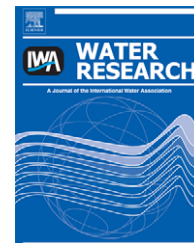


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Investigating dissolved air flotation performance with cyanobacterial cells and filaments

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ABSTRACT

Dissolved air flotation (DAF) performance with two different naturally occurring cyanobacterial morphologies was investigated with respect to the biomass removal efficiency, the toxin release to water and the coagulant demand by different water background natural organic matter (NOM). Coagulation (C)/Flocculation (F)/DAF bench-scale experiments (2 min coagulation at 380 s^{-1} with polyaluminium chloride (0.5–4 mg/L Al_2O_3 , the dose depending on the water NOM content); 8 min flocculation at 70 s^{-1} ; 8 min DAF with 5 bar relative pressure and 8% pressurised recycle) were performed with single cells of *Microcystis aeruginosa* and *Planktothrix rubescens* filaments spiked in synthetic waters with different NOM contents (hydrophobic vs. hydrophilic NOM; moderate (2–3 mgC/L) vs. moderate-high concentration (ca. 6 mgC/L)). For both morphologies, the results show no apparent cyanobacterial damage (since the water quality did not degrade in dissolved microcystins and the removal of intracellular microcystins matched the removal of chlorophyll *a*) and high biomass removal efficiencies (93–99% for cells and 92–98% for filaments) provided optimal coagulant dose for chlorophyll *a* removal was ensured. Charge neutralisation by the polyaluminium chloride was the main coagulation mechanism of the *M. aeruginosa* cells and most likely also of the *P. rubescens* filaments. The specific coagulant demand was severely affected by NOM hydrophobicity, hydrophobic NOM (with a specific $\text{UV}_{254\text{nm}}$ absorbance, SUVA, above 4 L/(m mgC)) requiring ca. the triple of hydrophilic NOM (SUVA below 3 L/(m mgC)), i.e. 0.7 vs. 0.2–0.3 mg Al_2O_3 /mg DOC.

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1. Introduction

The occurrence of cyanobacteria (blue-green algae) in reservoirs, lakes and rivers used as drinking water sources is a worldwide environmental health issue, due to the ability of some cyanobacterial streams to produce toxins, as well as taste and odour compounds, as secondary metabolites under particular conditions of growth. The most commonly occurring group of cyanobacterial hepatotoxins in freshwaters are

microcystins and as a result of the increasing concern with their health implications the World Health Organisation (WHO, 1998) has set a provisional guideline-value in drinking water of $1.0\text{ }\mu\text{g/L}$ for microcystin-LR (MC-LR), one of the most toxic and usual microcystin variant. Microcystins are potentially produced by common genera of cyanobacteria, such as *Microcystis*, *Planktothrix* and *Anabaena* (Sivonnen and Jones, 1999). *Microcystis* are unicellular or colonial while *Planktothrix* and *Anabaena* are naturally occurring filamentous cyanobacteria.

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Conventional drinking water treatment by coagulation, flocculation, sedimentation (C/F/S), filtration and chlorination is not a safe barrier against cyanobacteria and associated toxins. Depending on the cyanobacteria and on the operating conditions used, conventional treatment may control the cells removal, and *Microcystis* removals between 58% and 90% have been reported (Ando et al., 1992; Velzeboer et al., 1995; Chow et al., 1999). C/F/S is however ineffective to remove the dissolved toxins originated from the release of intracellular (or cell-bound) toxins to water due to cell ageing and/or external stress factors responsible for cell damage which may be caused by the treatment itself. Indeed, while Chow et al. (1998) and Drikas et al. (2001) found no release of intracellular toxins and taste and odour compounds by C/F/S, Himberg et al. (1989), Lam et al. (1995) and Hrudehy et al. (1999) reported the occurrence of cell lysis and subsequent release of such compounds to the water.

Cyanobacteria are low-density particles and some may float. Therefore, dissolved air flotation (DAF) of flocculated waters (i.e. C/F/DAF) has proven to be very effective for treating cyanobacterial-rich waters (Edzwald and Wingler, 1990; Yan and Jameson, 2004), more than the conventional C/F/S (Ribau Teixeira and Rosa, 2006a, 2007) or the floc blanket clarification (98% vs. 77%; Zabel, 1985; Hrudehy et al., 1999). Two recent studies compared the removal of *Microcystis aeruginosa* cells and MC-LR (the associated microcystin) by C/F/DAF and conventional C/F/S (Ribau Teixeira and Rosa, 2006a, 2007). The first study focused the key operating conditions for removing chlorophyll *a* from tap water spiked with *M. aeruginosa* cells and the second investigated the effect of the water background natural organic matter (NOM) on the process performance. C/F/S and C/F/DAF were able to remove the cells with no MC-LR increase in the treated water, and both were affected by the water NOM. However, C/F/DAF was less affected by the NOM concentration (tap water vs. natural waters) and type (natural waters before and after ozonation) and showed higher removal efficiencies of *M. aeruginosa* cells (above 92% in terms of chlorophyll *a*) using more cost-effective operating conditions including lower coagulant dose.

