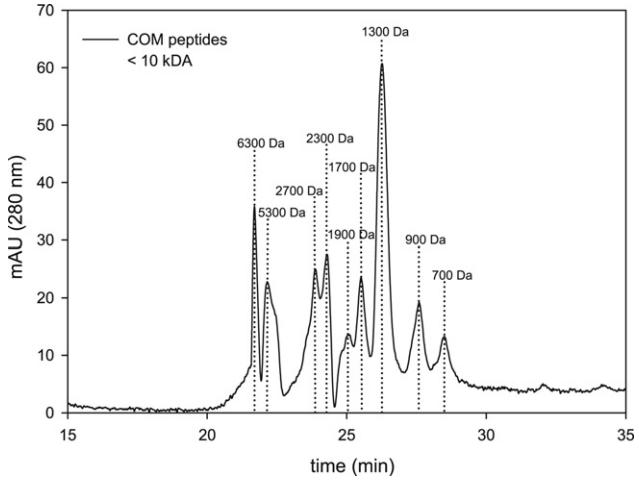

Fig. 3 – HPSEC chromatogram displaying apparent molecular weights of COM peptides <10 kDa.

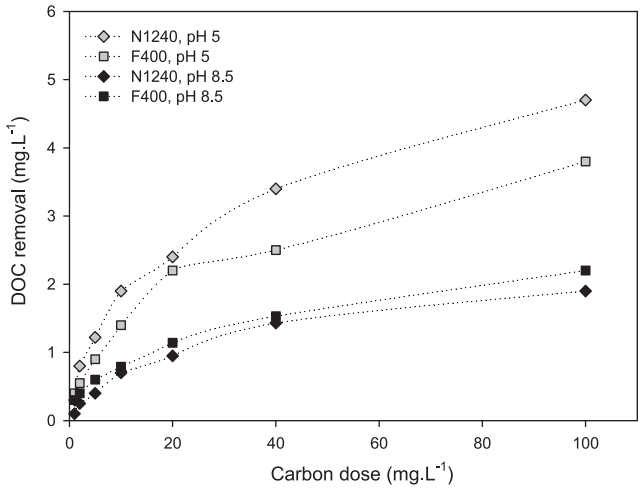

Fig. 4 – DOC removed as a function of carbon dose.

studies have also shown that the surface chemistry, in particular surface charge, of the activated carbon has a strong influence on the adsorption. Then, solution pH controls the charge and extent of (de)protonation of the adsorbate as well as the net charge of activated carbon pore surfaces (Knappe et al., 1998; Moreno-Castilla, 2004). In our study, at pH 5, the differences in total pore volume between studied carbons became apparent. This is in agreement with studies of Bjelopavlic et al. (1999) and Newcombe et al. (2002a) who reported consistent dependence of the adsorption of NOM on the pore volume of different carbons at acidic pH.

The result of IEF indicated that, at pH 5, the positive charge of COM peptides predominated. On the contrary, at pH 8.5, the COM peptides were negatively charged. It was due to their amphoteric character, as the range of determined peptides p*I* was 5.25–8.05. Similarly, at pH 5, the net charge of both GACs was positive and it was negative at pH 8.5, according to determined pH_{pzc} 5.5 for carbon N1240 and 6.7 for F400. The schema in Fig. 5 illustrates fundamental interactions at experimental conditions. Hydrophobic interaction is a major driving force of adsorption, but in case of the adsorption of proteins and peptides, the electrostatic interactions and hydrogen bonding are very important, so the adsorption is controlled by the surface charge (Burns et al., 1996; Yoon et al., 1999). During adsorption experiments at pH 5, the pH was lower than determined p*I* values of COM peptides. Increased adsorption onto both carbons at pH 5 (Fig. 4) can be explained by formation of hydrogen bonds between protonated functional groups of COM peptides and protonated surface groups of carbon. On the other hand, at pH 8.5, COM peptides were negatively charged due to their p*I* values (5.25–8.05). Therefore, the repulsive electrostatic interactions between deprotonated carboxyl groups of COM peptides and deprotonated surface acidic oxygen groups of the carbons reduced adsorption significantly. A similar concept of protein-surface interactions was described in several literature (Yoon et al., 1999; Zhou et al., 2007; Katiyar et al., 2010). This effect became more apparent in case of the carbon N1240. The extent of electrostatic and hydrogen bonding contribution to the adsorption is governed by the densities of surface acidic oxygen groups, such as carboxyl groups. The surface of the carbon N1240 is expected to contain more acidic oxygen groups than F400 due to its lower pH_{pzc} (Fig. 2), consequently, the effect of the repulsive electrostatic interactions can be more pronounced.

In this study, adsorption experiments were conducted only at low ionic strength (IS = 12.8 mM). However, a shielding effect of increased ionic strength diminishing electrostatic repulsions between carbon surface and adsorbate as well as lateral repulsions between NOM molecules was described in literature (Bjelopavlic et al., 1999; Campinas and Rosa, 2006). Since COM peptides carried similar charges in relation to GACs surface, positive at pH 5 and negative at pH 8.5, and all adsorption experiments were performed at high surface concentrations (22–300 mg g^{-1} F400, 19–395 mg g^{-1} N1240), an enhanced adsorption by shielding effect would be expected by increase in ionic strength.

3.3.2. *Change in MW distribution*

Chromatograms on Fig. 6 compare MWs of remaining COM peptides after adsorption by different doses of F400 and N1240

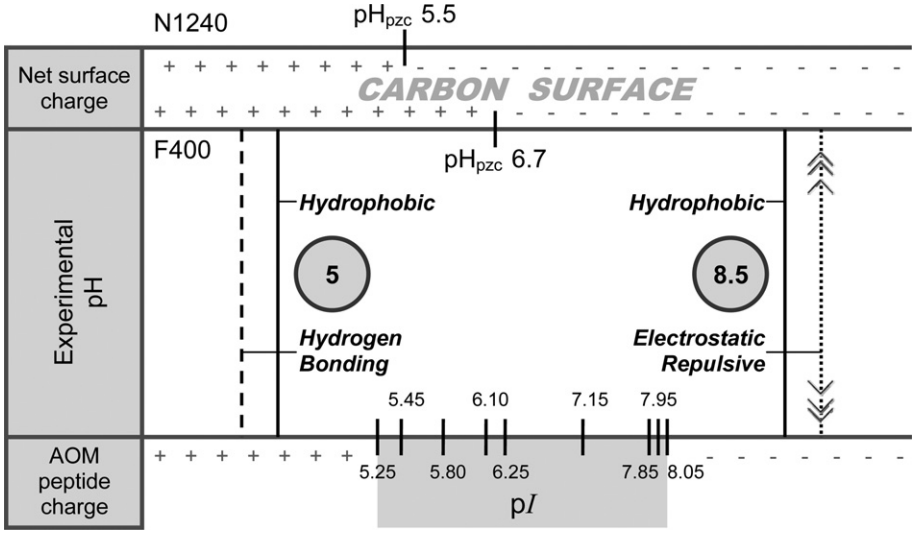

Fig. 5 – Fundamental interactions involved in COM peptides adsorption at experimental conditions.

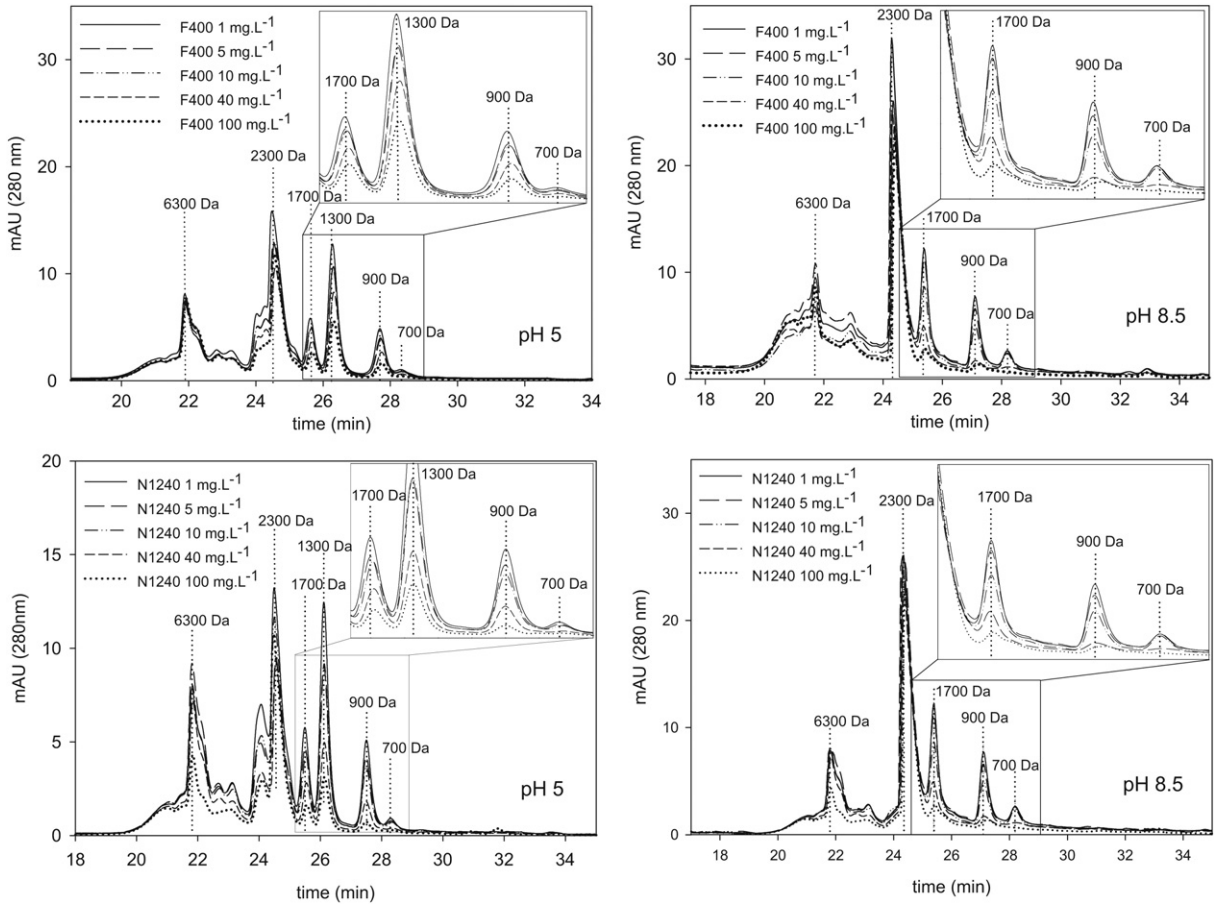

Fig. 6 – Molecular weight distributions of remaining COM peptides after adsorption on F400 and N1240 at different GAC doses at pH 5 and pH 8.5.

