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Impact of wastewater characteristics on the removal of organic micropollutants by *Chlorella sorokiniana*

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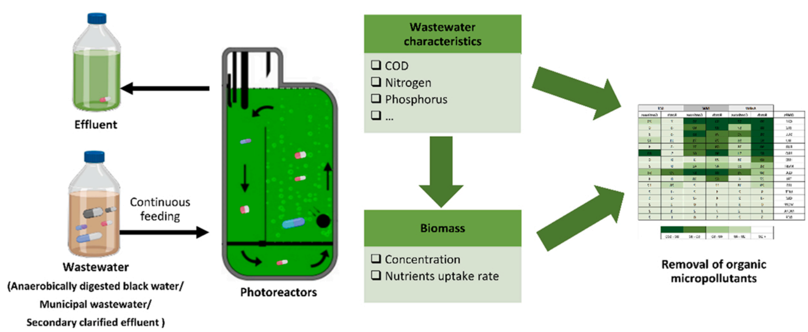
HIGHLIGHTS

- Wastewater with high nutrient and COD favored the removal of 11 tested OMPs.
- Poor nutrient concentrations in wastewater lead to poor removal of 13 tested OMPs.
- Biomass concentration and wastewater COD predominantly affected the removal of OMPs.
- Carbon uptake rate significantly affected the removal of OMPs.

GRAPHICAL ABSTRACT



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ABSTRACT

Microalgae-based technologies can be used for the removal of organic micropollutants (OMPs) from different types of wastewater. However, the effect of wastewater characteristics on the removal is still poorly understood. In this study, the removal of sixteen OMPs by *Chlorella sorokiniana*, cultivated in three types of wastewater (anaerobically digested black water (AnBW), municipal wastewater (MW), and secondary clarified effluent (SCE)), were assessed. During batch operational mode, eleven OMPs were removed from AnBW and MW. When switching from batch to continuous mode (0.8 d HRT), the removal of most OMPs from AnBW and MW decreased, suggesting that a longer retention time enhances the removal of some OMPs. Most OMPs were not removed from SCE since poor nutrient availability limited *C. sorokiniana* growth. Further correlation analyses between wastewater characteristics, biomass and OMPs removal indicated that the wastewater soluble COD and biomass concentration predominantly affected the removal of OMPs. Lastly, carbon uptake rate had a higher effect on the removal of OMPs than nitrogen and phosphate uptake rate. These data will give an insight on the implementation of microalgae-based technologies for the removal of OMPs in wastewater with varying strengths and nutrient availability.

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1. Introduction

Organic micropollutants (OMPs) are only partially removed by conventional wastewater treatment plants (WWTP), resulting in their accumulation into surface water [21,45]. Since many OMPs, consisting of pharmaceuticals, personal care products and pesticides, are biologically active and persistent, they can negatively affect aquatic organisms, such as zooplankton, aquatic vertebrates and invertebrates [2,62]. It is therefore crucial to develop technologies for removing OMPs from wastewater, that are effective and sustainable (i.e. low requirement of energy and materials). Microalgae-based technologies fit these requirements [27,47].

Microalgae-based technologies can efficiently remove a wide range of OMPs from different types of wastewater under various environmental conditions [27,40]. In batch experiments where *Chlorella sorokiniana* was grown on diluted anaerobically digested black water (AnBW; 683 mg $COD_{soluble}$/l; 24.5 mg PO_4^{3-}- P/l; 540 mg NH_4^+- N/l), 60–100% of metoprolol, paracetamol, diclofenac, and ibuprofen were removed [57]. A pilot high-rate algal pond (HRAP), inoculated with *Chlorella vulgaris*, removed 51–90% of ibuprofen, methylparaben, and oxybenzone from municipal wastewater (MW; 247.3 mg TOC/l; 1.4 mg PO_4^{3-}- P/l; 20.1 mg NH_4^+- N/l; 0.4 mg NO_3^- - N/l) under semi-batch mode [46]. Batch reactors inoculated with *Chlorella sp.* and *Scenedesmus sp.*, removed 99% and 95% of caffeine and ibuprofen respectively from mixed wastewater (25% urban wastewater + 75% groundwater; 60.3 mg $COD_{soluble}$/l, 0.4 mg PO_4^{3-}-P/l; 11.8 mg NH_4^+-N/l; 28.5 mg NO_3^--N/l) [33]. Ferrando & Matamoros [7] showed that an immobilised microalgae-based system removed 64–94% of sulfamethoxazole and 43–73% of mecoprop from modified ground water (5 mg PO_4^{3-}-P/l; 200 mg NO_3^--N/l) under continuous conditions. This shows that besides microalgae species, OMPs concentrations and operation conditions of reactors, wastewater characteristics might play an important role in the removal of OMPs.