While these studies were both performed with *M. aeruginosa* single cells, since these are an ideal surrogate for assessing the removal efficiency of the particles of problematic size range (3–10 µm) of protozoa (Vlaski et al., 1996) as well as of cyanobacteria, it is still not clear how do the naturally occurring filamentous cyanobacteria behave during the C/F/DAF treatment.

Edzwald and Wingler (1990) achieved C/F/DAF removal efficiencies above 98% for *Chlorella* and *Cyclotella*. Hrudehy et al. (1999) reported removal efficiencies of 40–80% for *Microcystis*, 90–100% for *Anabaena* and only 30% for *Planktothrix* in a Belgian DAF plant. Conversely, Yan and Jameson (2004) found no significant differences between the removal of *M. aeruginosa* cells or colonies and *Anabaena circinalis* filaments in a DAF unit.

With respect to cell integrity of filamentous cyanobacteria, Velzeboer et al. (1995) found no cell lysis of cultured *A. circinalis* by C/F/S with aluminium sulphate (5.3 mg/L Al; 1 min coagulation at 200 rpm; flocculation at 25 rpm during 14 min; 30 min sedimentation). Chow et al. (1998) verified that *A. circinalis* was susceptible to chemicals, but no increase of

microcystin was found in the water after C/F/S with ferric chloride (5.2 and 10.3 mg/L Fe).

Although *Planktothrix* sp. is frequently found in natural waters and may produce high concentrations of microcystins and anatoxin-a there are few water treatment studies with this filamentous cyanobacterium. Lahti et al. (2001), Schmidt et al. (2002) and Hoeger et al. (2005) agree on the effective removal of *Planktothrix* filaments by the conventional water treatment processes, but the first two studies report damage of cells and release of toxins to the water.

The objective of the present work is therefore to compare the C/F/DAF performance with commonly occurring cyanobacterial genera of different morphology. Filaments of *Planktothrix rubescens* and cells of *M. aeruginosa* were selected, the latter also as a surrogate of cyanobacterial colonies of problematic size. In addition to the biomass removal efficiency and the toxin release to water, this paper investigated the coagulant demand by a broad range of water background organic matrixes, both in terms of NOM hydrophobicity/hydrophilicity and dissolved organic carbon (DOC) concentrations (moderate and moderate-high concentration). Although the earlier study (Ribau Teixeira and Rosa, 2007) has shown that the presence of NOM exerts a significant coagulant demand for optimal chlorophyll *a* removal, only a narrow range of NOM nature (in the hydrophilic range) and a narrow range of concentration (low DOC concentration) were investigated, since they were limited to the characteristics of the assayed natural waters.

2. Material and methods

2.1. Chemicals and NOM model substances

Deionised water was used for the preparation of all stock solutions. Certified analytical grade potassium chloride (KCl) and calcium chloride (CaCl₂) salts were used to provide controlled water background ionic strength.

Salicylic acid (SA) and Aldrich humic acids (AHA) were used as the NOM surrogates for they represent the hydrophilic, low molar mass NOM vs. the hydrophobic, high molar mass NOM. These NOM model substances were obtained from commercial sources and have been used to provide consistent experimental conditions (Hong and Elimelech, 1997). The salicylic acid is a certified analytical grade reagent from VWR International (>99.0% purity, 138.12 g/mol) and was used with no further purification. A stock solution (1 g/L) of commercial AHA was prepared by dissolving it in deionised water and raising the pH to 8 through the addition of NaOH. AHA was then purified through a repeated precipitation with HCl to remove bound iron and decrease the ash content as described in Hong and Elimelech (1997).

2.2. Synthetic waters

Four synthetic waters of different DOC nature (hydrophilic and hydrophobic) and concentration, and constant background inorganic matrix were used (Table 1).

The water background ionic strength (IS) was provided by mono (KCl) and divalent (CaCl₂) salts, and was controlled at

Table 1 – Synthetic waters used in the C/F/DAF experiments.

