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Influence of microalgae on organic micropollutants removal from water by powdered activated carbon

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Abstract: This study investigates how two morphologically distinct microalgae, *Chlorella vulgaris* and *Arthrospira platensis*, at different growth phases, affect the adsorption of ibuprofen, caffeine, and diclofenac onto two powdered activated carbons (AC1 and AC2). Experiments were utilizing dechlorinated tap water (DCTW) matrix where microalgae were added. Experiments were performed with/without pre-chlorination and with/without filtration through 0.45 µm filter to assess the influence of total and dissolved algal organic matter. Organic micropollutants (OMPs) were analyzed using gas chromatography coupled with mass spectrometry. Results indicate that the effect of microalgae morphology on OMPs' removal efficiency is different for different carbons. Species and growth phase-dependent variations were observed in some cases. *A. platensis* in the stationary phase in the water reduced diclofenac removal by AC2 (from 99 % to range of 44 %–62 %), while *C. vulgaris* in the exponential phase reduced it to the range of 16 %–74 % in comparison to effectiveness of AC2 in DCTW without microalgae (99%). Removal of uncharged caffeine remained stable, suggesting minimal influence from algal matrices or AC variability. For negatively charged ibuprofen and diclofenac the observed effects were more variable and not always consistent, likely due to limitations in experimental methodologies.

Keywords: adsorption; algal organic matter; drinking water treatment; ibuprofen; caffeine; diclofenac.

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INTRODUCTION

The growing presence of organic micropollutants (OMPs) in freshwater systems poses a significant challenge to environmental sustainability and public health.[1] In a recent study, over 600 chemicals (pesticides, biocides, pharmaceuticals) were screened across various European streams.[2] This extensive study found that in three-quarters of the sites investigated across 22 European river basin the concentrations of these chemicals often surpassed the safety limits set to protect freshwater ecosystems from harmful pollution.[2] Some of the most frequently detected compounds (>25 % of sites) included 1,3-diphenylguanidine, sucralose, gabapentin-lactam, 1H-benzotriazole, candesartan, DEET, lamotrigine, oxypurinol, melamine, caffeine, and guanylurea.[2] Caffeine (CF) was particularly notable, with its presence confirmed in more than 25 % of the sampling sites, with a maximum concentration of more than 1 µg L^{-1} at several sites.[2] According to the review study by Luo *et al.*[3] average concentrations or range of ibuprofen (IB) concentrations in surface water were found to be 0.98, nd-8.0, nd-1417, 1.0–67, <15–414, 5.0–280, 0.3-100 and nd-77 ng L^{-1} for Canada, France, China, Greece, Korea, Taiwan, UK and US, respectively, while environmental concentrations of diclofenac (DCF) exceeding 100 ng L^{-1} have been detected in many water bodies around the world, including Antarctica (1,000 ng L^{1}).[4] These OMPs are extensively used and recognized as anthropogenic markers within the group of pharmaceuticals and personal care products. A typical water treatment plant uses flocculation, coagulation, filtration, and disinfection to remove suspended solids and microorganisms. However, they are not specifically engineered to address the removal of micropollutants.[5,6] Among the various strategies employed to mitigate the impact of OMPs, adsorption techniques using powdered activated carbon, granulated activated carbon and also including biological activated carbon have emerged as effective treatment methods.[5,7,8] The large surface area and high porosity of powdered activated carbon (PAC) provides numerous adsorption sites, enabling it to remove contaminants at very low concentrations.[9] Also, PAC's implementation requires minimal modifications to the existing infrastructure of drinking water treatment plants, and its dosage can be easily adjusted to accommodate variations in raw water quality.[5] However, the presence of natural organic matter (NOM) in surface waters particularly dissolved organic matter (DOM) introduces significant challenges to the adsorption capacity of PAC.[10] NOM is known to negatively impact the adsorption of organic micropollutants by, first, directly competing for adsorption sites, and secondly because NOM molecules between 200 and 700 Da can cause blockage of the AC pores.[11,12] Nam *et al.*[13] highlighted in their study on adsorption characteristics of hydrophilic and hydrophobic OMPs (initial concentration of 100 ng L^{-1}), including DCF and CF, that the presence of DOM in surface river water significantly decreases the adsorption capacity of PAC (doses of 1, 5, and 20 mg L^{-1}), especially for