Most studies on microalgae technologies for wastewater treatment focused on the removal of OMPs in one type of wastewater [36,42,54]. However, only very few studies have investigated the effect of wastewater characteristics on OMPs removal [57]. In our previous work where *Chlorella sorokiniana* was cultivated in batch bottles, it was shown that the dissolved organic matter (DOM) in AnBW can act as a photosensitizer, therefore inducing more ibuprofen removal by photodegradation than in artificial urine [57]. DOM can also influence the OMPs removal via enhancing or supressing microalgal biodegradation [10,56,61]. For example, Wang et al.[56] found that 0.3 g/l of glucose increased the removal of carbamazepine from 30% to 50% via the enhancement of carbamazepine biodegradation in the batch experiments with *Spirulina platensis*. Gatidou et al. [10] showed that 1 g/l of sodium acetate in artificial medium decreased benzotriazole removal from 20% to 80% via the suppression of benzotriazole biodegradation in the batch experiments with *Chlorella sorokiniana*. Compounds with similar structures in wastewater DOM might also affect the OMPs removal by similar mechanisms. On the other hand, many studies on the removal of OMPs from wastewater using microalgae and microalgae-bacteria consortium technologies have been conducted under batch mode [33,4,6]. Biological processes in WWTP are operated under continuous or semi-continuous modes [15]. Operational mode (batch, semi-batch or continuous mode) can remarkably affect the efficiency of microalgae-based OMPs removal [51]. Previous study showed that continuous mode (2–8 d HRT) achieved 50% higher removal of chlorpyrifos and pentachlorobenzene than batch mode for 14 days, when a microalgal consortium dominated by *Chlorella sp.* and *Scenedesmus sp.* was applied [32]. The authors proposed that the continuous mode of operation (2–8 d HRT) increased the contact time between OMPs and biomass, thus increasing the removal of OMPs. Therefore, understanding the removal of OMPs under continuous mode of operation is crucial for scaling up microalgae-based technologies for wastewater treatment and completing the picture of the potential of such technologies for OMPs removal.

In this study, the growth of *Chlorella sorokiniana* in three types of wastewater was followed and the effect of sixteen OMPs on its growth was assessed under batch and continuous mode of operation. Further, the removal efficiencies of these sixteen OMPs were investigated. Wastewater characteristics, biomass growth, and the overall removal of OMPs were correlated to elucidate which parameters predominantly affect the removal of OMPs.

2. Materials and methods

2.1. Cultivation medium

AnBW, MW and secondary clarified effluent (SCE) were selected as the cultivation media for the experiments since they have distinct characteristics in terms of soluble COD, nitrogen, phosphorus, and C/N/P molar ratio (Table 1). Dissolve inorganic nitrogen (DIN) in all wastewater only consist of ammonia and nitrate due to the absence of nitrite.

AnBW was collected from a UASB reactor treating vacuum-collected black water of a two-person household in Wageningen, The Netherlands. MW and secondary clarification effluent (SCE) were collected from the WWTP of Bennekom, The Netherlands. In this WWTP, municipal wastewater is treated by conventional activated sludge technology, followed by a settling tank and a sand filtration. Municipal wastewater refers to the influent of the WWTP, while secondary clarified effluent refers to the effluent of the settling tank.

After collection, all three types of wastewater were autoclaved at 121 ℃ for 90 min to remove potential human pathogen contamination. AnBW was further centrifuged at 4500 rpm for 5 min to remove suspended solids and prevent clogging of the tubings feeding the photobioreactors. All wastewater were stored at 4 ℃ under anaerobic conditions until use.

2.2. Microalgae species

Chlorella sorokiniana originated from the culture collection at the Netherlands Institute of Ecology (NIOO- KNAW), The Netherlands. It was maintained in M8a medium [23] at 35 °C under continuous average irradiation of 80 µmol m^{-2} s^{-1}.

2.3. Target OMPs

Sixteen OMPs were selected based on the diversity of therapeutical class, the measurability, the persistency in wastewater and aquatic ecosystems, and the guideline list of OMPs released by the Dutch Foundation for Applied Water Research (STOWA) [12,38]. The OMPs are caffeine (CAF), trimethoprim (TRI), propranolol (PRO), carbamazepine (CBZ), sulfamethoxazole (SUL), benzotriazole (BTZ), 4/5-methylbenzotriazole (MeBT), clarithromycin (CLA), irbesartan (IRB),

Table 1
Average characteristics (± standard deviation) of the three types of wastewater (n = 3).