DOC nature ^a	DOC concentration ^b	Synthetic waters after cyanobacterial spiking									
		Code	Cyanobacteria	chl_a (µg/L)	MC-LR eq. intra (µg/L)	DOC (mgC/L)	UV _{254nm} (1/cm)	SUVA (L/(m mg))	MC-LR eq. extra (µg/L)	pH	EC (µS/cm)
Hydrophobic (SUVA > 4 L/(m mgC))	Moderate-high (ca. 6 mgC/L)	w1	Cells	49.7	1.35	5.77	0.319	5.53	1.35	7.20	359
			Filaments	67.7	1.01	5.64	0.420	7.45	2.99	6.58	341
	Moderate (2–3 mgC/L)	w2	Cells	51.0	2.01	1.90	0.110	5.79	1.79	6.80	355
			Filaments	61.0	1.21	2.17	0.094	4.33	4.18	6.78	348
Hydrophilic (SUVA < 3 L/(m mgC))	Moderate-high (ca. 6 mgC/L)	w3	Cells	41.9	1.19	5.59	0.119	2.13	3.22	7.15	366
			Filaments	67.2	1.55	5.71	0.110	1.93	1.60	6.73	368
	Moderate (2–3 mgC/L)	w4	Cells	47.8	2.67	2.76	0.028	1.01	2.90	6.50	374
			Filaments	59.5	1.23	2.35	0.020	0.85	1.88	6.86	351

a Edzwald and Van Benschoten (1990) classification based on SUVA values, i.e. SUVA < 3 L/(m mgC) represents hydrophilic NOM whereas SUVA > 4 L/(m mgC) indicates hydrophobic NOM.

b EPA (1999) classification, i.e. moderate DOC concentration between 2.0 and 4.0 mgC/L, moderate-high DOC concentration between 4.0 and 8.0 mgC/L, and high DOC concentration above 8.0 mgC/L.

4.0 mM (1 mM IS KCl plus 3 mM IS CaCl₂), which corresponds to a moderately-hard water (AWWA, 2000).

DOC nature and concentration were controlled by adding different mixtures of the two NOM surrogates, AHA and SA. Hydrophobic and hydrophilic DOC (based on SUVA values above 4 L/(m mgC) and below 3 L/(m mgC) respectively (Edzwald and Van Benschoten, 1990)) and moderate and moderate-high DOC concentrations (2–3 mgC/L and ca. 6 mgC/L respectively (EPA, 1999)) were studied. The small differences observed in each water sample (w1, w2, w3 and w4, Table 1) are due to the cyanobacterial spiking input.

2.3. Cyanobacterial cells and filaments

Cultured cells and filaments were used. *M. aeruginosa* supplied by Pasteur Culture Collection (PCC 7820) was grown in laboratory in 10 L of modified BG11 medium ((Stanier et al., 1971); the modification consisted on replacing iron and ammonia citrate by iron sulphate), at 23–24 °C, under a light regimen of 12 h light, 12 h dark (ca. 5 µM photon/(m s)). Cultures were harvested at the late exponential phase of growth (Ribau Teixeira, 2005). The cultured material differs physically from naturally occurring field populations of *M. aeruginosa* for it is made up of small, regular colonies, and many single cells and pairs of cells which contain much less mucilage (mucopolysaccharides) than the natural material. The field populations may contain very large (macroscopic) colonies and greater amounts of mucilage surrounding them (Sivonnen and Jones, 1999; Drikas et al., 2001). MC-LR was the toxin variant identified in the PCC 7820 cultures of single cells used in the C/F/DAF experiments.

P. rubescens filaments were supplied by DVGW – Technologiezentrum Wasser Karlsruhe (TZW), within the TOXIC European Project “Barriers against cyanotoxins in drinking water”, and maintained in the laboratory according to TZW instructions. The growth medium mainly contains stock solution (KNO₃, K₂HPO₄, MgSO₄·7H₂O), soil extract, micro-nutrient solution and vitamins. Two microcystin variants, MC-LR and MC-RR, were identified in the *P. rubescens* cultures used in the C/F/DAF experiments.