hydrophobic molecules. Based on their findings, DOM alters the surface charge of PAC and competes with OMPs for adsorption sites on the PAC surface. The impact of NOM is especially pronounced in surface waters, where algal blooms form algal organic matter (AOM).[14] Total AOM includes both intracellular and extracellular dissolved organic matter originating from algae. The presence of high-molecular-weight fractions (e.g. MW range 1,100-10,000 Da) in algal-rich waters, need higher PAC doses to achieve effective removal than the dose which is needed for lower MW range (20-1,100 Da). When present, charge balance and interaction potential of diverse amphoteric groups in low molecular weight NOM and proteinaceous low-aromaticity substances can cause their effective adsorbability, while high MW moieties have the tendency to block the pores of AC.[11] While PAC can effectively adsorb hydrophobic components of AOM, it has limited adsorption capacity for polysaccharides and proteins, which are the main components in AOM.[15] In the 1990s, microalgae research in drinking water focused on optimizing physical removal processes to prevent algal toxin release, later shifting to disinfection by-product (DBP) formation after chlorination.[16,17] Chlorination, used as disinfection method during drinking water treatment, disrupts algal cells, releasing intracellular organic matter which reacts with chlorine to form DBPs, which can also block active sites on PAC or alter its surface properties.[18,19] AOM is a complex DBP precursor in water treatment, with its composition, influenced by algal species and environmental conditions, affecting DBP formation potential and nature.[17] However, there is a lack of information regarding the influence of pre-chlorination on AOM structure and its consequences for subsequent activated carbon treatment steps. Similarly, how the presence of total and dissolved AOM originating from different microalgae growth phases and species influences OMP removal through adsorption on PAC is not fully understood. According to Tian *et al*.[20] AOM levels from green algae *Chlorella vulgaris* and *Scenedesmus obliquus* during the stationary phase increased under simulated conditions mimicking karst environments, with more cell metabolites being released. The stationary phase was characterized by a decrease in amino groups and an increase in aromatic protein-like compounds, while the exponential phase exhibited higher levels of amino structural material. When it comes to influence of morphology onto efficiency of water treatment for microalgae removal, Yu *et al*. [21] study emphasized the significance of divalent ions in removal of algae during a two-step process including first, preoxidation with $KMnO_4$, followed by coagulation with polyaluminium chloride. It was shown that complexation of divalent ions improved the removal of algae, and filamentous *Pseudanabaena* sp. was more efficiently removed compared to unicellular *Chlorella* sp. Ma *et al*.[22] investigated how microalgal morphology affects their removal efficiency in conventional coagulation–sedimentation–filtration water treatment processes, finding that smaller algae with irregular shapes, such as thorns

or flagellae were more difficult to remove. Laksono *et al*.[23] studied ultrafiltration membrane fouling by AOM substances and found that biopolymers, building blocks, and hydrophobic organic carbon significantly affected membrane performance. Algae lysing notably increased fouling severity. They also observed that microalgae cell shape and size influenced fouling: helical cells primarily blocked membrane pores, causing strong fouling, while rectangular and cylindrical cells caused milder, combined fouling.[23] Furthermore, related to investigating on how growth phase affect treatment efficiency, Park *et al*.[24] found that toxic colonial cyanobacterial species *Microcystis aeruginosa* at the stationary growth phase had higher cell density and extracellular organic matter, and both the exponential and stationary phases could be fully inactivated by chlorination during drinking water treatment, with faster toxin degradation occurring at the stationary phase. But based on our knowledge no research has examined how pre-chlorination in distinct growth phases influence the efficacy of PAC for OMP adsorption. For this lab-case study, we chose unicellular *Chlorella vulgaris* and filamentous *Arthrospira (Spirulina) platensis* in two growth phases (exponential and stationary) due to their distinct morphological differences and well-documented growth characteristics. Three widely prevalent in surface waters contaminants with varying hydrophilicities represented by different distribution coefficient (log D) values were chosen — ibuprofen (log D 0.45), caffeine (log D 0.28), and diclofenac (log D 1.37). Log D refers to corrected log P (octanol water partitioning coefficient) at epH 7.4 (Table SI).

The aim of this study is to assess how the distinct morphological differences and growth phases of microalgae *C. vulgaris* and *A. platensis* impact the adsorption of three different OMPs under various conditions, including the presence of total (tAOM) and dissolved algal organic matter (dAOM) and the effects of pre-chlorination. Although microalgae are present as mixed cultures in nature, we studied them separately to isolate their individual effects. By filling this gap, we seek to get more fundamental knowledge which might result in improved treatment strategies for water containing microalgae and emerging micropollutants in the future.