	AnBW	MW	SCE
pH	10.1 ± 0.5	7.6 ± 0.2	8.5 ± 0.4
$COD_{soluble}$ (mg/l)	1570 ± 31	681 ± 5	31 ± 3
TSS (mg/l)	206 ± 21	168 ± 4	< DL
VSS (mg/l)	191 ± 19	158 ± 3	< DL
Alkalinity (mg $CaCO_3$/l)	875 ± 13	185 ± 2	94 ± 0
TN (mg/l)	1912 ± 18	83 ± 2	4 ± 0
NH_4^+-N (mg/l)	1291 ± 31	63 ± 3.1	0.2 ± 0.0
NO_3^--N (mg/l)	<DL	< DL	2 ± 0
DIN (mg/l)	1291 ± 31	63 ± 3	2 ± 0
TP (mg/l)	152 ± 4	9 ± 0.2	0.2 ± 0.0
PO_4^{3-}-P (mg/l)	100 ± 4	8 ± 0.2	0.2 ± 0.0
N/P molar ratio	29/1	17/1	23/1

DL = Detection limit.

metoprolol (MET), diclofenac (DCF), ibuprofen (IBU), furosemide (FUR), hydrochlorothiazide (HYD), mecoprop (MCPP), and 2-methyl-4-chlorophenoxyacetic acid (MCPA). The OMPs were spiked in wastewater according to Wu et al. [58]. We spiked each OMP in the cultivation media to a final concentration of 6 μg/l. Caffeine was already present in the non-spiked AnBW and MW, thus reaching a final concentration of respectively 183 ± 3 and 25 ± 0 μg/l.

2.4. Experimental set-up

Four 380 ml flat panel photobioreactors (PBRs) were inoculated with 133 ± 4 μg chlorophyll a/l of *C. sorokiniana*. Two replicate PBRs were fed with OMPs spiked wastewater (treatment reactors), while the other two replicate PBRs were fed with non-spiked wastewater (control reactors). Each PBR had a light path of 14 mm, and an illuminated area of 0.027 m^2. Optimal temperature (35 °C) and pH (6.8 ± 0.1) for the growth of *C. sorokiniana* were automatically controlled [52]. The content of each PBR was homogeneously mixed by bubbling air enriched with 10% CO_2 at a flow rate of 400 ml/min. During the AnBW and MW experiments, the light regime followed a sinus curve with a maximum average light intensity (400–800 nm) of 150 μmol m^{-2} s^{-1} and a 16:8 (light: dark) cycle. In the SCE experiment, the maximum average light intensity was 100 μmol m^{-2} s^{-1} to prevent light inhibition on microalgal growth.

The PBRs were initially operated in batch mode until the end of the exponential growth phase of microalgae. Then a continuous mode of operation (0.8 d HRT) was applied until the end of the experiment, when a steady state was reached. Steady state is defined as the period when the dry weight and chlorophyll a are stable for a minimum of five consecutive days, with a maximum standard deviation up to 5%.

2.5. Analytical methods

Algal biomass was daily quantified by dry weight and chlorophyll a during the continuous mode of operation. Dry weight was measured by a standard method of Rice & American Public Health Association [44], and chlorophyll a was measured by a PhytoPAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). Both measurements were performed in duplicate.

The elemental composition of dried biomass was determined in duplicate during steady state. For the analysis of biomass C and N content, a dried biomass sample was placed into a small tin cup and measured in an organic elemental analyzer (Flash 2000, Interscience Breda). For the analysis of P, the dried biomass was combusted at 550 ℃ for 30 min and digested with 10 ml persulfate (2.5%) at 121 ℃ for 30 min. The digested supernatant was used for P measurement by a PhosVer® 3 Phosphate Reagent Powder Pillow (Hach Lange, The Netherlands). For dissolved inorganic nutrients (NH_4^+-N, NO_3^--N, NO_2^--N, and PO_4^{3-}-P), 2 ml of AnBW or MW samples, or 10 ml of SCE samples, were filtered with a 0.2 μm cellulose acetate filter (VWR, The Netherlands), diluted with demi water to a final volume of 10 ml, and measured using a Seal QuAAtro39 AutoAnalyzer (SEAL, Analytical Ltd., Southampton, UK).