M. aeruginosa cells or *P. rubescens* filaments were spiked in the synthetic waters until a concentration of ca. 50 µg/L in

chlorophyll *a* (chl_a) was achieved, corresponding to WHO’s alert level 2 (cyanobacterial biomass of 100,000 cells/mL or 50 µg/L chl_a; Bartram et al., 1999). Alert level 2 describes an established toxic bloom with high biomass, and indicates an increase in the risk of human health effects and the need for effective water treatment systems (Bartram et al., 1999). The cyanobacterial-rich synthetic waters stayed overnight at room temperature before use. As found by Vlaski et al. (1996), spiking similar biomass of cells and particularly filaments from the original culture suspensions was a difficult task, and it was therefore impossible to provide to all experiments cyanobacterial suspensions with 50 µg/L chl_a (Table 1).

2.4. C/F/DAF experiments

C/F/DAF experiments were carried out in a bench-scale laboratory-made apparatus as described by Ribau Teixeira and Rosa (2006a), and the standard experimental procedure used has been explained. This apparatus has a 2 L pressure chamber and a 3 L calibrated cylinder, with a paddle for C/F mixing. The calibrated cylinder was partially filled with synthetic water spiked with cyanobacterial cells or filaments, and the pressure chamber contained synthetic water with no cyanobacterial biomass, to resemble the full-scale C/F/DAF operation with pressurised recycle. Prior to the release of the pressurised volume into the calibrated cylinder, a rapid (coagulation) and a slow (flocculation) mixing were performed using the operating conditions responsible for the best C/F/DAF data previously found for *M. aeruginosa* single cells (Ribau Teixeira and Rosa, 2006a), namely: (i) coagulation at a velocity gradient (G_C) of 380 s⁻¹ for 2 min using a polyaluminium chloride (aluminium polyhydroxichlorosulphate with 60–70% relative basicity from Elf Atochem; stock solution with 850 mg/Al₂O₃), coagulant dose ranging from 0.5 to 1.5 mg/L Al₂O₃ for the synthetic waters with hydrophilic DOC (w3 and w4) and 1.5–4 mg/L Al₂O₃ for the synthetic waters with hydrophobic DOC (w1 and w2); (ii) flocculation at 70 s⁻¹ G_F for 8 min; (iii) DAF during 8 min using 5 bar of relative pressure and 8% of pressurised recycle (computed from the ratio between the pressurised volume released to the cylinder over the initial volume in the cylinder × 100). The experiments

were carried out at room temperature (20 ± 2 °C) and neutral pH (6.5–7.2). This is a common pH in natural waters and there are evidences of cell lysis at acid pH, with subsequent release of toxins to the water (Velzeboer et al., 1995; Ribau Teixeira and Rosa, 2006b). All experiments were performed in duplicate and each duplicate sample was analysed in triplicate (following a random sampling of the six analytical measurements of each parameter of water quality). When the extremes defined by the means of the two duplicates for the cells and the filaments crossed, the significance of the difference was analysed by a one way ANOVA test ($\alpha = 0.05$). In the following experimental conditions no statistical significant ($\alpha = 0.05$) difference between the averages was found: water 2, dose 1.5 mg/L Al_2O_3 for chl_a and DOC; water 3, dose 1.5 mg/L Al_2O_3 for $\text{UV}_{254\text{nm}}$. In the remaining tested experimental conditions the ANOVA test showed statistical difference between the averages of *P. rubescens* filaments and *M. aeruginosa* cells.

2.5. Analytical methods

Samples were analysed for pH (at 25 °C in a Crison Basic 20+ pH meter), electrical conductivity (EC, at 20 °C in a Crison GLP32 conductivity meter), DOC and $\text{UV}_{254\text{nm}}$ absorbance using standard methods of analysis (Eaton et al., 2005), as well as for chl_a and microcystins.

DOC and $\text{UV}_{254\text{nm}}$ absorbance were determined in filtered samples (through a 0.45 μm acrodisk filter). DOC was analysed as non-purgeable organic carbon in a Shimadzu TOC 5000A analyser (50 ppb–4000 ppm, using the high-temperature combustion method). $\text{UV}_{254\text{nm}}$ absorbance was measured using a Beckman DU 640B UV/VIS spectrophotometer.

For chl_a analysis, the samples were filtered through Whatman GF/F filter paper and the chlorophylls were extracted using 10 mL acetone (90%). The optical densities of the extracts were measured at 665 and 750 nm using a Beckman DU 640B UV/vis spectrophotometer, and the chl_a concentration was computed from Lorenzen equations (Lorenzen, 1967).