at pH 5 and pH 8.5. The results of MW distribution of remaining COM peptides in solution after adsorption onto F400 and N1240 were similar for both studied carbons. This was presumably caused by the fact that the textural properties of both carbons, in terms of microporosity and mesopore surface, were not significantly different to affect MW distribution. COM peptides of MW 700, 900, 1300 and 1700 Da were adsorbed proportionally to carbon doses. Even with high carbon doses, a negligible amount of COM peptides with MW higher than 2700 Da was adsorbed. With 40 mg L^{-1} of F400, almost all COM peptides with MW of 700, 900 and 1700 Da were removed at pH 8.5. Similarly, with 100 mg L^{-1} of F400, a significant amount of COM peptides with MW of 1700, 1300, 900 and 700 Da was removed at pH 5. These results demonstrated that among COM peptides <10 kDa, the low MW peptides were preferentially adsorbed. This observation is similar to conclusions of authors who studied adsorption of different NOM fractions (Ebie et al., 2001; Li et al., 2003a). However, at pH 8.5, the COM peptides of the lowest MW (<1700 Da) were adsorbed more quantitatively compared to pH 5 (Fig. 6). With regard to the fact that the overall adsorption of COM peptides was higher at pH 5 (Fig. 4), this could be caused by the pore blockage phenomenon. At pH 5, the higher adsorption of high MW COM peptides in mesopores could block micropore openings and restrict their accessibility for low MW COM peptides.

3.4. *Adsorption of herbicides*

Isotherm tests were conducted with ALA and TBA in COM-free water using both GACs N1240 and F400. The initial concentrations of both herbicides used were 100 μg L^{-1}. The adsorption capacity of the carbon N1240 was higher for both herbicides which can be related to the total pore volume. This corresponded to the Freundlich adsorption parameters for the 7 day single-solute herbicide isotherms which are summarized in Table 2. Moreover, the obtained K_f parameters for both carbons indicated that the adsorption capacity for ALA was up to tenfold higher in comparison with TBA during adsorption experiments. With regard to the similar molecular weight of ALA and TBA, this can be attributed to the different chemical

Table 2 – Freundlich parameters for ALA and TBA adsorption onto fresh GACs.

Adsorbate	GAC	K_f (μg/mg)(L/μg)$^{1/n}$	1/*n*
ALA	N1240	29.4	0.94
	F400	12.6	0.93
TBA	N1240	4.9	0.29
	F400	2.7	0.36

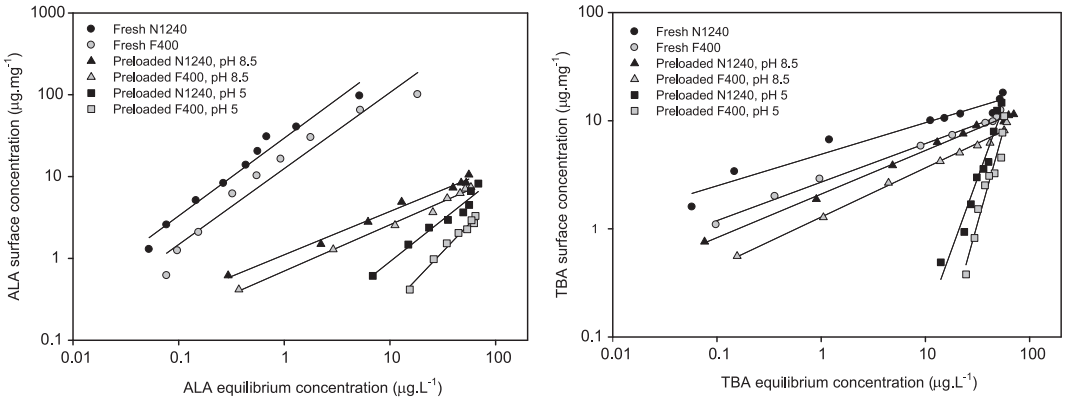

Fig. 7 – Adsorption isotherms of ALA and TBA onto two GACs in the absence and presence of competing COM peptides at pH 5 and pH 8.5.

structure of these herbicides. Attractive interactions between delocalized π electrons of aromatic rings, contained in ALA structure, and π electrons of polyaromatic basal planes of the carbon have been reported by several authors (Newcombe and Drikas, 1997; Moreno-Castilla, 2004). On the other hand, TBA contains two amino groups, which can obstruct adsorption onto activated carbon due to their hydrophilic character (McCreary and Snoeyink, 1980).

Equilibrium adsorption experiments with ALA and TBA were also performed onto carbons preloaded with COM peptides <10 kDa at pH 5 and pH 8.5. The impact of COM peptides preloading on TBA and ALA adsorption at pH 5 and pH 8.5 is shown by the isotherms plotted in Fig. 7. The corresponding Freundlich parameters are not shown since the Freundlich model did not fit the adsorption isotherms obtained for carbons preloaded by COM peptides (particularly at pH 5). ALA and TBA uptake by preloaded carbons was lower than uptake by fresh carbons. The reduction in adsorption was significantly greater for ALA than for TBA. The difference between the adsorption of ALA and TBA onto preloaded carbons is negligible compared to the difference in adsorption capacity of both herbicides onto fresh carbons. Moreover, the reduction in adsorption was significantly greater at pH 5 compared to pH 8.5, which corresponded to the increased adsorption of COM peptides at pH 5. Probable explanation of greater impact of COM peptides preloading on ALA adsorption is that COM peptides adsorbed to the oxygen functional groups by hydrogen bonding became secondary adsorption centers, which retained other COM peptides. This reduced the accessibility of the aromatic ring of ALA to the hydrophobic parts of the carbon surface. Since, the major adsorption driving forces of ALA are π–π dispersion interactions between the aromatic ring of ALA and the aromatic structure of the graphene layers.

According to HPSEC chromatograms visualized in Fig. 6, the COM peptides of 700, 900, 1300 and 1700 Da were identified as the most relevant components in competition for adsorption sites with ALA and TBA. The mechanism of direct site competition of low molecular weight NOM components was reported in several studies with different MW fractions of NOM (Pelekani and Snoeyink, 1999; Ebie et al., 2001). On the contrary, COM peptides with MW of 2300 and 6300 Da which were not quantitatively adsorbed proportionally to carbon doses are supposed to cause pore blockage effect, since both carbons in this study represented relatively microporous carbons. This is consistent with the study of Newcombe et al. (2002a, 2002b), who reported that the higher molecular weight compounds restricted access to the adsorption sites for smaller NOM compounds in microporous carbons, while access was not restricted in mesoporous carbons.

4. Conclusions

This study investigated the competitive adsorption on granular activated carbons between two herbicides (ALA and TBA) and COM peptide fraction <10 kDa, which represented the hardly separable COM protein fraction during chemical water treatment. Moreover, the effect of surface charge on the adsorption of COM peptides was studied at pH 5 and pH 8.5. The extent of electrostatic and hydrogen bonding contribution to the adsorption was also investigated.

Electrostatic interactions and hydrogen bonding proved to be important mechanisms of COM peptides adsorption. The solution pH controlled the charge of peptides, as well as the net charge of activated carbon pore surfaces. The extent of electrostatic repulsion could be predicted by peptides characterization by means of p*I* and by the determination of net surface charge of used carbon. At acidic pH, the formation of hydrogen bonds between protonated acidic oxygen groups of peptides and carbon surface contributes to the adsorption.

The adsorption of TBA and ALA onto activated carbon was influenced by their different chemical structures. The impact of COM peptides preloading on herbicides adsorption was significantly greater for ALA in comparison with TBA. As expected, the reduction in adsorption was significantly greater at pH 5 compared to pH 8.5, which corresponded to the increased adsorption of COM peptides at pH 5. Adsorbed COM peptides were supposed to become secondary adsorption centers which could consequently prevent the migration of herbicides to a large portion of carbon surface. The low molecular weight COM peptides were adsorbed to a greater extent than the larger peptides, due to size exclusion effects. The majority of the competition between COM peptides and both herbicides can be attributed to COM peptides of MW from

700 to 1700 Da, where the hepatotoxic microcystins may be included.

It is generally acknowledged that the pore size distribution plays a major role in the adsorption of NOM onto activated carbon. Nevertheless, the adsorption of charged COM peptides showed the influence of electrostatic effects related to the charge of the adsorbate and of the carbon surface. The selection of the carbon with broad pore size distribution can lead to a higher removal of micropollutants coexistent with COM in drinking water sources. Moreover, the knowledge of surface charge dependence on solution pH is fundamental for the selection of the proper activated carbon.