Prior to OMPs measurement, 5 ml of microalgal biomass samples were daily collected from the PBRs. After centrifugation (4500 rpm, 10 min), 3 ml of supernatant was used for solid phase extraction [58]. Solid phase extraction recoveries of sixteen OMPs were 40–103% with a standard deviation up to 10% (Table S1).

OMPs, except for IBU and FUR, were measured by a liquid chromatograph coupled to a triple quadruple mass spectrometer (LC-MSMS) as described in Wu et al. [58]. CAF (transition; 195.0 -> 138.0), TRI (291.0->230.0), PRO (260.0 -> 116.0), CBZ (237.0 -> 194.0), SUL (254.0 -> 92.0), BTZ (120.0 -> 65.3), MeBT (134.0 -> 77.2), CLA (748.5 -> 158.0), IRB (429.2 -> 207.1), MET (268.0 -> 116.0), DCF (296.0 -> 214.0), and HYD (296.0 -> 268.8) were measured in positive ionisation model, while MCPP (213.0 -> 141.0) and MCPA (199.0 -> 141.0) were measured in negative ionisation mode.

IBU and FUR were measured by an ultra-high performance liquid equipped with a tandem mass spectrometer as described in van Gijn et al. [13]. IBU (205.0 ->161.2) and FUR (328.9 -> 285.0) were measured in negative ionisation mode.

The measurement error was 10% for MCPA and 5% for other OMPs in our study (data not shown). Thus, removal lower than 10% was regarded as negligible for MCPA, and lower than 5% for all other OMPs.

2.6. Statistical analyses

The statistical analyses were conducted to elaborate on the effect of wastewater characteristics ($COD_{soluble}$, DIN, and PO_4^{3-}-P), kinetic parameters (chlorophyll a, dry weight and growth rate), and nutrient (C/N/P) uptake rate of the biomass on the removal of OMPs during steady state. Since the removal of MET, CBZ, MCPP, MCPA, and DCF was negligible in all wastewater, these five OMPs were not included in the statistical analyses.

The nutrient uptake rate of the biomass was calculated based on the C/N/P ratios of biomass: the C/N/P ratios were 179/22/1 for AnBW, 265/26/1 for MW, and 675/26/1 for SCE, respectively. The Principal Component Analysis (PCA) showed the dimension reduction of the OMPs data. The first two principal components PC1 and PC2 were used to represent the OMPs removal data. To determine the limiting conditions on OMPs removal, the Redundancy Dimensional Analysis (RDA) was used with PC1 and PC2 of OMPs removal. Similarly, RDA was performed with the nutrient uptake rate of the biomass and PC1 and PC2 of OMPs removal. All the statistical tests were conducted using OriginPro, Version 2022b, OriginLab Corporation, Northampton, MA, USA.

3. Results and discussion

3.1. Microalgal growth

The experiments with *C. sorokiniana* were started in batch mode to achieve exponential growth and therefore high biomass. When growth rate decreased mostly due to nutrient depletion, continuous mode was applied therefore continuously supplying nutrients at an HRT of 0.8 d. After a few days steady state was achieved, indicating that the growth rate of *C. sorokiniana* was constant.

The dry weight of *C. sorokiniana* in AnBW in the treatment (with OMPs) reached 4367 ± 42 mg/l at the end of the batch mode (day 6), and 1714 ± 32 mg/l in continuous mode during steady state (day 10–15) (Fig. 1a). In comparison with the control (no OMPs), the dry weight was 15% higher at the end of the batch mode, but 14% lower during steady state. A similar trend was observed for chlorophyll a, even though the standard deviation during steady state was much larger (10% for control, 11% for treatment, Fig. S1a).

In MW, the dry weight was 1845 ± 131 mg/l at the end of the batch mode in the treatment and 1371 ± 75 mg/l during steady state of continuous (day 6–15). The dry weight and chlorophyll a were similar in both treatment and control (Fig. 1b, S1b).

In SCE, a much lower dry weight and chlorophyll a was obtained than with AnBW and MW (Fig. 1c, S1c) due to the low nitrogen and phosphorus concentrations in the medium (Table 1). During steady state (day 5–10), the dry weight in the treatment (573 ± 14 mg/l) was 16% lower than control (4% standard deviation), while the chlorophyll a in the treatment was only 11% lower and with low deviation (1%).