EXPERIMENTAL

The experiments aimed to assess how different microalgae types (*C. vulgaris* and *A. platensis*) and growth phases (exponential and stationary) influence the adsorption efficiency of powdered activated carbon for removing OMPs, focusing on the effects of tAOM and dAOM. The number of algae used in the experiments ranged from 2 to $8x10^8$ cells L^{-1}. The dAOM is the fraction remained after glass fiber filtration (GF filter 0.6 µm, Macherey-Nagel, Germany), and subsequent membrane filtration (MF filter 0.45 µm, Sartorious, US). Furthermore, effects of usually applied pre-chlorination in waterworks were investigated. 1 mg L^{-1} Cl_2 was added into the matrix containing algae for 30 min and afterwards dechlorination was performed. In this way, it was possible to investigate how the potential change of both, chlorinated tAOM and chlorinated dAOM structure and content affect the OMPs' adsorption. Adsorption tests were

performed on a shaker (KS 501-IKA, Germany) with shaking intensity of 180 rpm. Nominal concentration of selected OMPs was 1.5 - 2 µg L^{-1}. Applied dose (5 mg L^{-1}) of two selected powdered activated carbons was 2 h in contact with water sample that is close to real application rather than reaching equilibrium. The scheme of experimental methodology is shown in Supplementary as Fig S1. Adsorption tests were performed in 1 L bottles in a way that 0.7 L of each prepared matrix spiked with OMP (nominal Co= 1.5-2 µg L^{-1}) was shaken with 5 mg L^{-1} of AC1 or AC2 for 2 h. Afterwards, samples are filtered through glass-fiber filter (0.6 µm, Macherey-Nagel, Germany) and afterwards through 0.45 µm membrane filter (Sartorious, US) to remove PAC. First 300 mL of aliquot was discarded. From 400 mL collected, 250 mL was taken for further gas chromatography–mass spectrometry (GC/MS) analysis. Analysis of the OMPs was performed by the in-house developed GC/MS method.[25] Solid-phase extraction (60 mg, Oasis® HLB, Waters™, Massachusetts, USA) was used to prepare water samples for the analysis of selected OMPs. Details of the tested OMPs sample preparation and analysis are given in the Supplementary material under the Chapter Analytical method. Full method validation performed in DCTW is given in Supplementary material in Table SIII. For this study five water matrices were used for investigation (Fig. S1): i) Dechlorinated tap water (DCTW) without algae addition; ii) DCTW with total algal organic matter (tAOM); iii) DCTW with dissolved algal organic matter (dAOM); iv) Pre-chlorinated tAOM and v) Pre-chlorinated dAOM. All the details related to materials (water matrices, microalgae selection and cultivation, OMP stock solution preparation and PAC) used are given in Supplementary, Chapter Material. All adsorption tests were performed once within set I, and after nine months, some of them were repeated (set II). Repeatability of experiments without microalgae addition within both batches was tested for experiment testing adsorption on AC2 in DCTW (matrix (i), Fig.S1)), in 5 and 3 replicates, respectively. Repeatability of experiments with microalgae addition (matrix (ii), Fig. S1) was tested once in triplicate for stationary phase of each microalgae type. Quality control procedures of experimental methodology and GC/MS measurement are shown in Supplementary material under the Chapter Quality control procedures. Dissolved organic carbon (DOC) method characterization and obtained values for each of the tested matrices are presented in Supplementary Table SIV and SV.

RESULTS AND DISCUSSION

How microalgal morphology and growth phase affect the OMPs removal from different water matrices by two activated carbons is presented in Fig 1-3.

Ibuprofen

The removal of ibuprofen (IB) with and without the presence of different species of microalgae, in their different growth phases (exponential *vs.* stationary), in the presence of different type of AOM (tAOM vs. dAOM), and with /without chlorination is presented in Fig 1 (*A. platensis*, Fig. 1a and 1c vs. *C. vulgaris*, Fig. 1b and 1d). For the clarity of the following discussion we adopted the term "significant difference" between two removals if the error bars do not overlap and if the closest results of two cases which are compared are different more than 30 % (acceptance limit for quality control samples of the Environmental Protection Agency (EPA) Method 525.3)).[26]