Acknowledgments

The research project has been funded by the Grant Agency of AS CR under the project No. IAA200600902, by the Czech Science Foundation under the project No. P105/10/P515 and Institutional Research Plan No. AVOZ20600510. The authors acknowledge the financial assistance on this project.

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Conference contribution published in Proceedings of the Conference "6th IWA International Conference for Young Water Professionals - IWA YWPC 2012", 10-13 July, 2012, Budapest, Hungary.

Effect of cellular peptides of *Microcystis aeruginosa* on granular activated carbon adsorption of herbicides

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Abstract

This study is aimed at the adsorption of organic micropollutants onto granular activated carbon (GAC) and the effect of cellular organic matter (COM) produced by *Microcystis aeruginosa* on this process. Adsorption experiments were performed for two herbicides with distinct chemical structure (alachlor and terbuthylazine) and two types of GAC with different surface charge (Norit 1240 and Filtrasorb 400). Both adsorbents were used fresh and preloaded with COM peptides of molecular weight < 10 kDa at pH 5 and pH 8.5. It was found that the adsorption of COM peptides was higher at pH 5 compared to pH 8.5. Adsorption of ALA and TBA onto preloaded carbons was lower than adsorption onto fresh carbons. Significantly lower efficiency of ALA and TBA adsorption was observed at pH 5, which corresponded to greater adsorption of COM peptides at acidic pH. The peptides of MW 700, 900, 1300 and 1700 Da were identified as the COM components responsible for competition with herbicides.

Keywords

Activated carbon; adsorption; cellular peptides; herbicides; *Microcystis aeruginosa*

INTRODUCTION

Herbicides constitute a major class of synthetic organic pollutants especially in agricultural areas. Due to their broad application and specific physico-chemical properties they are commonly found in surface waters, including water sources used to supply water treatment plants (Badriyha et al., 2003). Adsorption onto granular activated carbon (GAC) is one of the most suitable technologies for removing health dangerous herbicides from drinking water. Unfortunately, the adsorption capacity and the effectiveness of GAC adsorbers can be negatively affected by the competitive adsorption of natural organic matter (NOM) (Ebie et al., 2001; Li et al., 2003a), since NOM is usually present in raw water in much higher concentration ($mg.L^{-1}$) than problematic herbicides ($\mu g.L^{-1}$) (Bjelopavlic et al., 1999). NOM can generally compete through two different ways according to its molecular weight (MW). Direct competition for adsorption sites is attributed to compounds with MW similar to that of target micropollutants which can enter the same pores (Newcombe et al., 2002a; Quinlivan et al., 2005). Larger NOM can decrease adsorption due to pore blockage when it tends to adsorb in the entrance to the smaller pores (Pelekani and Snoeyink, 1999). Generally, NOM includes humic substances and algal organic matter (AOM). AOM can be further divided into extracellular organic matter (EOM) produced by metabolic processes of algae and cyanobacteria, and cellular organic matter (COM) released during decay of their cells (Hoyer et al., 1985; Takaara et al., 2007). In terms of composition, AOM can be characterized as protein and non-protein organic matter (carbohydrates) (Pivokonsky et al., 2006; Henderson et al., 2010).

The main objective of this study was to evaluate the competitive adsorption between herbicide alachlor (ALA) and terbuthylazine (TBA) and COM produced by *Microcystis aeruginosa*. For this purpose, COM peptides with MW < 10 kDa were chosen, since the previous study showed that this fraction was relatively difficult to remove by coagulation/flocculation processes (Pivokonsky et al., 2009) and can be expected to affect adsorption of herbicides. Additional objectives were to reveal the effect of solution pH and consequent surface charge conditions on GAC adsorption.

MATERIALS AND METHODS

Adsorbents

Two granular activated carbons were selected for this study, namely Filtrasorb 400 (Chemviron Carbon, Belgium) and Norit 1240 (Norit Americas Inc., USA), referred hereafter as F400 and N1240. Adsorbents were washed with ultrapure water to remove ash, dried at 110 °C overnight and stored in a desiccator until use.

Textural characterization. Textural properties (specific surface area, mesopore surface area, total pore volume and micropore volume) of carbons were evaluated from 77 K nitrogen physical adsorption isotherms obtained with the volumetric instrument ASAP2020 (Micromeritics, USA). The specific surface area (S_{BET}) was evaluated by the BET method (Brunauer et al., 1938), mesopore surface area (S_{meso}) and micropore volume (V_{micro}) by the t-plot method with Lecloux-Pirard master isotherm (Lecloux et al., 1979) and pore-size distribution by the advanced BJH method (Barrett et al., 1951).

Surface charge determination. Surface charge of adsorbents was determined by potentiometric titration using an Orion 960 Autotitrator (Thermo Scientific, USA) according to the methodology of Bjelopavlic et al. (1999).

Adsorbates

Alachlor (ALA) of 99.2 % purity and terbuthylazine (TBA) of 98.8 % purity were supplied by Sigma-Aldrich (USA). These herbicides present common surface water contaminants. ALA contains benzene ring, while TBA consists of triazine heterocycle and two amino groups. They have similar molecular weight (ALA 269.8 Da, TBA 229.7 Da) and both are relatively hydrophobic (aqueous solubility at 20 °C: ALA 148 $mg.L^{-1}$, TBA 8.5 $mg.L^{-1}$). Gas chromatography with electron capture detector (Agilent Technologies 6890N, USA) and a DB-XLB capillary column (30 m × 0.32 mm i.d. × 0.17 µm film thickness) (Agilent Technologies, USA) was used for the analysis of ALA and TBA. Target herbicides were extracted from water phase by SPE columns with C18 sorbent (Agilent SAMPLI Q C18, Agilent Technologies, USA) and eluted by acetone prior to analysis.

COM preparation

The cyanobacterium *M. aeruginosa*, Kutz. (Zapomelova 2006/2) supplied by the Institute of Botany (Acad. Sci. Czech Republic) was used in this study. The cellular organic matter (COM) samples were prepared according to the methodology described elsewhere (Pivokonsky et al., 2006, 2009).

COM characterization

DOC analysis. Dissolved organic carbon (DOC) was analyzed using a Shimadzu TOC-V_{CPH} analyzer (Shimadzu Corporation, Japan). All samples were filtered through a 0.22 μm membrane filter (Millipore, USA).

Determination and isolation of COM protein portion. The COM was characterized in terms of the amount of protein (DOC_P) and non-protein (carbohydrates) (DOC_{NP}) organic matter. Proteins were isolated from COM using $(NH_4)_2SO_4$ as a protein precipitant. The protein precipitate was separated from the dissolved organic matter by filtration through a 0.22 μm membrane filter (Millipore, USA), and DOC_{NP} was analyzed in the filtrate. The protein portion DOC_P was calculated as follows:

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

where DOC_P is the amount of protein DOC, DOC_T the total DOC of the COM, and DOC_{NP} the amount of non-protein (carbohydrates) DOC.

Molecular weight fractionation. COM protein fraction was characterized in terms of molecular weight (MW) distribution. Centrifugation (4000 rpm, *T* = 40 minutes) was used to drive the COM proteins through Amicon Ultra-15 centrifugal filters of 100, 50, 30, 10 and 3 kDa NMWL (Millipore, USA). The MW distribution was expressed as DOC portion of each MW fraction. The low MW fraction of COM peptides < 10 kDa was collected as an experimental material for adsorption tests. The MW fractionation by high performance size exclusion chromatography (HPSEC) was performed using Agilent Bio SEC-5 100 Å and 300 Å (7.8 × 300 mm, 5 μm) columns in series (separation range 100 – 1 250 000 Da). The HPLC system (Agilent 1100 series, Agilent Technologies, USA) was coupled with a diode array

detector (DAD) operated at 280 nm. The data analysis was performed using Agilent Technologies Chemstation software (Agilent Technologies, USA). The details of methodology are described in the literature (Pivokonsky et al., 2006).

Determination of isoelectric point. The isoeletric point (p*I*) of COM peptides < 10 kDa was determined by isoelectric focusing (IEF) and was carried out with a Multiphor II electrophoresis system (Pharmacia, Sweden). The IEF gel (7.5 %) was prepared using ampholines of p*I* 2.5-5.0 and 3.5-10.0 (Pharmacia, Sweden). A standard calibration curve with broad-p*I* protein calibration kit, p*I* 3.0-10.0 (Pharmacia, Sweden), was used to determine the isoelectric points. Gels were stained with silver staining kit (Bio-Rad Silver Stain, USA) and activity-stained with 0.005 M guaiacol.

Adsorption experiments

Batch adsorption tests with ALA and TBA (initial concentration 100 $\mu g.L^{-1}$) were conducted with fresh GAC and GAC preloaded with COM peptides of MW < 10 kDa. Synthetic water was prepared by herbicide stock solution and ultrapure water (alkalinity adjusted to 1.45 $mmol.L^{-1}$). Sodium azide was added to eliminate the biological activity. In the case of experiment with fresh GAC, the jars were agitated for 7 days to reach adsorption equilibrium. During preloading tests at pH 5 and pH 8.5, fresh GAC was agitated with COM peptides (initial DOC 6 $mg.L^{-1}$) for 7 days. DOC samples were taken after preloading to determine the surface concentration of COM peptides. Herbicide stock solution was then spiked into the solution to obtain the initial concentration of 100 $\mu g.L^{-1}$ and the jars were agitated for the next 7 days. After that the liquid-phase herbicide concentration and DOC were measured. The rest of the filtrates was concentrated using a vacuum rotary evaporator (Laborota 4002 control, Heidolph, Germany) prior to HPSEC analysis.