M. aeruginosa and *P. rubescens* suspensions were analysed for extra and intracellular microcystins (MC extra and MC intra, respectively), before and after treatment to check the release of microcystins into water, using the standard operation procedures developed by Meriluoto and Spoof (2005a,b,c) for, respectively, the extraction of MC intra, the concentration of MC extra in water samples and its analysis by high-performance liquid chromatography with photodiode-array detection (HPLC-PDA), with the adaptations described in Ribau Teixeira and Rosa (2006b). A HPLC-PDA Dionex Summit system was used with a C18 column (Merck Purospher STAR RP-18 endcapped, 3 μm particles, LiChroCART 55 \times 4 mm). All microcystin variants detected were quantified as MC-LR and the overall concentration was expressed in MC-LR equivalent concentration (MC-LR eq.).

3. Results and discussion

Fig. 1 shows the normalised residuals (C/C_i , where C and C_i are the final and the initial concentrations, respectively) of particulate matter (chl_a and MC intra) after C/F/DAF with different coagulant doses and with the four synthetic waters

studied (the hydrophobic NOM on the left, Fig. 1a and the hydrophilic NOM on the right, Fig. 1b). Fig. 2 displays the analogous data for the dissolved matter (DOC, $\text{UV}_{254\text{nm}}$ and MC extra). Figures show the average values and the variation (error bars) of the six measurements taken for each analytical parameter.

Results point out an easier removal of *P. rubescens* filaments by C/F/DAF compared to the removal of *M. aeruginosa* cells, for all synthetic waters studied (Fig. 1). *P. rubescens* is a filamentous cyanobacterium producing long filaments whereas *Microcystis* sp. cells usually have diameters between 3 and 7 μm (Yan and Jameson, 2004).

In addition, the coagulant dose severely impacted the normalised residuals of chl_a, particularly for the *M. aeruginosa* cells (Fig. 1). Since the cyanobacterial suspension stability is associated with electrostatic repulsions between the negatively charged cyanobacterial particles (cells or filaments), steric effects and adsorbed macromolecules or extracellular organic matter (Edzwald, 1993), particle destabilisation is very important and coagulation conditions that produce flocs or particles of little or no charge should be provided for efficient DAF (Malley and Edzwald, 1991; Ribau Teixeira and Rosa, 2006a). Moreover, the coagulant dose effect on the chl_a removal depended on the cyanobacterial morphology and on the studied water. For the synthetic water with hydrophobic NOM and moderate-high DOC concentration (water 1) the coagulant dose increase from 1.5 to 4 mg/L Al_2O_3 did not affect the removal of the *P. rubescens* filaments but it strongly enhanced the removal of the *M. aeruginosa* cells, whereas a severe adverse effect was found on the removal of cells from the water with the hydrophobic NOM and moderate DOC concentration (water 2). This means that the concentration decrease of hydrophobic NOM was associated with a coagulant overdosing effect on the removal of *M. aeruginosa* cells, and the same happened to a lower extent for hydrophilic NOM but for lower coagulant concentrations. As far as the *P. rubescens* filaments are concerned, overdosing limitations were only apparent in water 4 (hydrophilic NOM, moderate DOC concentration). This different behaviour of cells and filaments may be due to a mechanism of particle destabilisation. Actually, Bernhardt and Clasen (1991) reported that the coagulation of smooth and more or less spherical algal cells occurs largely by charge neutralisation, while filamentous algae, large algae or species with bristles on their cell surface could be dealt by sweep coagulation. The adverse effect of coagulant overdosing is usually associated with the charge neutralisation mechanism whereas no such phenomenon affects the sweep coagulation. For moderate DOC concentration (waters 2 and 4), 3 and 4 mg/L Al_2O_3 exceed the water coagulant demand. As a result, the coagulant positive charges exceed the negative charges present in water and particle restabilisation occurs (Liu and Chin, 2009). If sweep coagulation was the main mechanism responsible for the high particle removals observed, higher coagulant dose should be needed for precipitation of the metal salts and entrapment of the cyanobacteria on the sweep flocs. Furthermore, an increased concentration of compounds in the water should not require a compulsory increase of the coagulant dose (Jiang and Graham, 1996), or the same coagulant dose should not yield higher removal efficiencies (Fig. 1). Charge neutralisation was also the mechanism