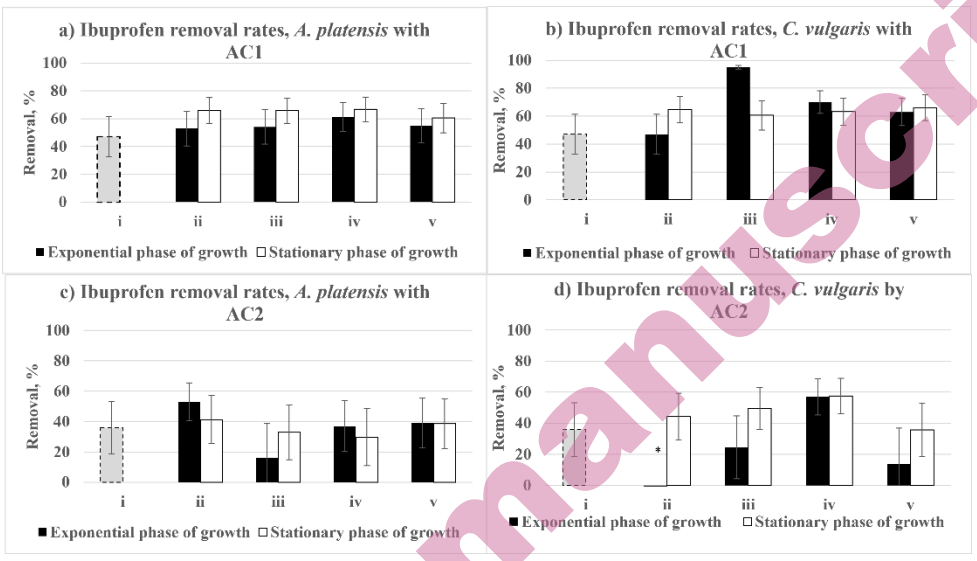

Figure 1. Efficiency of IB removal using PACs (dose 5 mg L^{-1}). i) – DCTW without microalgae addition (average value of five replicates). ii) – non-chlorinated matrix with tAOM; iii) - non-chlorinated matrix with dAOM; iv) - pre-chlorinated with tAOM; v) - pre-chlorinated with dAOM. * no removal efficiency; Letters a-d indicate ibuprofen removal rates for *A. platensis* with AC1 (a), *C. vulgaris* with AC1 (b), *A. platensis* with AC2 (c), and *C. vulgaris* with AC2 (d). Error bars show the error of the relative removal. The number of cells in matrices containing *A. platensis* in exponential and stationary phase of growth were $8x10^8$ and $4x10^8$ cells L^{-1}, respectively. For *C. vulgaris* these numbers in exponential and stationary phase of growth were $2x10^8$ and $5x10^8$ cells L^{-1}, respectively.

In DCTW without algae addition the removal efficiency of IB was 47±14 % by AC1 (Fig 1a and 1b, i). In matrix containing *A. platensis* in both phases of growth the removal efficiency ranged between 53±13 % and 66±9 % (Figure 1a, ii) showing no significant change in comparison to DCTW without algae addition (47±14 %, Fig. 1a, i). Similarly, in the second set of experiments (Table SVI), matrix (ii), which contained *A. platensis* in the exponential phase of growth, no significant difference compared to DCTW (51±3 % vs 77±6 %, respectively) was observed, indicating good repeatability after nine months. A significant increase of removal efficiency was noticed in the presence of *C. vulgaris* in exponential phase in dAOM matrix (Fig. 1b, iii) in comparison to DCTW matrix (95±1 % vs 47 ±14 %). Similarly, significant difference (48 %) in removal of IB between non-chlorinated tAOM (removal 47±14 %) and dAOM (removal 95±1 %) in matrix containing *C. vulgaris* in exponential phase was also noticed (Fig. 1, b, ii and iii), indicating difference between competition mechanisms between tAOM and dAOM matrices under given conditions. The set of repeated experiments after nine months did not include any of these tests, so there was no additional confirmation of the effect of AOM, originating from *C. vulgaris* in exponential phase. Same