RESULTS AND DISCUSSION

Adsorbent characterization

The main characteristics of selected GACs, such as the type of precursor and activation agent, specific surface area (S_{BET}), mesopore surface area (S_{meso}), total pore volume (V_{total}), micropore volume (V_{micro}), microporosity (V_{micro}/V_{total}) and the pH of the point of zero charge

(pH_{pzc}) are listed in Table 1. Regarding S_{BET} and V_{micro}, adsorbents showed almost negligible differences. On the other hand, they differed in microporosity (41 % for N1240 vs. 50 % for F400) and S_{meso} which was higher for N1240 (536 $m^2.g^{-1}$) than for F400 (416 $m^2.g^{-1}$). The results of several studies demonstrated that larger NOM molecules were preferentially adsorbed in mesopores. Higher portion of mesopores in adsorbent can reduce the blockage of micropores. Therefore, these small pores stay attainable for micropollutants (Pelekani and Snoeyink, 1999; Ebie et al., 2001).

Table 1. The characteristics of adsorbents

General characteristics	F400	N1240
producer	Chemviron Carbon, Belgium	Norit Americas Inc., USA
precursor	bituminous coal	bituminous coal
activation agent	steam	steam
form	granular	granular
Textural properties		
S_{BET} ($m^2.g^{-1}$)	1025	1110
S_{meso} ($m^2.g^{-1}$)	416	536
V_{total} ($cm^3.g^{-1}$)	0.59	0.70
V_{micro} ($cm^3.g^{-1}$)	0.30	0.29
(V_{micro}/V_{total}) (%)	50	41
Surface charge		
pH_{pzc}	6.7	5.5

The values of pH_{pzc} mentioned in Table 1 were determined by potentiometric titration. It is known that at pH lower than pH_{pzc}, activated carbon displays overall positive charge and at pH higher than pH_{pzc}, the overall charge is negative. The negative charge results from the dissociation of surface oxygen complexes of acid character such as carboxyls, phenols or lactones. The positive charge is usually assigned to basic surface complexes like pyrones or chromenes, to nitrogen functionalities or to the existence of electron-rich regions within the graphene layers which accept protons from the solution (Moreno-Castilla, 2004). The value of pH_{pzc} for N1240 was 5.5 and 6.7 for F400. The lower pH_{pzc} of N1240 indicated the higher portion of acidic oxygen groups present on its surface.

COM characterization

DOC analysis of COM samples showed that 60.2 % of DOC_T was composed of protein components (DOC_P) and the rest 39.8 % was made up of non-protein components (DOC_{NP}).

According to the fractionation using Amicon Ultra-15 Centrifugal filters, it was found that COM peptides with MW < 10 kDa represented 20.4 % of the whole COM protein portion. Complete percentage distributions of all MW fractions were as follows: > 100 kDa 45.6 %; 50-100 kDa 18.6 %; 30-50 kDa 9.9 %; 10-30 kDa 5.8 %; 3-10 kDa 1.0 % and < 3 kDa 19.4 %. The HPSEC analysis of COM peptides < 10 kDa proved the presence of peptides with MW of 700, 900, 1300, 1700, 1900, 2300, 2700, 5300 and 6300 Da. Their corresponding p*I* values were 5.25; 5.45; 5.80; 6.10; 6.25; 7.15; 7.85; 7.95 and 8.05.

Adsorption of COM peptides of *Microcystis aeruginosa*

Adsorption experiments at pH 5 and pH 8.5 were conducted with COM peptides of MW < 10 kDa (initial DOC 6 $mg.L^{-1}$). This fraction of COM was selected to represent hardly separable residual dissolved organic matter remaining in a coagulation pre-treated drinking water (Pivokonsky et al., 2006). Residual DOC concentrations were measured after a 7-day preloading. The dependence of solution DOC on a carbon dose is displayed in Figure 1. Generally, the higher adsorption of COM peptides was seen on carbon N1240 compared to F400. It can be attributed to higher available pore volume and mesopore surface area of carbon N1240, see Table 1. The results also showed the overall lower adsorption of target COM peptides at basic (pH 8.5) compared to acidic conditions (pH 5). The solution pH influences the surface charge of both adsorbents as well as the charge of COM peptides due to their amphoteric character (Moreno-Castilla, 2004). As the determined range of p*I* was 5.25-8.05, the COM peptides showed prevailing positive charge at pH 5 and negative charge at pH 8.5. The same situation occurred in the case of both adsorbents. According to their pH_{pzc} (see Table 1), they were positively charged at pH 5 and negatively charged at pH 8.5. Thus, the decreased adsorption of COM peptides < 10 kDa at pH 8.5 could be caused by the strong repulsive electrostatic interactions between negatively charged functional groups of COM peptides (eg. carboxyls) and deprotonated surface groups of the carbons. On the contrary, increased adsorption of COM peptides at pH 5 could be caused by the formation of hydrogen bonds between protonated surface groups of carbon and functional groups of COM peptides. As a result, the hydrophobic interactions, which are the major driving force of adsorption, were intensified. A similar concept of protein-surface interactions was described in the literature (Burns et al., 1996; Yoon et al., 1999).

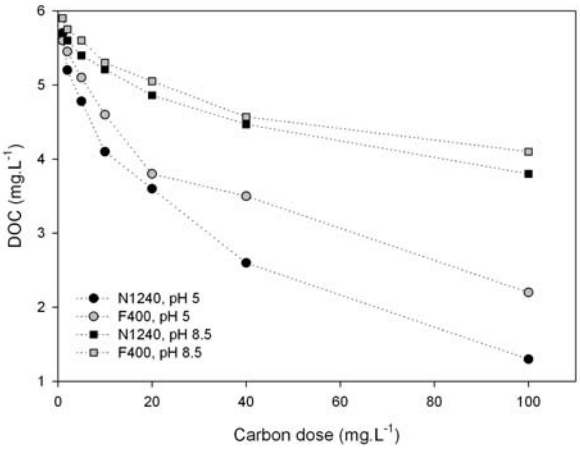
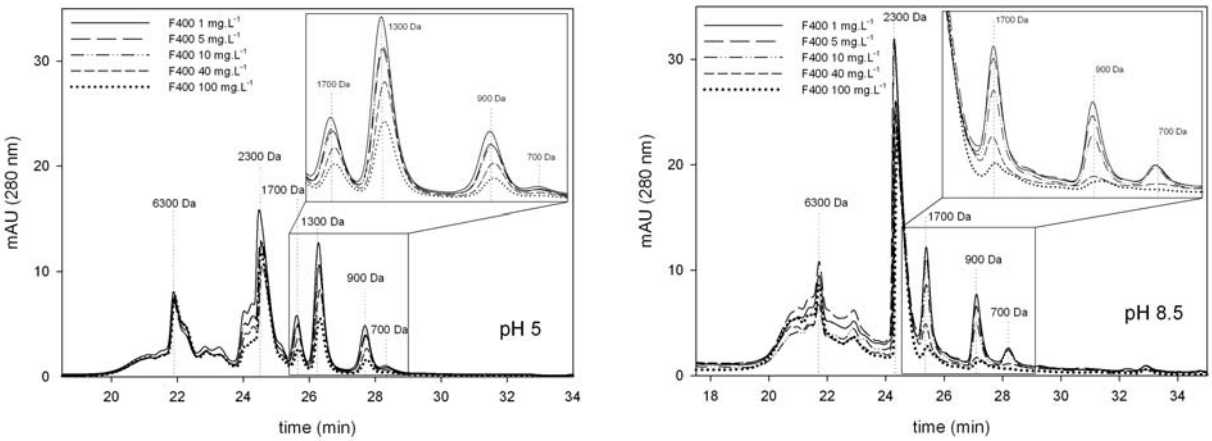


Figure 1. The relationship between solution DOC and carbon dose

HPSEC analysis

Figure 2 a,b shows the HPSEC plots of apparent MW distribution of COM peptides < 10 kDa after adsorption at different pH. The final MW distributions of remaining COM peptides were similar for both carbons. Apparently, the slight differences in textural properties of both carbons were probably not able to affect MW distributions to such extent that there were noticeable changes in chromatograms. The results obtained on F400 were depicted as a representative example.



a) **b)**

Figure 2 a, b. The HPSEC chromatograms of COM peptides remaining after adsorption on F400 at a) pH 5 and b) pH 8.5

The HPSEC analysis indicated that the adsorbents removed a range of MW compounds. However, evident removal proportional to GAC dose was seen only for COM peptides with low MW of 700, 900, 1300 and 1700 Da. As shown in Figure 2 a,b, only a small portion of

COM peptides of MW > 2300 Da was adsorbed. These could be probably organic molecules that had poor affinity for the carbon surface (Li et al., 2003a, 2003b) or there was not enough appropriate surface area available (Pelekani and Snoeyink, 1999).

Adsorption of herbicides

Figure 3 illustrates the single solute isotherms for ALA and TBA onto fresh adsorbents. Up to tenfold higher capacity was obtained for ALA than TBA in the case of both GAC. This could be due to presence of -NH- groups in TBA, which resulted in more hydrophilic character of its molecule and consequent restricted adsorption onto GAC (McCreary and Snoeyink, 1980; Ormad et al., 2008). Moreover, adsorption of ALA was supported by attractive π-π interactions between the benzene ring of ALA and the aromatic structure of the carbon graphene layers (Newcombe and Drikas, 1997; Bjelopavlic et al., 1999; Moreno-Castilla, 2004). The results also showed that GAC N1240 had slightly higher capacity for target herbicides than F400, which can be related to the higher total pore volume (see Table 1**)**.