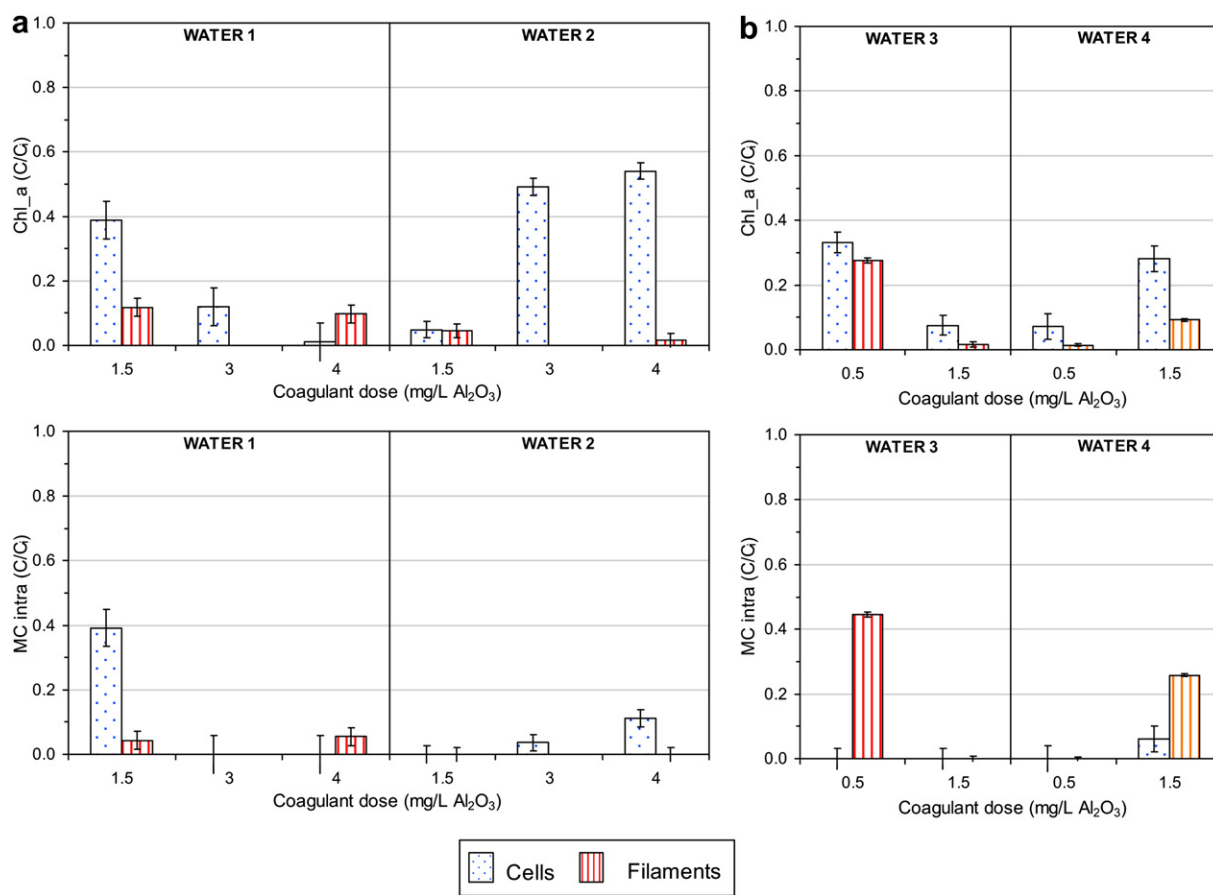


Fig. 1 – Normalised residuals of chl_a and MC intra after C/F/DAF treatment of water samples with hydrophobic (a) or hydrophilic (b) NOM.

used by Jiang et al. (1993) to explain the coagulation of algal cells and dissolved organic species (extracellular material and humic substances). These authors reported that appreciable metal hydroxide precipitation occurred only for monomeric and/or polyaluminium coagulant doses higher than 10 mg/L Al₂O₃, far above the doses used in the present study.

Moreover, pre-polymerised coagulants act mostly by charge neutralisation (Koether et al., 1997). Pre-polymerisation of the metal salt coagulant is mostly to enhance the charge interaction mechanism of colloid destabilisation by slowing down the hydrolysis of the metal salt (Jiang and Graham, 1996). Pernitsky and Edzwald (2003) examined the solubility of polyaluminium chlorides using ca. 39 mg/L Al₂O₃ and showed that a pH higher than 6–6.5 produced an aluminium precipitate. With these rather high coagulant doses (more than 10 times higher than the range used in the present study) and at pH values above 6–6.5, enmeshment of colloids and adsorption of NOM may therefore rule the coagulation mechanism. From these conclusions and the results obtained in this study, the most feasible mechanism for the coagulation of *M. aeruginosa* cells is charge neutralisation and is likely the main mechanism also for the coagulation of *P. rubescens* filaments.