OMPs positively affected the microalgal growth in batch mode in AnBW, but not in MW and SCE. During steady state, OMPs appeared to slightly inhibit the microalgal growth in AnBW and SCE. This is difficult to validate due to the large standard deviation between control and treatment for both dry weight and chlorophyl a. This was not the case for MW, where clearly no inhibition was found. In batch mode, *C. sorokiniana* was exposed to the lower concentration of OMPs due to the higher removal efficiency in comparison with steady state in

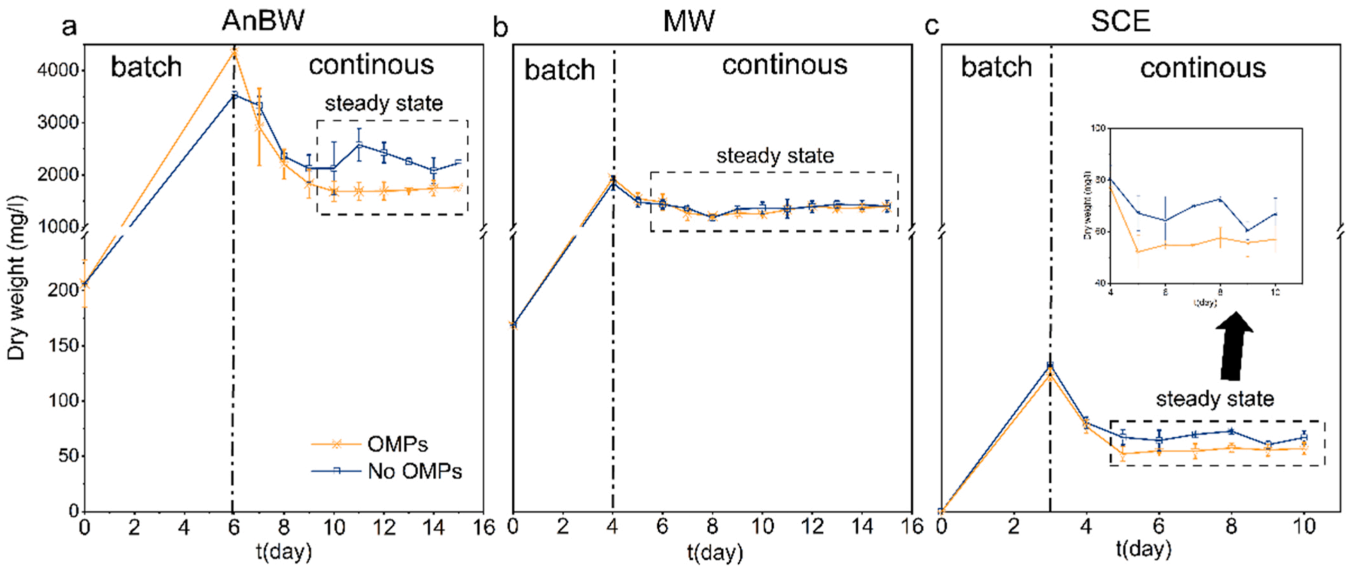

Fig. 1. Dry weight of *Chlorella sorokiniana* in time in AnBW (a), MW (b) and SCE (c).

continuous mode (Fig. 2). Mao et al. [29] found that azithromycin stimulated the growth of *Chlorella pyrenoidosa* at low concentration (0.5, 1 µg/l), while inhibited the growth at high concentration (5–100 µg/l). Possibly, this stimulation at low concentration of OMPs also occurred in batch mode. During steady state, the higher concentration of OMPs inhibited the growth possibly by intervening the synthesis of protein in chloroplasts, as it has been demonstrated previously [24,37,59]. In MW, DOM may have reduced the bioavailability of OMPs by complexation of

	AnBW		MW		SCE	
OMPs	Batch	Continous	Batch	Continous	Batch	Continous
CAF	99	92	93	93	7	29
BTZ	88	57	89	69	-3	0
SUL	91	79	76	83	-3	3
IBU	92	56	75	71	11	32
FUR	90	38	69	74	-3	4
PRO	82	51	90	64	5	83
HYD	69	18	29	3	0	0
MeBT	51	39	47	42	0	2
CLA	50	75	88	87	79	58
TRI	27	4	62	38	0	4
IRB	29	18	11	2	28	17
MET	9	2	4	2	-1	2
CBZ	-3	2	4	-2	-2	1
MCPP	0	3	-3	0	-2	2
MCPA	3	-2	7	2	-1	2
DCF	5	2	5	0	1	2

< 20	20 - 40	40 - 60	60 - 80	80 - 100

Fig. 2. Heatmap of OMPs removal (%) in AnBW, MW and SCE. The removal in batch mode refers to the removal at the end of batch mode (day 6, 4 and 3 for AnBW, MW and SCE, respectively). The removal in continuous mode refers to the average removal during steady state. The standard deviations of removal in batch and continuous mode are shown in Table S2.