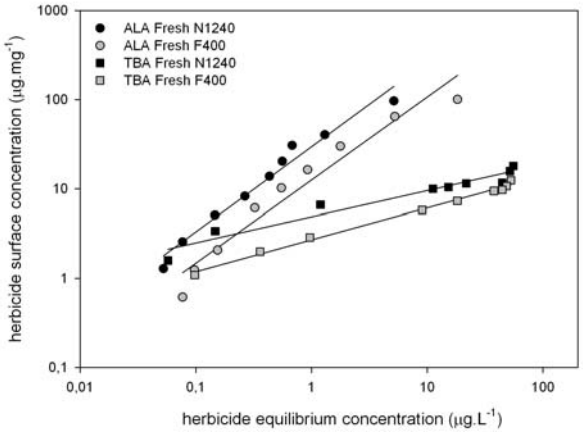


Figure 3. Adsorption isotherms of ALA and TBA onto fresh N1240 and F400

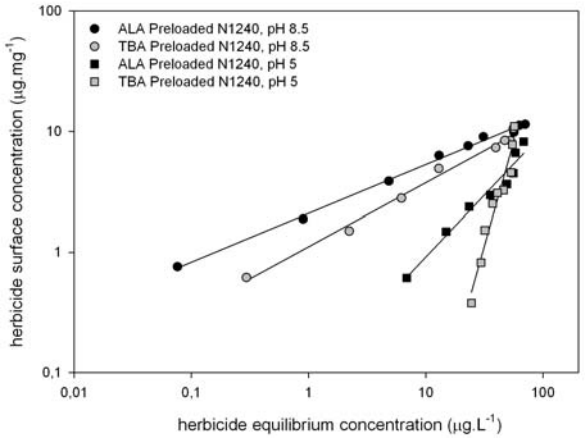


Figure 4. Adsorption isotherms of ALA and TBA onto preloaded N1240 at pH 5 and pH 8.5

Adsorption tests were also performed with carbons preloaded with COM peptides of MW < 10 kDa at pH 5 and pH 8.5. It was find out that ALA and TBA removal by both preloaded carbons was lower than removal by fresh carbons. Figure 4 displays the effect of preloading on herbicide adsorption by carbon N1240 as a representative example. It follows from the comparison of Figure 3 and 4 that the decrease in herbicide adsorption onto preloaded GAC

was more pronounced at pH 5 compared to pH 8.5, which corresponded to greater adsorption of COM peptides < 10 kDa at acidic pH (see Figure 1).

Taking into account the HPSEC analysis results displayed in Figure 2 a,b, COM peptides with low MW between 700 and 1700 Da were determined to participate in direct site competition with ALA and TBA. Analogous results of the role of low MW NOM in competitive adsorption were reported in several studies (Pelekani and Snoeyink, 1999; Ebie et al., 2001). The higher MW peptides (2300 and 6300 Da) are supposed to cause pore blockage, since both carbons used in experiments represented relatively microporous adsorbents, which is consistent with the study of Newcombe et al. (2002a, 2002b).

CONCLUSIONS

The competitive adsorption of herbicides (ALA and TBA) and COM peptides < 10 kDa on granular activated carbons (F400 and N1240) were examined in this study. According to the results observed, the conclusions are as follows:

1) The extent of competitive effect of COM peptides on herbicide adsorption is affected by the pore size distribution and surface charge of GAC and COM peptides.
2) Adsorption capacity of both carbons was higher for ALA compared to TBA, which was attributed to their different chemical structure.
3) The removal of herbicides was lower on preloaded carbons compared to fresh carbons, which was caused by the competitive adsorption of COM peptides.
4) The reduction in adsorption of herbicides onto preloaded carbons was higher at pH 5 compared to pH 8.5, which corresponded to the increased adsorption of COM peptides at acidic pH.
5) The low MW COM peptides were adsorbed to a greater extent than peptides with higher MW. The majority of competition between herbicides and COM peptides was attributed to peptides with MW from 700 to 1700 Da.

Acknowledgements

This research project was funded by the Czech Science Foundation under project No. P105/11/0247 and Institutional Research Plan No. AV0Z20600510.

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Adsorpce pesticidů na granulovaném aktivním uhlí při úpravě vody: vliv organických látek produkovaných fytoplanktonem

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Abstrakt

Příspěvek se zabývá výzkumem kompetitivního působení organických látek produkovaných fytoplanktonem (AOM - Algal Organic Matter) na adsorpci pesticidů (alachlor, terbuthylazin) na granulovaném aktivním uhlí - GAC (Norit 1240, Filtrasorb 400). Laboratorní adsorpční experimenty byly provedeny s GAC nezatíženým a předem zatíženým celulárním podílem AOM (COM – Cellular Organic Matter). Byla prokázána rozdílná účinnost adsorpce obou sledovaných pesticidů, kterou lze přisuzovat jejich odlišné chemické struktuře. Prokázán byl i negativní vliv předchozího zatížení GAC COM, které vedlo k významnému snížení účinnosti adsorpce pesticidů. Důvodem je především kompetitivní adsorpce nízkomolekulární frakce COM (proteinová složka s MH < 10 kDa) v mikropórech GAC. Dále bylo zjištěno, že na adsorpci pesticidů má vliv i pH roztoku (kompetitivní vliv COM se více projevil při pH 5 než při pH 9), velikostní distribuce pórů aktivního uhlí (zastoupení mikropórů a mezopórů) a jeho povrchový náboj (mění se v závislosti na pH roztoku).

klíčová slova:

aktivní uhlí, adsorpce, pesticidy, organické látky produkované fytoplanktonem (AOM – Algal Organic Matter), celulární organické látky (COM – Cellular Organic Matter)

1 ÚVOD

Pesticidy představují díky širokému použití a specifickým fyzikálně-chemickým vlastnostem velmi významnou skupinu polutantů povrchových vod (Bruzzoniti a kol., 2006). Pro jejich odstranění se využívá řada metod. Mezi nejúčinnější patří kromě membránových procesů také adsorpce na aktivním uhlí. Účinnost adsorpce je závislá především na vlastnostech použitého aktivního uhlí (specifický povrch, velikostní distribuce pórů, chemismus povrchu) a chemické podstatě adsorbované látky (hydrofobicita, polarita, funkční skupiny, aromaticita, dvojné vazby). Významnou roli hraje také charakter upravované vody, především pH, iontová síla a složení vody.

Surová voda obsahuje celou řadu organických příměsí přirozeného původu – NOM (Natural Organic Matter), mezi které patří zejména huminové látky a organické látky produkované fytoplanktonem – AOM (Algal Organic Matter). Organické látky, které se neodstraní při koagulaci, se mohou kompetitivně adsorbovat na povrch aktivního uhlí a snižovat tak adsorpční kapacitu pro pesticidy. Adsorbovatelnost jednotlivých frakcí NOM se značně liší především v závislosti na jejich molekulové hmotnosti a chemickém složení. Díky rozdílné velikosti mohou NOM adsorpci organických mikropolutantů ovlivňovat dvěma hlavními mechanismy – přímou kompeticí o adsorpční místa na povrchu aktivního uhlí a blokací pórů (Newcombe a kol., 2002; Li a kol., 2003a). Nízkomolekulární NOM, které mají podobné molekulové hmotnosti jako molekuly pesticidů, mohou při adsorpci kompetitivně obsazovat stejná adsorpční místa. Vysokomolekulární látky naopak blokují transportní póry aktivního uhlí, tím zabraňují vnitřní difúzi a následné adsorpci pesticidů na úrovni mikropórů. Mikropóry s vysokou adsorpční kapacitou pro nízkomolekulární látky tak zůstávají prakticky nedostupné (Li a kol., 2003b).

Kompetitivnímu působení NOM, představovaných zejména huminovými látkami, na adsorpci pesticidů byla v literatuře věnována značná pozornost (Pelekani a Snoeyink, 1999; Matsui a kol., 2003). Studium adsorpce AOM na aktivním uhlí však bylo až doposud opomíjeno. AOM, tvořené především proteiny a polysacharidy, však mohou za určitých podmínek představovat většinový podíl NOM (Hoyer a kol., 1985). AOM lze rozlišit na látky extracelulárního a celulárního původu. Extracelulární látky (EOM - Extracellular Organic Matter) jsou výsledkem metabolické aktivity organismů. Jedná se především o polysacharidy,

oligosacharidy, monosacharidy a částečně také o proteiny a peptidy (Pivokonský a kol., 2006). Celulární látky (COM, Cellular Organic Matter) naproti tomu vznikají autolýzou buněk a jejich podíl ve vodách roste se stárnutím a odumíráním kolonií fytoplanktonních organismů. Hlavními složkami této skupiny jsou především proteiny, dále nabité i neutrální polysacharidy, nukleové kyseliny či lipidy (Fogg, 1983; Henderson a kol., 2008). Vzhledem k tomu, že především nízkomolekulární COM jsou vodárenskou koagulací obtížně odstranitelné (Pivokonský a kol., 2009; Takaara a kol., 2010), mohou negativně ovlivňovat následnou adsorpci pesticidů na aktivním uhlí.

2 MATERIÁLY A METODIKA

Jako absorbent byly v experimentech použity dva typy GAC – Norit 1240 (Norit Americas Inc., USA) a Filtrasorb 400 (Chemviron Carbon, Belgie) (frakce 0,8 – 1,0 mm). Oba typy GAC jsou vyrobeny z černého uhlí a aktivovány vodní parou. Specifický povrch (S_{BET}), objem mikropórů (V_{mikro}), celkový objem pórů (V_{total}), povrch mezopórů (S_{mezo}) a podíl mikropórů (V_{mikro}/V_{total}) byly pro oba typy GAC stanoveny pomocí fyzikální adsorpční izotermy dusíku (při 77 K) prostřednictvím volumetrického přístroje ASAP2020 (Micromeritics, USA). Specifický povrch byl vyhodnocen BET metodou (Brunauer a kol., 1938). Objem mikropórů a specifický povrch mezopórů byly hodnoceny pomocí t-plot metody a Lecloux–Pirard master izotermy (Lecloux a Pirard, 1979). Celkový povrchový náboj GAC byl stanoven potenciometrickou titrací (Bjelopavlic a kol., 1999).