Figs. 1 and 2 also show the important role of the water background NOM on the coagulant demand and on the overall performance of C/F/DAF. For hydrophobic waters (water 1 and

water 2), a DOC coagulant demand effect is observed particularly for single cells (Figs. 1a and 2a), and the optimal coagulant dose for chl_a removal is 4 mg/L Al₂O₃ for water 1 (moderate-high DOC concentration, 5.8 mgC/L, Fig. 1a) and 1.5 mg/L Al₂O₃ for water 2 (moderate DOC concentration, 1.9 mgC/L, Fig. 1a). In addition, in water 2, coagulant doses above 1.5 mg/L Al₂O₃ produce overdosing problems both in terms of particulate and dissolved matter removal (Figs. 1a and 2a). In hydrophilic waters (water 3 and water 4), the same effect of DOC coagulant demand is observed but for lower coagulant concentrations: the optimal coagulant dose for chl_a removal is 1.5 mg/L Al₂O₃ for moderate-high DOC water (water 3) and 0.5 mg/L Al₂O₃ for water 4 (Fig. 1b). With these coagulant doses and conditions, removals higher than 92% were obtained for chl_a and MC intra, for both morphologies (Fig. 1). The specific coagulant demand, i.e. the coagulant required for chl_a removal over the water DOC, was computed for the four analysed waters (Table 2).

The results show that the coagulant demand depends on the DOC nature and the ratio is approximately 3, i.e. hydrophobic DOC requires ca. the triple dose required by hydrophilic DOC. This is due to an increased consumption of coagulant by the DOC in hydrophobic waters (C/C_i for DOC is lower in water 1 and water 2 than in water 3 and water 4, Fig. 2), associated with the weaker acidic groups of the