DOM and OMPs, and further mitigate the potential toxic effect of sixteen OMPs. Tong et al. [50] showed that commercial DOM reduced the inhibition of tetracycline to *Coelastrella sp.* by the binding of tetracycline to the DOM. In SCE, the poor removal of OMPs in batch mode (Fig. 2) resulted in a higher exposure concentration of OMPs than in AnBW, thereby allowing for a higher inhibitory effect on the removal of OMPs. These results indicate that the effect of OMPs on microalgal growth was influenced by both OMPs removal and DOM in wastewater.

3.2. OMPs removal

Generally, eleven out of sixteen OMPs were removed from AnBW and MW, except for MET, CBZ, MCPP, MCPA, and DCF (Fig. 2). On the contrary, only CAF, IBU, PRO, CLA and IRB (five out of sixteen) were removed from SCE.

The compounds (CAF, BTZ, SUL, IBU, FUR, and PRO) showed the highest removal (82–99%) in batch mode with AnBW, while a decrease was observed for all these compounds after switching to continuous mode, except for CAF for which removal remained almost 100%. In MW, the removal of these OMPs ranged from 69% to 93% in batch mode. In comparison with AnBW, BTZ and PRO removal decreased less, and the removal of other OMPs remained constant or showed limited increase (<7%) upon changing from batch to continuous mode. The observed decrease of the removal of OMPs from batch to continuous mode was paralleled with a decrease of biomass, as lower biomass means less enzymes and less adsorption surface available for the removal of OMPs. A lower decrease in the removal of CAF, BTZ, SUL, IBU, FUR, and PRO was observed in MW from batch to continuous mode due to a lower decrease (25% in dry weight) of biomass than in AnBW (61% in dry weight). In SCE, the removal of most OMPs was negligible in batch mode, and upon switching to continuous mode, increased for three compounds (CAF, IBU, and PRO) to more than 20%. Most likely, the low biomass in SCE resulted in a negligible removal of most OMPs (Fig. 1c), while for CAF, IBU and PRO, the removal capacity of *Chlorella sorokiniana* was enhanced by acclimatizing to these OMPs, even at low biomass concentrations [18]. Additionally, aromatic compounds containing nitrogen, such as CAF and PRO, may serve as extra nitrogen sources for maintaining the growth of *Chlorella sorokiniana*, therefore leading to a remarkable removal of DIN in SCE. Luther [28] found that *Scenedesmus obliquus* grew by using nitro- and ammonia substituted aromatic compounds (amino naphthalene, 4- amino naphthalene-1-sulfonic acid, 4-aminobenzoate, 4-nitroanilene, and 2-nitrobenzoate) as nitrogen sources in the absence of inorganic nitrogen sources. The removal efficiencies of these six OMPs in our experiments with AnBW and MW are within previously reported removal efficiencies in other microalgae-based systems, such as flasks and pilot-scale HRAP [9,10,14,19,34,54,4].

In comparison, HYD, MeBT, CLA, TRI, and IRB showed less removal in batch mode with AnBW. Furthermore, the removal of these OMPs in AnBW decreased after switching from batch to continuous mode, except for CLA, for which the removal increased. In MW, all these compounds showed a decrease of removal when switching from batch to continuous mode. HYD and IRB showed less removal than AnBW, and MeBT was removed similarly in both wastewater. Specially, CLA in batch mode with MW (88%) and SCE (79%) showed higher removal than AnBW. TRI removal in batch mode with MW was 35% higher than AnBW. Most likely, nutrient limitation (Fig. S2) during the experiment with MW stimulated the production of peroxidase and PY450 enzymes [11,48]. These enzymes are responsible for TRI removal [1,3], and PY450 enzymes can remove CLA by adding hydroxyl group on its cladinose ring [49]. A higher removal of CLA and TRI was therefore achieved in batch mode with MW.