K laboratorním adsorpčním experimentům byla použita voda s KNK upravenou pomocí 0,125 M $NaHCO_3$ na hodnotu $KNK_{4,5}$ = 1,45 mmol·l^{-1}. Za cílové mikropolutanty byly vybrány pesticidy s odlišnou chemickou strukturou. Jedná se o alachlor (ALA) s relativní molekulovou hmotností 269,8 a terbuthylazin (TBA) o relativní molekulové hmotnosti 229,7 (oba Sigma-Aldrich, USA).

Vzorek AOM používaný v experimentech byl získán z uměle vypěstované kultury sinice *Microcystis aeruginosa*, Kutz. (Zapomelova 2006/2, Kolekce kultur, Botanický ústav AV ČR, v.v.i., Praha). Pro adsorpční experimenty byl využit celulární podíl vzorku (COM). Metodika kultivace sinice *M. aeruginosa* a metodika izolace a stanovení COM jsou podrobně popsány

ve studii Pivokonského a kol. (2006). Koncentrace COM byly stanovovány jako DOC po zfiltrování přes 0,22 µm membránový filtr (Millipore, USA) na TOC analyzátoru (TOC-V_{CHP}, Shimadzu, Japonsko).

Adsorpční izotermy pro ALA a TBA byly sestrojeny pomocí rovnovážných adsorpčních experimentů, kdy byly vodné vzorky pesticidů o koncentraci 40 až 100 $\mu g \cdot l^{-1}$ míchány s různými dávkami GAC (0,5 až 100 $mg \cdot l^{-1}$). Experimenty byly prováděny po dobu 7 dní, kdy došlo k ustavení adsorpční rovnováhy. Po skončení experimentů byly vzorky filtrovány přes membránový filtr 0,22 µm (Millipore, USA) a extrahovány metodou SPE na kolonkách Agilent SAMPLI Q C18 (Agilent Technologies, USA).

Kompetitivní adsorpce (COM vs. pesticidy) byla posuzována pomocí experimentů s GAC předem zatíženým COM. Adsorpční experimenty byly prováděny při hodnotách pH 5 a pH 9. GAC (0,5 až 100 $mg \cdot l^{-1}$) bylo nejprve zatíženo po dobu 7 dní adsorpcí COM (iniciální koncentrace DOC = 8 $mg \cdot l^{-1}$). Následně byly ke vzorkům přidány roztoky pesticidů (40 až 100 $\mu g \cdot l^{-1}$) a adsorpční experimenty probíhaly dalších 7 dní až do dosažení rovnovážného stavu. Vzorky pro analýzu zbytkových koncentrací DOC byly odebírány vždy na počátku, 7. a 14. den experimentů.

Zbytkové koncentrace pesticidů byly stanoveny pomocí plynové chromatografie s detekcí elektronového záchytu (GC-ECD) (Agilent Technologies 6890N, USA). Použita byla kapilární křemenná kolona DB-XLB (Agilent Technologies, USA), jako mobilní fáze helium s konstantním průtokem 1 $ml \cdot min^{-1}$.

COM byly dále charakterizovány s využitím frakcionace na sorpčních pryskyřicích Supelite DAX-8 (Sigma-Aldrich, USA), Amberlite 252RF H (ZSE Praha s r.o., ČR) a Amberlite IRA-958 (Sigma-Aldrich, USA), které umožňují rozdělit organické látky na hydrofobní kyselé (HPOA), hydrofobní zásadité (HPOB), hydrofobní neutrální (HPON), hydrofilní kyselé (HPIA), hydrofilní zásadité (HPIB) a hydrofilní neutrální (HPIN). Koncentrace jednotlivých oddělených frakcí byla vyjadřována jako DOC a určována z rozdílu DOC před a po adsorpci na danou pryskyřici (Marhaba a kol., 2003).

Zastoupení zjevných molekulových hmotností (MH) proteinů obsažených v COM bylo stanoveno metodou SEC (Size Exclusion Chromatography) na přístroji Agilent 1100 series s DAD detektorem (Agilent Technologies, USA). Použity byly kolony Agilent Bio SEC-5 100 Å a Agilent Bio SEC-5 300 Å (obě Agilent Technologies, USA). Jako mobilní fáze byl zvolen fosfátový pufr 0,15 M Na_2HPO_4 (pH 7) s průtokem kolonou 1 ml·min^{-1}. Při analýze byla použita vlnová délka 280 nm (Pivokonský a kol., 2006).

3 VÝSLEDKY A DISKUZE

3.1 Povrchové charakteristiky GAC

Zjištěné strukturální vlastnosti obou adsorbentů shrnuje tab. 1. Hodnoty specifického povrchu (S_{BET}) a objemu mikropórů (V_{mikro}) jsou u obou GAC velmi podobné. Pro GAC Norit byla zjištěna hodnota S_{BET} = 1110 m^2·g^{-1} a V_{mikro} = 0,29 cm^3·g^{-1}, pro GAC Filtrasorb S_{BET} = 1025 m^2·g^{-1} a V_{mikro} = 0,30 cm^3·g^{-1}. Rozdíly v těchto povrchových charakteristikách lze z hlediska ovlivnění adsorpce považovat za minimální. Rozdíl v hodnotách celkového objemu pórů (V_{total}) a podílu mikropórů (V_{mikro}/V_{total}) u obou GAC je naopak z hlediska kompetitivního vlivu COM na adsorpci pesticidů důležitý. V_{total} je u GAC Filtrasorb 0,59 cm^3·g^{-1}, u GAC Norit 0,70 cm^3·g^{-1}. V_{mikro}/V_{total} u GAC Filtrasorb tvoří cca 50 %, v případě GAC Norit je tato hodnota nižší, cca 41 %.

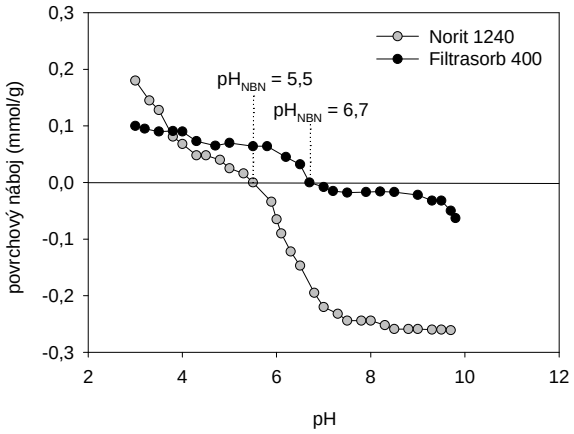
Tab. 1 Charakteristiky povrchu GAC Norit 1240 a Filtrasorb 400

	Norit 1240	Filtrasorb 400
S_{BET} (m^2·g^{-1})	1110	1025
V_{total} (cm^3·g^{-1})	0,70	0,59
V_{mikro} (cm^3·g^{-1})	0,29	0,30
S_{mezo} (m^2·g^{-1})	536	416
V_{mikro}/V_{total} (%)	41	50

Mikropóry (d < 2 nm) představují nejvýznamnější část celkového povrchu aktivního uhlí. Samotná adsorpce mikropolutantů je lokalizována právě v nich, proto může být větší podíl mikropórů ve struktuře GAC Filtrasorb předpokladem jeho vyšší adsorpční účinnosti pro pesticidy. Oba adsorbenty se liší i v zastoupení mezopórů. Hodnoty povrchu mezopórů (S_{mezo}) jsou u GAC Norit 536 m^2·g^{-1} a u GAC Filtrasorb 416 m^2·g^{-1}. Z výsledků některých studií vyplývá, že frakce NOM s vyšší MH se adsorbují právě v mezopórech. Větší zastoupení

mezopórů ve struktuře aktivního uhlí tedy může omezit blokační vliv NOM na úrovni mikropórů (Pelekani a Snoeyink, 1999).

Hodnoty celkového povrchového náboje obou typů GAC v závislosti na pH jsou patrné z obr. 1. Nulového bodu náboje je u GAC Filtrasorb 400 dosaženo při pH 6,7, u GAC Norit 1240 již při pH 5,5. Ze získaných hodnot pH nulového bodu náboje (pH_{NBN}) je patrný kyselejší charakter GAC Norit 1240 ve srovnání s Filtrasorb 400. Kyselé vlastnosti aktivního uhlí jsou přisuzovány zejména přítomnosti kyslíkatých funkčních skupin (karboxylová, fenolová, laktonová, quinonová) (Moreno-Castilla, 2004). Při disociaci těchto skupin ve vodných roztocích dochází k odštěpení H^+ iontů a povrch adsorbentu tak získává celkový negativní náboj. Na vytvoření náboje na povrchu aktivního uhlí se však v reálných vodných roztocích kromě funkčních skupin podílí i specifická adsorpce iontů z roztoku, která je závislá na jeho iontové síle (Newcombe, 2006).