matrix showed a slight but still not significant difference between non-chlorinated algae containing matrix dAOM (removal 95±1 %, Fig. 1, b, iii) and pre-chlorinated algae containing matrix with dAOM (removal 63±10 %, Fig. 1, b, v) which, if present could have indicated potential effect of pre-chlorination on competitiveness of the AOM moieties. However, this finding was also not possible to confirm for particular pair of tests since only one of the tests was repeated. In the matrix containing *C. vulgaris* in the stationary phase of growth (Fig. 1, b, ii, iii, iv, v) removal of IB by AC1 remained constant (61±11 % to 66±9 %). When it comes to the other activated carbon, AC2, similar removal efficiency of IB in DCTW matrix without algae addition (36±17 %, Fig. 1, c, d, i) in comparison with AC1 (47±14 %) was observed. In a matrix containing *A. platensis* in both growth phases, no significant change was observed compared to DCTW without algae addition (36±17 %) (Fig. 1, c, ii-v). For the same matrices containing *A. platensis* in the stationary phase no significant differences were observed (removal rates ranged between 30±19% and 41±16%, Fig. 1, c, ii-v). Likewise, in most of the tests repeated after nine months for the same algae and growth phase, no significant differences were found between the removals observed in set I and II (Arth-s, AC2, Table SVI). Only one of four tests had significant differences (Arth-s, matrix iv, Table SVI). Also, tests in stationary phase did not show significant decreases of removal in comparison to DCTW matrix without algae except the matrix iv (Arth-s, AC2, Table SVI). In the non-chlorinated tAOM matrix containing *C. vulgaris* in exponential growth phase no removal was observed (Fig. 1, d, ii), indicating high potential for competition of the tAOM when compared to DCTW without algae addition (36±17 %). However, this was not confirmed in repeated tests after nine months when removal rate was 54±12 % (Chlor-e, matrix ii, AC2, Table SVI). Similarly, significant removal rate differences were noticed between non-chlorinated tAOM (Fig. 1, d, matrix ii, no removal) and pre-chlorinated tAOM (Fig. 1, d, iv, 57±12 %), indicating that pre-chlorination can positively affect adsorption. However, during the second set of experiments opposite effect was observed between non-chlorinated tAOM (54±12 %) and pre-chlorinated tAOM (no removal), indicating contradicting effect of higher potential for competition in pre-chlorinated samples. For matrices containing *C. vulgaris* in the exponential growth phase, a slight difference was observed between pre-chlorinated tAOM (57±12 %) compared to pre-chlorinated dAOM (14±23 %) (Fig. 1, d, iv, v). Repeated experiments (Table SVI, Chlor-e, AC2, matrix iv and v) showed somewhat higher removal efficiency in pre-chlorinated dAOM matrix (28±19 %) in comparison to pre-chlorinated tAOM matrix (no removal). For matrices containing *C. vulgaris* in the stationary growth phase, the removal rates by AC2 stabilize between 36±17 % and 58±11 % (Fig. 1, d, ii-v) with no significant difference. Although differences between growth phases were observed,only for non-chlorinated tAOM matrix containing *C. vulgaris* (Fig. 1, d, ii) the impact of

algal growth phase cannot be conclusively confirmed. In the exponential growth phase no removal was observed while in the stationary phase an increase of 44±15 % was noticed. The difference in cell number between exponential and stationary phases exceeded 50 % (Fig. 1 b, d), which is well above the known 20–30 % uncertainty of the hemocytometer counting method. While these differences reflect realistic physiological states of algae, they may have also contributed to observed variability in IB removal. Therefore, due to differences in both physiological composition and biomass quantity, the effect of growth phase cannot be reliably isolated in this case. Repeated experiments again did not confirm such an effect showing no difference between measurements in two phases of growth (54±12 % and 56±12 %, Table SVI). In the second set of experiments, the difference in cell density between growth phases was within the 20–30% measurement uncertainty, suggesting that the growth phase did not have a meaningful effect on IB removal (3 x10^8 and 4x10^8 cell L^{-1}, Table SVI).

Caffeine

Results for caffeine (CF) removal across all matrices and both microalgae species are shown in Fig. 2.

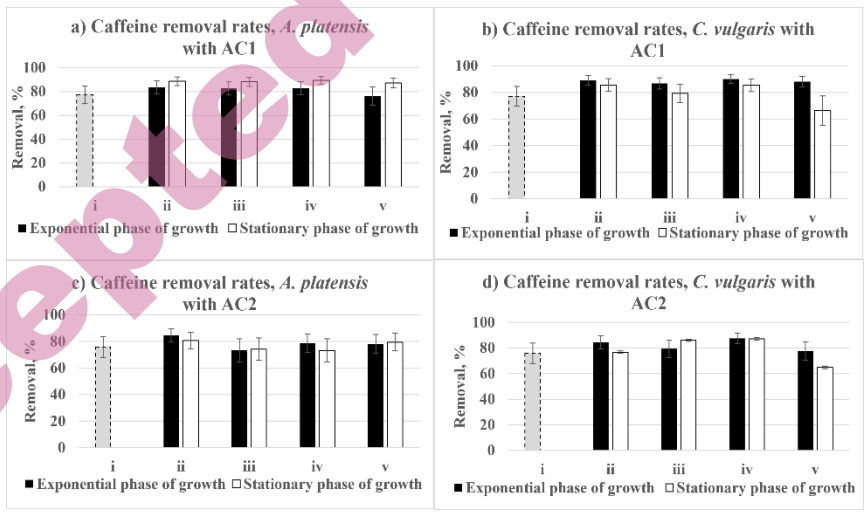

Figure 2. Efficiency of CF removal using PACs (dose 5 mg L^{-1}). i) – DCTW without microalgae addition (average value of five replicates). ii) – non-chlorinated matrix with tAOM; iii) - non-chlorinated matrix with dAOM; iv) - pre-chlorinated with tAOM; v) - pre-chlorinated with dAOM. Letters a-d indicate caffeine removal rates for *A. platensis* with AC1 (a), *C. vulgaris* with AC1 (b), *A. platensis* with AC2 (c), and *C. vulgaris* with AC2 (d). Error bars show the error of the relative removal. The number of cells in matrices containing *A. platensis* in exponential and stationary phase of growth were 8x10^8 and 4x10^8 cells L^{-1}, respectively. For *C. vulgaris* these numbers in exponential and stationary phase of growth were 2x10^8 and 5x10^8 cells L^{-1}, respectively.