MET, CBZ, MCPP, MCPA, and DCF, were poorly removed in all three types of wastewater. The poor removal efficiencies of MET, CBZ and MCPP were in line with previous studies ([57,9,58]). MCPA has a similar recalcitrant structure as MCPP, which includes an aromatic ring with a carboxylic side chain [41]. This might explain the poor removal of MCPA. Poor removal of MCPA in sewage was also observed in batch experiments with four different green algal species (*Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, *Chlorella pyrenoidosa*, and *Chlorella vulgaris*) under fluorescent light [63]. In contrast, 89% of MCPA removal in agricultural run-off was achieved in a full-scale semi-closed PBR inoculated with a mixed community of bacteria, microalgae, protozoa and small metazoan, under natural light conditions [8]. This removal was attributed to photodegradation and biodegradation. The photodegradation of MCPA required the light with wavelength of lower than 290 nm [39]. MCPA therefore was not removed by photodegradation under visible light (400–700 nm) in our study. DCF removal on the other hand has been shown to be completely removed by photodegradation under white fluorescence light [57,58]. Under natural light conditions, 20–60% of DCF removal was observed in multiple pilot-scale HRAP [9,34,53]. The contradicting results between this study and others are because visible light (400–700 nm) in this study is unable to induce DCF photodegradation [20,43]. Therefore, applying a light source with the same spectrum as sunlight can be a solution for optimising the removal of MCPA and DCF in this study.

3.3. Effect of wastewater characteristics and biomass composition on OMPs removal

RDA was applied to show that OMPs removal was influenced by wastewater type (MW, SCE and AnBW), as shown by wastewater characteristics, kinetic parameters of the reactors and biomass characteristics (Fig. 3).

The coefficients of RDA showed that dry weight (RDA1: 0.96) and soluble COD (RDA1: 0.79) had the highest positive impact on the total removal of OMPs (Fig. 3a), whereas the concentration of DIN (RDA1: 0.41 and RDA2: −0.84) and PO_4^{3-}-P (RDA1: 0.41 and RDA2: −0.84) were not as effective. The similar tendencies in dry weight and the removal of most OMPs in AnBW and MW from batch to continuous mode manifested the positive impact of biomass concentration on the removal of OMPs. Therefore, increasing biomass levels in microalgae-based photobioreactors, either by increasing the HRT or by decoupling HRT from SRT, appears to be an important way to further optimize the removal of OMPs. Furthermore, soluble COD in wastewater can affect OMPs removal by complexation of DOM and OMPs in microalgae-based systems [50,5]. DOM, such as humic acid, reduced the removal of triclosan by *Cymbella sp.* because the complexation of humic acid and triclosan reduced the availability of triclosan to microalgal cells for subsequent biodegradation [5]. This negative effect was also observed in the removal of tetracycline by *Coelastrella sp.* [50]. These negative results contradicted with the outcome of our study. Possibly, other mechanisms induced the positive effect of soluble COD on OMPs removal. Humic substances in DOM can function as surfactant and emulsifier [22], which can increase the accessibilities of OMPs to the biomass and the subsequent intracellular biodegradation [47]. Humic acid contains quinone moieties, and can act as electron shuttle to enhance the electron transfer between electron donors and electron acceptors [26,31]. He et al. [17] found that this mechanism was responsible for enhancing the removal of metoprolol, naproxen, and diclofenac (electron donors) by DOM from constructed wetland in aerobic enrichment cultures. Due to the similarities of OMP removal pathways by microalgae and bacteria [35], this process can play a role in our microalgae-based systems.

The biomass uptake rate of C/N/P played a significant role in total removal of OMPs. The mole of C in the biomass (RDA2: 0.82) showed a higher impact than the mole of N (RDA2: 0.76) and PO_4^{3-}-P (RDA2: 0.74) on OMPs removal (Fig. 3b). This is also shown by the variable importance plot (VIP) of each parameter on the total removal of OMPs (Fig. S3a; S3b). The biomass in MW showed higher mole C uptake rate than AnBW (Table S3). This was probably because of the accumulation of carbohydrate and lipid in *Chlorella sp.*, induced by limited nutrient availability in MW [16]. Conjunction with carbohydrate, such as