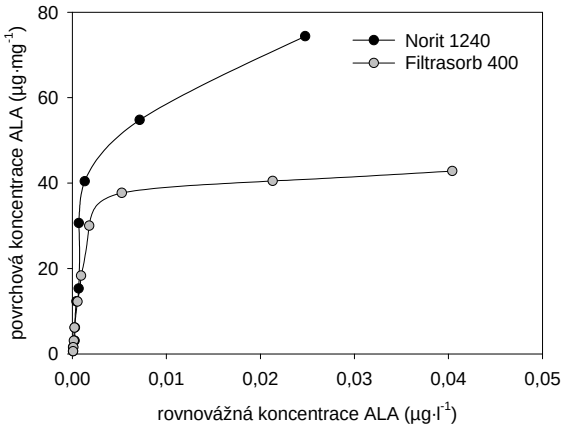


Obr. 1 Povrchový náboj GAC Norit 1240 a Filtrasorb 400

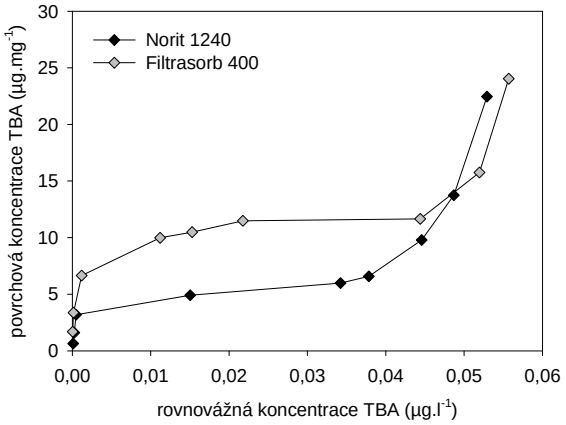
3.2 Adsorpční izotermy a účinnost adsorpce pesticidů

Na obr. 2 a 3 jsou znázorněny adsorpční izotermy pro oba pesticidy. Izoterma vynesená pro ALA na obou GAC svým charakterem odpovídala Freundlichově typu (obr. 2), který je nejčastěji používaným matematickým popisem adsorpce ve vodných systémech. Častý bývá i sigmoidální průběh izotermy (Moreno-Castilla, 2004), kterému odpovídala adsorpční

izoterma zkonstruovaná pro TBA na obou GAC (obr. 3). Z průběhu izoterem vyplývá, že adsorpční kapacita obou GAC je vyšší pro alachlor než pro terbuthylazin.



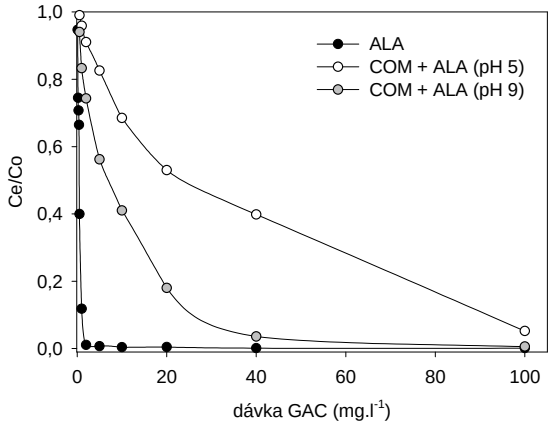
Obr. 2 Adsorpční izoterma pro ALA na Norit 1240 a Filtrasorb 400
(inic. koncentrace C_0 = 62 µg·l^{-1})



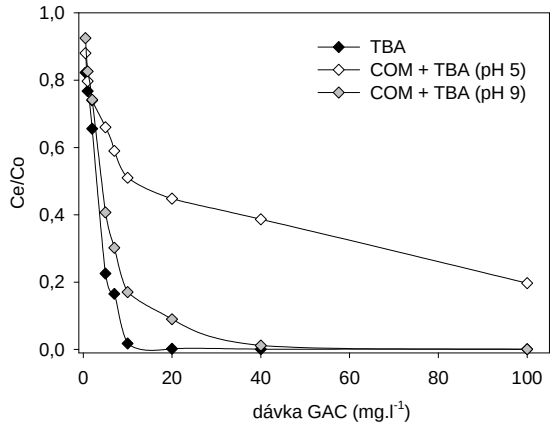
Obr. 3 Adsorpční izoterma pro TBA na Norit 1240 a Filtrasorb 400
(inic. koncentrace C_0 = 66 µg·l^{-1})

Na obr. 4 a 5 jsou znázorněny výsledky rovnovážné adsorpce ALA a TBA na GAC Filtrasorb 400. Ze získaných výsledků je opět patrná vyšší účinnost adsorpce ALA oproti TBA. Rozdílná účinnost adsorpce pesticidů je způsobena jejich odlišnou chemickou strukturou. V molekule TBA hrají důležitou roli amino-skupiny. Jejich vysoká afinita k vodě může bránit v adsorpci TBA z vodného roztoku (McCreary a Snoeyink, 1980). Ve struktuře ALA je pro adsorpci zásadní přítomnost benzenového jádra. Mechanismus adsorpce je založen na

působení přitažlivých interakcí mezi delokalizovanými π-elektrony benzenového jádra a polykondenzovanými uhlíkatými jádry ve struktuře aktivního uhlí. Tento typ přitažlivých interakcí bývá společně hydrofobními interakcemi a vodíkovými vazbami řazen k základním specifickým interakcím působícím při adsorpci na aktivním uhlí (Newcombe a Drikas, 1997; Bjelopavlic a kol., 1999; Moreno-Castilla, 2004).



Obr. 4 Rovnovážná adsorpce ALA na Filtrasorb 400 nezatíženém a zatíženém COM (8 mg·l^{-1}) při pH 5 a pH 9 (C_e–rovnov. koncentrace; C_0–inic. koncentrace)



Obr. 5 Rovnovážná adsorpce TBA na Filtrasorb 400 nezatíženém a zatíženém COM (8 mg·l^{-1}) při pH 5 a pH 9 (C_e–rovnov. koncentrace, C_0–inic. koncentrace)

3.3 Vliv COM na adsorpci pesticidů

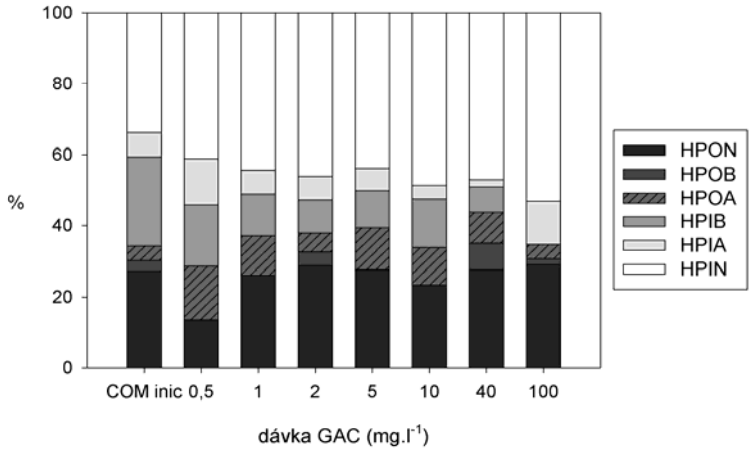
Porovnáním adsorpční účinnosti GAC nezatíženého a předem zatíženého COM byl na zatíženém adsorbentu zjištěn pokles účinnosti adsorpce pro oba sledované pesticidy.

Omezení adsorpce bylo zjevné při pH 5 i pH 9. Důvodem je kompetitivní působení molekul COM, které se v průběhu předzatížení mohou naadsorbovat na povrch GAC. Obdobná situace je popisována i v případě NOM (Pelekani a Snoeyink, 1999; Matsui a kol., 2003). V podstatě existují 2 typy kompetitivního působení, přímá kompetice o adsorpční místa na úrovni mikropórů u nižších molekulových hmotností a blokace pórů adsorbentu u vyšších molekulových hmotností (Newcombe a kol., 2002; Li a kol., 2003a).

Porovnáním výsledků adsorpce pesticidů na GAC zatíženém COM při různém pH (obr. 4 a 5) bylo zjištěno, že k většímu omezení adsorpce došlo při pH 5. V případě ALA byl nejvýraznější pokles účinnosti adsorpce (v porovnání s experimenty na nezatíženém GAC) o 90 %, u TBA 49 %. V případě experimentů při pH 9 byl zaznamenán pokles 73 % u ALA a 18 % pro TBA. Důvodem je větší afinita COM k povrchu GAC při nízkých hodnotách pH, kdy jsou minimalizovány odpudivé elektrostatické interakce mezi povrchem GAC a COM. COM se tak na GAC kvantitativněji naadsorbují a sníží počet adsorpčních míst pro pesticidy. Obdobný efekt byl popsán i v případě NOM huminového charakteru (Bjelopavlic a kol., 1999). Vzhledem k tomu, že elektrostatické interakce jsou pouze jedním z možných mechanismů, který se při adsorpci na aktivním uhlí uplatňuje, dochází pravděpodobně k projevu i dalších typů interakcí. Příkladem může být tvorba vodíkových můstků, hydrofobní interakce nebo tzv. polymer bridging (Gregory, 2006).

3.4 Frakcionace COM na sorpčních pryskyřicích

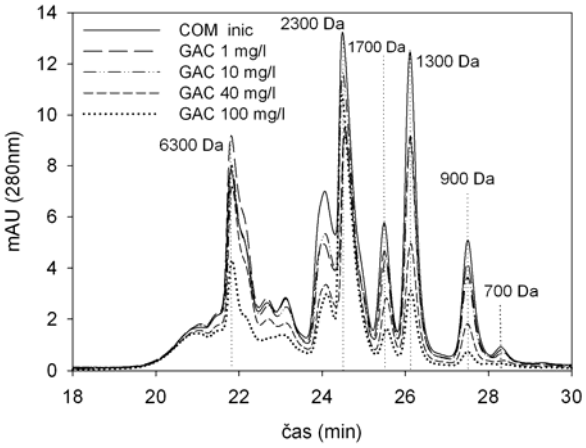
Z výsledků frakcionace vzorků COM po adsorpčních experimentech (obr. 6) vyplývá, že se vzrůstající dávkou GAC se zvyšuje relativní zastoupení hydrofilní neutrální frakce (HPIN) reprezentované polysacharidy. Zároveň klesá relativní zastoupení hydrofilní bazické frakce (HPIB) tvořené zejména proteiny. Získané výsledky naznačují, že právě amfoterní proteinové složky COM se adsorbují na GAC ve větší míře než neutrální polysacharidy a částečně tak inhibují adsorpci pesticidů. Elektrostatické interakce, které se podílejí na adsorpci látek na aktivním uhlí, se tedy uplatňují s větší mírou na proteinech, které mohou nést náboj, než na neutrálních polysacharidech. Z výsledků je patrné, že při adsorpci proteinů COM bude hrát významnou roli hodnota jejich izoelektrického bodu a zároveň hodnota bodu nulového náboje povrchu GAC.