Caffeine removal rates in DCTW without microalgae were 77±7 % for AC1 and 76±8 % for AC2. Removal rates remained essentially stable (from 66±11 % to 90±3 % for AC1 and from 65±11 % to 87±4 % for AC2) with no significant differences in removal across the matrices and conditions (Fig. 2). In the second set of experiments removals were 93±2 % and 92±3 % for AC1 and AC2, respectively (Table SVI). Algae addition caused the change of rates in the ranges of 76±4 % to 88±8 % for AC1 and 64±12 % to 92±3 % for AC2. Only one sample with *A. platensis* in stationary phase showed high discrepancy with a removal rate of 19±26 %.

Diclofenac

Results for diclofenac removal across all matrices and both microalgae species are shown in Fig. 3.

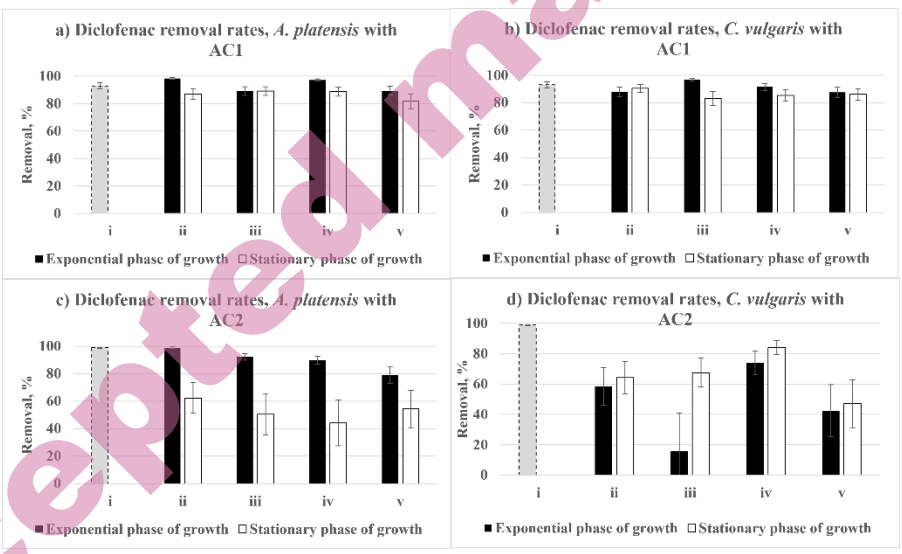

Figure 3. Efficiency of DCF removal using PACs (dose 5 mg L^{-1}). i) – DCTW without microalgae addition (average value of five replicates). ii) – non-chlorinated matrix with tAOM; iii) - non-chlorinated matrix with dAOM; iv) - pre-chlorinated with tAOM; v) - pre-chlorinated with dAOM. Letters a-d indicate DCF removal rates for *A. platensis* with AC1 (a), *C. vulgaris* with AC1 (b), *A. platensis* with AC2 (c), and *C. vulgaris* with AC2 (d). Error bars show the error of the relative removal. The number of cells in matrices containing *A. platensis* in exponential and stationary phase of growth were $8x10^8$ and $4x10^8$ cells L^{-1}, respectively. For *C. vulgaris* these numbers in exponential and stationary phase of growth were $2x10^8$ and $5x10^8$ cells L^{-1}, respectively.

In experiments using DCTW without microalgae, removal rates for DCF were 93±2 % for AC1 and 99±0.3 % for AC2 (Fig. 3, i). A second set of experiments after nine months (Table SVI) showed repeatable removals of 90±3 % and 85±4 %, respectively. None of the factors (morphology, growth phase - even difference