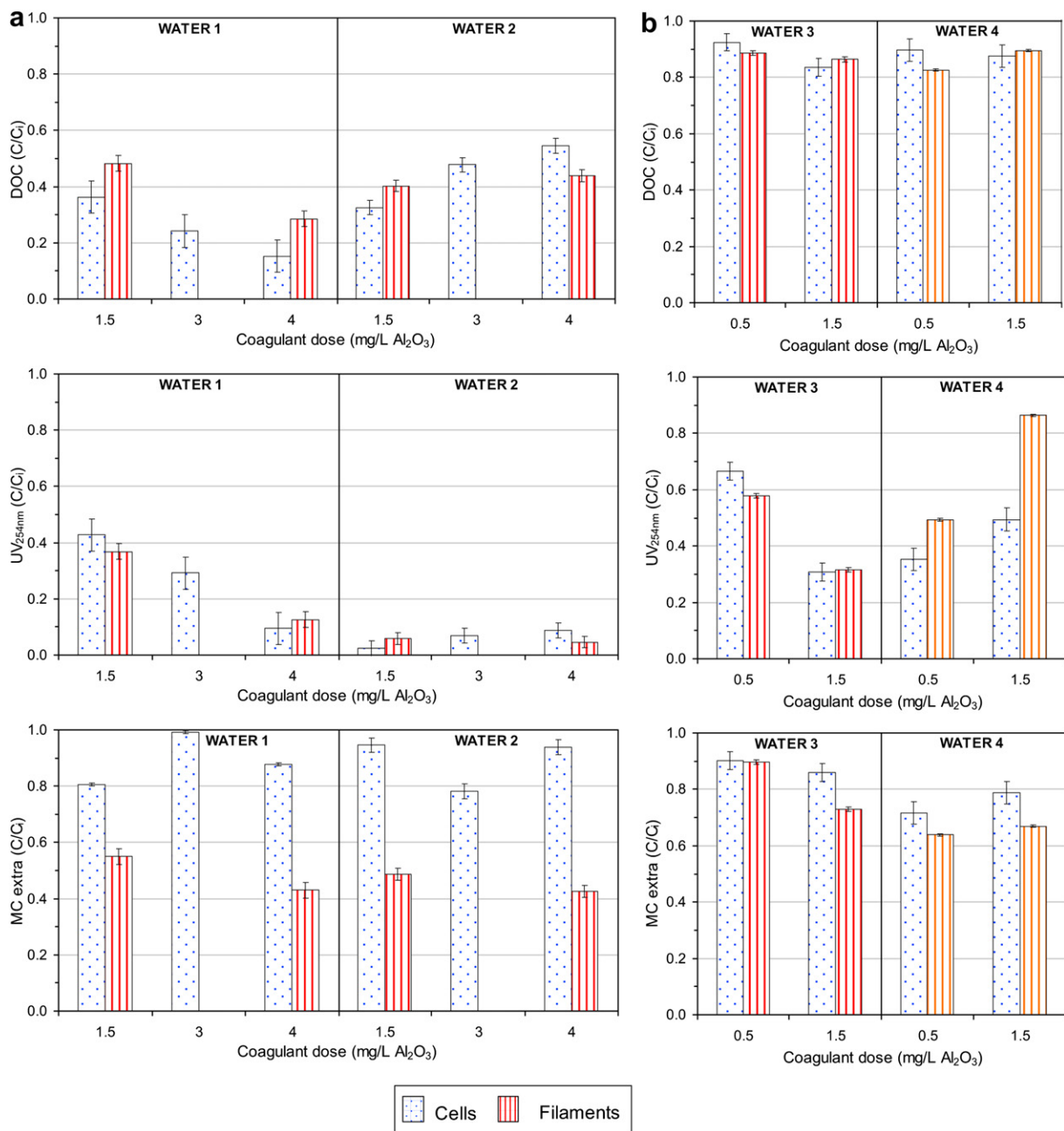


Fig. 2 – Normalised residuals of DOC, UV_{254nm} and MC extra after C/F/DAF treatment of water samples with hydrophobic (a) or hydrophilic (b) NOM.

hydrophilic fraction, with much higher values of their isoelectric point (Sharp et al., 2006) when compared with the hydrophobic fraction of NOM. Actually, due to its increased number of anionic binding sites, the hydrophobic NOM is more easily removed by charge neutralisation with cationic aluminium hydrolysis products than the hydrophilic NOM. Higher NOM removals were thus achieved for hydrophobic waters than for hydrophilic waters, both in terms of DOC and UV_{254nm} absorbing substances (humics) (Fig. 2).

A major issue is the maintenance of cell integrity during C/F/DAF treatment. The trends of the MC-LR and chl_a removals match (Fig. 1), which indicates an apparent removal

of intact cells. This feature is corroborated by MC extra data in Fig. 2, which shows no degradation of the water quality in terms of dissolved toxins: C/C_i is always below unity. No apparent release of toxins to the water was therefore observed for cells and filaments in the studied conditions, as already obtained for C/F/DAF of single cells of *M. aeruginosa* in tap water and in natural waters (Ribau Teixeira and Rosa, 2006a, 2007, respectively). However, Lahti et al. (2001) and Schmidt et al. (2002) reported some damage of *P. rubescens* filaments with subsequent release of toxins (MC-RR intra) to the water. Furthermore, while no significant removal of MC extra was observed in the experiments with *M. aeruginosa* cells

Table 2 – Specific coagulant demand for chl_a removal.

DOC character	DOC concentration	Code	mg coagulant/mg DOC
Hydrophobic (SUVA > 4 L/(m mgC))	Moderate-high (ca. 6 mgC/L)	w1	0.70
	Moderate (2–3 mgC/L)	w2	0.69
Hydrophilic (SUVA < 3 L/(m mgC))	Moderate-high (ca. 6 mgC/L)	w3	0.26
	Moderate (2–3 mgC/L)	w4	0.20

regardless of the water DOC nature and concentration, rather high and unexpected (Velzeboer et al., 1995; Hrudey et al., 1999; Drikas et al., 2001) removals of MC extra were found in the experiments performed with *P. rubescens* filaments. This behaviour was most likely associated with the interactions developed between the easily destabilised filaments (easier than cells), the water hydrophobic DOC and the hydrophobic microcystin molecules.

4. Conclusions

The present study investigated the DAF efficiency for removing different naturally occurring cyanobacterial morphologies, namely single cells of *M. aeruginosa* and filaments of *P. rubescens*, from four synthetic waters with different NOM contents (moderate vs. moderate-high DOC content, hydrophilic vs. hydrophobic NOM).

C/F/DAF process showed high removal efficiencies of cyanobacterial cells and filaments (90–100% for MC intra and 92–99% for chl_a), for both hydrophobic and hydrophilic waters, provided the specific coagulant dose was ensured. In addition, no apparent cell damage and release of microcystins to water were observed with the operating conditions tested. The results indicated charge neutralisation by the poly-aluminium chloride used as the main coagulation mechanism of the *M. aeruginosa* cells and most likely of the *P. rubescens* filaments. Finally, the specific coagulant demand was severely affected by the DOC nature, hydrophobic DOC requiring ca. the triple of hydrophilic DOC, i.e. 0.7 vs. 0.2–0.3 mg Al₂O₃/mg DOC.

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