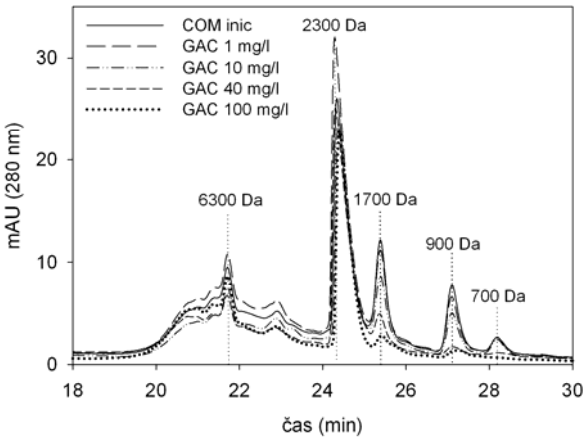
Obr. 6 Frakcionace COM na sorpčních pryskyřicích

3.5 Stanovení zjevných molekulových hmotností proteinů COM

Z výsledků SEC analýzy (obr. 7 a 8) vyplývá, že se v závislosti na dávce GAC kvantitativně adsorbují zejména proteinové látky o nižší molekulové hmotnosti (700 – 1700 Da), a to v případě nízkého i vysokého pH. Účinnost adsorpce pro proteinové látky o vyšší molekulové hmotnosti (2300 - 6300 Da) je podstatně nižší.



Obr. 7 Zastoupení zjevných MH proteinů COM adsorbovaných na Norit 1240 při pH 5



Obr. 8 Zastoupení zjevných MH proteinů COM adsorbovaných na Filtrasorb 400 při pH 9

Nízkomolekulární frakce COM mají podobnou MH jako pesticidy a mohou tak vstupovat do mikropórů, kompetitivně se zde adsorbovat a snižovat adsorpční účinnost GAC pro pesticidy. Proteinové látky s vyšší MH se díky své velikosti do úzkých mikropórů nedostanou, adsorbují se proto především v mezopórech.

4 ZÁVĚR

1) Míra kompetitivního vlivu COM na adsorpci pesticidů je ovlivněna velikostní distribucí pórů GAC a jeho povrchovým nábojem. Ten se u obou GAC mění v závislosti na pH roztoku. Pro GAC Norit 1240 byla stanovena hodnota pH_{NBN} = 5,5, zatímco pro Filtrasorb 400 na 6,7.
2) Adsorpční kapacita a účinnost obou typů GAC je vyšší pro ALA než pro TBA. Rozdílnou účinnost adsorpce obou pesticidů lze přisuzovat jejich odlišné chemické struktuře. Zkonstruované adsorpční izotermy měly navíc odlišný průběh – izoterma Freundlichova typu pro ALA a izoterma sigmoidálního charakteru pro TBA.
3) Předchozí zatížení GAC COM vedlo k poklesu adsorpční účinnost pro oba pesticidy. Adsorpce pesticidů byla výrazněji omezena při pH 5 než při pH 9. Důvodem je větší afinita COM k povrchu GAC při nízkých hodnotách pH, kdy dochází k minimalizaci odpudivých elektrostatických interakcí mezi povrchem GAC a COM.
4) Bližší charakterizace COM ukázala, že proteiny COM se na kompetici při adsorpci podílejí více než polysacharidy. Kompetitivní adsorpce se účastní zejména nízkomolekulární

proteiny (MH 700 - 1700 Da), které mohou díky své velikosti zaujímat stejná adsorpční místa jako pesticidy.

Poděkování

Výzkum, jehož dílčí výsledky jsou publikovány v tomto příspěvku, je řešen v rámci grantového projektu GA ČR P105/11/0247 a výzkumného plánu AV0Z20600510. Autoři děkují za poskytnutou finanční podporu.

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8 SUMMARY AND CONCLUSIONS

The dissertation thesis evaluated the adsorption of algal organic matter (AOM) onto activated carbon (AC) during drinking water treatment, which has been published as a main part of the study in Publication 1. In the context of increasing anthropogenic eutrophication of the environment and more frequent occurrence of algal blooms also in raw water sources that are used for the production of potable water, the use of alternative technologies for removing algal products from water represents a very current topic for waterworks. During the study, emphasis was placed on the removal of undesirable cellular peptides produced by *Microcystis aeruginosa*, a cyanobacterium commonly dominating algal blooms in water reservoirs also in the Czech Republic. The selection of these low-molecular weight substances representing target pollutants for the study was well-founded due to their difficult removability by conventional water treatment based on coagulation/flocculation and their presumptive negative impact on the adsorption of other organic compounds present in raw water, namely on micropollutants. These hypotheses were confirmed mainly in Publication 3 and 4.

Several series of laboratory operation were performed during the study with the gist consisted of adsorption experiments with different types of activated carbon and adsorbate analyses before and after the treatment. Emphasis was placed on the characterisation of both ACs (Publication 1 and 4) and peptides (Publication 1, 2 and 4), as well as on the identifying the influence of solution properties (pH, ionic strength) on the adsorption of peptides (Publication 1, Publication 5, Publication 6). On the basis of the results of equilibrium and kinetic experiments, the mechanisms and interactions of AOM removal and competitive adsorption were discussed mainly in Publication 1 and 4.

It was confirmed that, in the case of properly optimised adsorption process, adsorption onto activated carbon is an efficient method for removing natural and anthropogenic organic substances which are resistant to coagulation/flocculation water treatment. Specific conclusions of the publications, which were essential for this thesis, can be summarised as follows:

1) The peptide adsorption onto activated carbon is highly pH dependent and increases with decreasing pH value. The highest adsorption capacities were reached at pH 5 due to the

electrostatic attraction between functionalities of the AC and the peptides and/or thanks to the conformation changes in the structure of peptide molecules. Furthermore, a high portion of secondary micropores and mesopores in AC structure contributes to the peptide removal because these pores provide a fundamental portion of the specific surface area available for adsorption of natural polymers with MW < 10 kDa (Publication 1).

2) Adsorption experiments at different electrolyte concentrations demonstrated that ionic strength significantly impacts the adsorption of organic pollutants. In case of the peptides, an increasing ionic strength can enhance their adsorption by screening the repulsive forces, or by strengthening the attractive ones in the adsorption system, all of that depending on the type of carbon used and pH applied (Publication 1).
3) The characterisation of *Microcystis aeruginosa* products revealed that COM forms a significant portion of DOC mainly in the stationary phase of algal growth and that it is predominantly hydrophilic with low SUVA and also richer in peptides/proteins than EOM. The amount of peptides/proteins produced by cyanobacterium *Microcystis aeruginosa* was one of the highest between the studied microorganisms (Publication 2) and forms up to 63 % of COM compared to 37 % of non-protein material (Publication 1, Publication 4).
4) The HPSEC analysis of the changes in peptide/protein composition after coagulation tests under optimum reaction conditions confirmed the findings of earlier studies that high-MW proteins of MW > 10 kDa are completely removed, whereas low-MW peptides of MW between 1 and 10 kDa remain in the solution after the treatment (Publication 3) and other methods should be used to remove them. Moreover, the peptide fraction of MW < 10 kDa, which was consequently used in adsorption experiments, comprises a significant portion of the whole COM peptides/proteins (up to 21 %) and it is very likely that it also includes harmful cyanobacterial microcystins (Publication 1, Publication 4).
5) The analyses of pore size distribution and surface charge of ACs demonstrated that the textural dissimilarities of the adsorbents are dependent on the type of raw precursor used for their manufacture. The net surface charge of activated carbons can be both positive and negative depending on the solution pH value. Adsorbents also display different pH values of the point of zero charge (pH_{pzc}), i.e. the pH value where the net charge is zero, which depends on the content and the type of surface functional groups (Publication 1, Publication 4).

6) The characterisation of COM properties important in terms of adsorption showed that peptides have an amphoteric character and contain different functional groups, e.g. –OH, –COOH, –SH, $=NH^{2+}$, $–NH^{3+}$. Thanks to de-/protonation of the ionizable groups, COM peptides carry both positively ($=NH^{2+}$, $–NH^{3+}$) and negatively charged sites ($–COO^{-}$) which enable the existence of electrostatic interactions with charged groups on carbon surface during the adsorption. The overall results of HPSEC analyses demonstrated that low molecular weight COM peptides of MW < 4.5 kDa are adsorbed onto AC to a greater extent than the larger ones (Publication 1, Publication 4).
7) The adsorption capacities of ACs for organic micropollutants were higher in the case of alachlor compared to terbuthylazine. With regard to the similar MW of ALA and TBA, this can be attributed to the different chemical structures of these herbicides. The adsorption of TBA can be obstructed particularly due to its more hydrophilic character. Moreover, the removal of herbicides is negatively affected by the presence of COM in water through the competitive adsorption of peptides with low MW because they can access the same adsorption sites as micropollutants (Publication 4, Publication 5 and Publication 6).
8) In addition to van der Waals forces and hydrophobic interactions that are the driving forces for adsorption onto AC, a formation of H-bonds and electrostatic interactions were confirmed to play an essential role during the removal of organic pollutants onto activated carbon (Publication 1, Publication 4).

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This thesis should be cited as:

Kopecká, I., 2014. Adsorption of organic compounds onto activated carbon in water treatment process. Ph.D. Thesis. Charles University in Prague, CR.

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