in cell number between exponential and stationary phases often exceeded 50 %, chlorination or AOM type) had a significant effect on DCF removal with AC1 for both *A. platensis* and *C. vulgaris*, as removal rates ranged from 82±5 % to 99±0.5 % (Fig. 3, a) and 83±5 % to 97±1 % (Fig. 3, b), respectively, across all matrices. Good repeatability of experiments was achieved after nine months, when removal values ranged between 69±9 %–90±3 % (which is in line with the acceptance limit for quality control samples)[27]. All repeated matrices with AC1 showed insignificant difference (≤30 %) compared to first adsorption set (Table SVI). In the case of AC2 use it was shown that the stationary phase of *A. platensis* significantly decreased DCF removal rates in all tested matrices in comparison to removal in DCTW without algae (they are from 44±17 to 62±11 %, Fig. 3, c, ii-v), but the difference in between tested matrices remains insignificant. It should be noted that the result may have been influenced by the approximately twofold lower cell number in the stationary phase of growth, and therefore the effect of the growth phase cannot be clear. In the repeated experiments with *A. platensis* in the stationary phase after nine months, similar results were obtained. Pre-chlorinated tAOM (matrix iv) showed the highest removal drop compared to DCTW without microalgae (30±21 % *vs.* 85±4 %, respectively, Table SVI). Contrarily, for *C. vulgaris*, significant differences in DCF removal range with AC2 (Fig. 3, d) were observed when microalgae were in the exponential growth phase (the removal rate dropped sharply from 99±0.3 % in DCTW to 58±12 % in non-chlorinated tAOM and 16±25 % in the non-chlorinated dAOM matrix (Fig 3. d, i, ii, iii). However, this was not clearly confirmed between the tAOM and dAOM matrix in the second set of experiments after nine months (Table SVI, matrices ii and iii showed 74±8 % and 39±18 % respectively). In this case, the difference in cell number between exponential growth phases during the first and second set was within the 20–30 % measurement uncertainty, without expectation of algal cell influence. Similarly, a significant difference was not possible to show in the matrix containing *C. vulgaris* in the exponential growth phase for AC2 removal efficiency in the pre-chlorinated dAOM matrix (43±17 %) in comparison to pre-chlorinated tAOM (removal efficiency 74±8 %) (Fig. 3, d, iv, v). Similar effect was obtained in the repeated experiment (Table SVI) as well as in the case when water matrix contained *C. vulgaris* in the stationary phase between pre-chlorinated tAOM and dAOM (84±5 % *vs.* 47±16 %, respectively, Fig. 3, d, iv, v). Repetition for this pair of tests was not performed.

Performance of activated carbons in matrices without algae differed to some extent in comparison to literature findings. Thus, Westerhoff *et al.*[27] reported lower removals of 16% and 39% for IB and DCF respectively (PAC dose 5 mg L^{-1}, contact time 4 h) in surface waters spiked with 100 ng L^{-1} OMPs, while, similarly to our results. Nam *et al.*[13] observed high removal (>90 %) of DCF from distilled water, while in surface water removal under the same experimental conditions (at

Co=100 ng L^{-1}, PAC dose 5 mg L^{-1}, contact time 4 h), was around 80 %. Snyder *et al*.[28] found that IB, CF and DCF average removal from four surface water was 15 %, ~70 % and ~40 %, respectively, at a PAC dose of 5 mg L^{-1} (Co=100 ng L^{-1}, contact time 4 h). The reasons for those differences in comparison to our study are most likely due to the use of different matrices (DCTW vs. surface water) and a higher OMPs initial concentration in this study (1.5-2 µg L^{-1} *vs.* 100 ng L^{-1}). Furthermore, PAC type, dose and different contact time can also influence results. High removal efficiency (~80 %) of hydrophilic CF (logD 0.28) by PAC is similar to results of Nam *et al*.[13] and Piel *et al*.[29] It could be due to a strong affinity of heterocyclic-N group in his molecular structure for the carbonaceous surfaces, and specific sorbate–sorbent interactions.[13] Notably, the removal of IB was less efficient compared to that of CF and DCF, indicating potential differences in adsorption behavior based on the compounds' molecular properties (Table SI). In all cases, effects depended on the type of carbon and on the type of substance which was tested. Microalgae morphology and growth had an effect in some cases, but their influence was more context-dependent and secondary influenced by the type of PAC used. In general, it was observed that addition of microalgae increased the value of DOC by more than 13 %, which corresponds to the measurement accuracy of the method for measuring DOC (Table SIV and SV). Rare significant differences for IB removal were observed in the presence of *C. vulgaris* during the exponential growth phase in the first set of single experiments for AC1, particularly between matrices containing tAOM and those with only dAOM. In the case of AC2, AOM had significant effects in certain instances but not clearly confirmed in repeated experiments. The removal of CF remained stable and was not affected by the investigated matrix influences. The removal of DCF significantly decreased in presence of algae only when AC2 is used in matrices containing *A. platensis* in the stationary growth phase and in some cases when *C. vulgaris* was in exponential growth phase. One can hypothesize that the opposite effects of tAOM and dAOM obtained by filtration (increase of IB removal by AC1 and decrease of DCF removal by AC2 in matrix iii containing *C. vulgaris* in the exponential growth phase), might be caused by the chemical composition of the dAOM and characteristics of OMPs. In this phase of growth *C. vulgaris*, has high lipid (20–38 %) and protein (42–58 %) content.[30] *A. platensis*, has lower lipid (5–20 %) and higher protein (50–70 %) content.[31,32] This may cause different interactions in between dAOM and PAC adsorption sites as well as formation of complexes of dAOM and OMPs of different characteristics. The lipid fraction of DOC may promote IB and DCF focus through complex formation, while proteinaceous compounds on AC can release amino acids, increasing competition for adsorption sites, especially for hydrophobic OMPs like DCF (*A. platensis*, stationary phase, AC2). Aromatic rings in IB and DCF also enable electron donor-acceptor complexes and π-π interactions with AC functional groups, enhancing