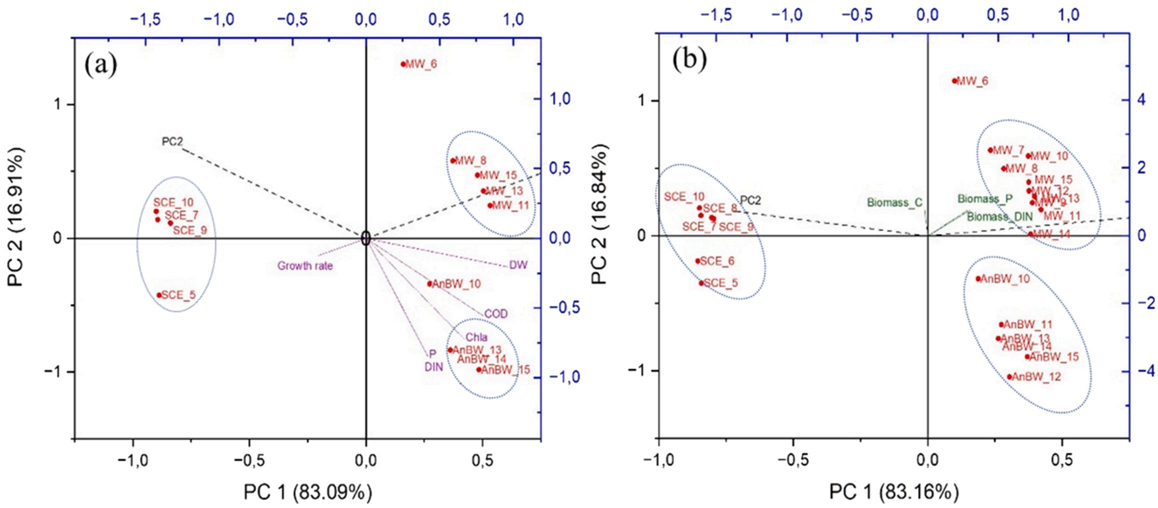

Fig. 3. RDA analysis of PC1 and PC2 of OMPs removal with wastewater characteristics and kinetic parameters (a), nutrient uptake rate of the biomass (b). Sampling points are indicated as red dots in the graphs. The wastewater characteristics and kinetic parameters are shown in rays. The angles between different rays represent their correlations, and a sharper angle shows a stronger correlation.

glucose, is an important step of removal of some OMPs, such as IBU and BTZ [25,30]. Possibly, the accumulated carbohydrate accelerated this procedure and resulted in a higher removal. Carbohydrates, such as glucose, can also act as co-substrate for the removal of OMPs (e.g. tetracycline and bisphenol A) and lead to a higher removal in the batch experiments with *Chlorella sorokiniana* [55]. However, the addition of 0.5 g/l glucose completely inhibited the removal of ciprofloxacin in flask experiments with *Chlamydomonas Mexicana* since the easily available carbon source (glucose) inhibited the synthesis of enzymes available for ciprofloxacin removal [60]. This inhibitory effect of carbohydrate might also occur in the removal of some OMPs in our study. Overally, a combination of these mechanisms can result in the significant impact of carbon uptake rate of the biomass to the overall removal of OMPs.

4. Conclusion

In batch mode, eleven out of sixteen OMPs were highly removed from AnBW and MW, whereas most OMPs showed poor removal (<11%) in SCE, except CLA and IRB. The removal of most OMPs decreased in AnBW and MW when the operation was switched from batch to continuous mode. However, removal percentages remained above 60% for most of these 11 OMPs. The reduced biomass concentrations during continuous mode seem to be the most important factor in this decrease in OMPs removal as less enzymes and adsorption surface are available for the removal of OMPs. An increase in the removal of CAF, IBU and PRO in SCE was observed when switching from batch to continuous mode. It appears that the exposure of microalgae to these compounds leads to an acquired removal capacity for these specific chemicals.

Statistical analyses showed that the total removal of OMPs during steady state of continuous mode was directly affected by the wastewater type. Specifically, the soluble COD of wastewater, dry weight and carbon uptake rate of the biomass positively influenced the total removal of OMPs.

To conclude, wastewater characteristics, such as soluble COD, and microalgae nutrient and carbon uptake rate, play an important role in the removal of OMPs by microalgae-based technology. To achieve a more efficient removal of OMPs, more biomass is needed in the bioreactors, which can be achieved by changing operational conditions (hydraulic and sludge retention times).

CRediT authorship contribution statement

Kaiyi Wu: Conceptualization, Data curation, Methodology, Visualization, Writing – original draft. **Merve Atasoy:** Methodology, Formal analysis, Writing – review & editing. **Hans Zweers:** Methodology, Writing – reviewing & editing. **Huub Rijnaarts:** Conceptualization, Supervision, Writing – review & editing, Alette Langenhoff: Conceptualization, Visualization, Supervision, Writing – review & editing, **Tânia V. Fernandes:** Conceptualization, Methodology, Visualization, Funding acquisition, Supervision, Writing – reviewing & editing.

Declaration of Competing Interest

The authors declare no competing known interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.131451.

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