removal.[33] Negatively charged IB and DCF seem to be more affected by those matrix effects changes than neutral caffeine. Those interactions can further change when AOM structure is changed by pre-chlorination. According to Pivokonsky *et al.*[11] chlorination can lead to the reduction in adsorption due to the increased hydrophilicity and molecular size changes. In this study, a slight adsorption decrease of DCF was observed, but also a consistent increase in adsorption in the case of IB, which was not sufficiently supported by the confirmatory experiments, which often yielded contradictory results. Inconsistent effects in repeated experiments likely resulted from uncertainties in matrix preparation and DOC composition, including variations in DOC quality between sets. The second set of measurements showed good reproducibility, with removal efficiency differences over 30% in few cases, but significant measurement uncertainties prevent confirming all effects. Impacts on charged molecules are evident but inconsistent.

CONCLUSION

This study evaluated the impact of the morphological characteristics and growth phases of *C. vulgaris* and *A. platensis* on the adsorption of IB, CF and DCF from water under various conditions, including the presence of total and dissolved algal organic matter and pre-chlorination. It was confirmed that different carbons have different ability to compensate for matrix variability. The results confirm that the removal efficiency of the uncharged CF remained stable across all tested conditions, suggesting minimal influence from algal matrices or PAC variability. For negatively charged IB and DCF, the observed effects were more variable. Significant differences were observed between algal matrices and PAC types, particularly for *C. vulgaris* in the exponential growth phase and *A. platensis* in the stationary phase. These effects were not consistently reproduced, likely due to variability in living systems, including changes in DOC structure and composition causing inconsistent interactions. The findings underscore the complexity of adsorption in living systems, where DOC variability and PAC interactions hinder reproducibility. Inconsistent effects highlight the need for further investigation into DOC behavior and its interactions. Future studies should assess the process in real surface waters with natural organic matter and algae, under varying environmental and seasonal conditions, to evaluate its robustness and scalability for practical application.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/13180, or from the corresponding author on request.

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И З В О Д

УТИЦАЈ МИКРОАЛГИ НА УКЛАЊАЊЕ ОРГАНСКИХ МИКРОПОЛУТАНАТА ИЗ ВОДЕ АКТИВНИМ УГЉЕМ У ПРАХУ

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У овом раду испитан је утицај две врсте микроалги различите морфологије *Chlorella vulgaris* и *Arthrospira platensis*, у различитим фазама раста, на адсорпцију ибупрофена, кофеина и диклофенака на 2 врсте активног угља у праху. Дехлорисана чесменска вода са додатком микроалги је коришћена као матрикс. Такав матрикс у експериментима је коришћен са/без предхлорисања и са/без филтрације кроз филтер од 0,45 μм како би се проценио утицај укупне и растворене алгалне органске материје. Одабрани органски микрополутанти (ОМП) анализирани су гасном хроматографијом са масеном спектрометријом. Резултати показују да је ефекат морфологије микроалги на ефикасност уклањања ОМП различит за различите угљеве. У неким случајевима примећене су варијације зависне од врсте микроалги и фазе раста. Присуство *A. platensis* у стационарној фази у води смањило је уклањање диклофенака активним угљем 2 (са 99 % на опсег од 44 %–62 %), док је присуство *C. vulgaris* у експоненцијалној фази изазвало пад на опсег од 16 %–74 % у поређењу са ефикасношћу активног угља 2 у дехлорисаној чесменској води без микроалги (99%). Уклањање неутралног кофеина је стабилно, што указује на минималан утицај алгалног матрикса или типа активног угља. За негативно наелектрисане ибупрофен и диклофенак, уочени ефекти су били променљиви и недоследни услед ограничења у експерименталним методологијама.

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