Journal of Hazardous Materials 406 (2021) 124284

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Research Paper

Removal of pharmaceutical and personal care products (PPCPs) from waterbody using a revolving algal biofilm (RAB) reactor

Si Chen [a], Jiahui Xie [a], Zhiyou Wen [a,b,*]

[a] Department of Food Science and Human Nutrition, Iowa State University, 536 Farmhouse Lane, Ames 50011, Iowa, USA
[b] Gross-Wen Technologies Inc., 404 Main Street, Slater 50244, Iowa, USA

ARTICLE INFO

Keywords:
Revolving Algal Biofilm
Pharmaceutical and personal care products
Accumulation
Degradation
Extracellular polymeric substances
Microbial community

ABSTRACT

The occurrence of Pharmaceutical and Personal Care Products (PPCPs) in the aquatic environment has raised concerns due to their accumulation in the ecosystem. This study aims to explore the feasibility of using a Revolving Algal Biofilm (RAB) reactor for PPCPs removal from waterbody. Five model PPCP compounds including ibuprofen, oxybenzone, triclosan, bisphenol A and N, N-diethyl-3-methylbenzamide (DEET) were mixed and added to the culture medium. It shows that PPCP removal efficiencies of the RAB reactor ranged from 70% to 100%. The degradation of PPCPs by the RAB reactor contributed > 90% PPCP removal while < 10% PPCPs removal was due to accumulation in the algal biomass. The nutrients removal performance of the RAB reactor was not affected by exposing to the PPCPs. The extracellular polysaccharides content of the biomass increased when exposing to PPCPs, while the extracellular proteins content remained constant. The Chl a content maintained constant in the PPCPs-treated biomass, but decreased in the biomass without PPCP treatment. It was also found that the microbial consortium of the RAB reactor was enriched with PPCPs degradation microorganisms with the progressing of feeding PPCPs. Collectively, this work demonstrates that the RAB system is a promising technology for removing PPCPs from wastewater.

1. Introduction

Pharmaceutical and Personal Care Products (PPCPs) are used to treat human and animal diseases and improve quality of life (Lin et al., 2016). The PPCPs in urban receiving water are from individual households, hospitals and animal farms. The presence of PPCPs in water, even at trace amounts, has caused great concerns due to their threat to the ecosystem (Maruya et al., 2016). For example, Asia vultures were near extinction because of the acute toxicity following the consumption of diclofenac contaminated animals (Arnold et al., 2013). The feminization of male fishes has been observed after exposure to estrogenic substances (Kidd et al., 2007).

The conventional activated sludge process was commonly used to remove PPCPs from wastewater. Some PPCP compounds such as triclosan could be removed at > 90% by the activated sludge process (Kapelewska et al., 2018). However, the removal efficiency of PPCPs by the conventional activated sludge processes was highly variable depending on multiple factors such as PPCP compounds (Kapelewska et al., 2018), seasonality (Mohapatra et al., 2016) and treatment processes (Mohapatra et al., 2016). For example, the average removal rate of triclosan was 90.1% in the activated sludge process, while the average removal rate of DEET was only 38.9% in the same facility (Kapelewska et al., 2018). The removal of metoprolol reached 80% during the summer season, while it was less than 30% during the winter season (Mohapatra et al., 2016). The average removal of ranitidine was 54% in the facultative lagoon, compared with nearly 100% removal with the cyclic activated sludge technique (Mohapatra et al., 2016).

In addition to the conventional activated sludge process, alternative methods for PPCPs removal have been explored, such as adsorption (Yoo et al., 2018), advanced oxidation (Liang et al., 2019) and membrane separation (Ganiyu et al., 2015). These methods, however, have various disadvantages. For example, in the adsorption and membrane separation processes, inappropriate disposal of PPCPs-loaded absorbents and concentrated wastewater can cause secondary contamination (Xu et al., 2017), while advanced oxidation has a high operation cost (Comninellis et al., 2008).

Microalgae-based technologies are ecofriendly approaches to remove pollutants from the wastewater, fixing carbon dioxide and producing algae byproducts. The algae-based treatment processes have been researched for their performance of removing PPCP compounds

* Corresponding author at: Department of Food Science and Human Nutrition, Iowa State University, 536 Farmhouse Lane, Ames 50011, Iowa, USA
E-mail address: wenz@iastate.edu (Z. Wen).

https://doi.org/10.1016/j.jhazmat.2020.124284
Received 11 May 2020; Received in revised form 30 August 2020; Accepted 12 October 2020
Available online 21 October 2020

(Wang et al., 2017b). For example, a high-rate raceway pond was developed to remove PPCPs from wastewater with 0–90% removal efficiencies depending on specific compounds (Matamoros et al., 2015). A tubular photo-bioreactor was reported to remove more than 48% of pharmaceutical compounds from toilet water (Hom-Diaz et al., 2017). These existing algae-based PPCPs removal technologies were based on suspended culture systems, in which the algal cells grow in the suspension at low cell densities (< 0.5 g/L) (Gross et al., 2013). To harvest the biomass, the algal culture has to be concentrated through sedimentation followed by centrifugation, which results in a high operation cost (Milledge and Heaven, 2013).

A Revolving Algal Biofilm (RAB) reactor has been developed to reduce the biomass harvest cost (Gross et al., 2013, 2015a; Gross and Wen, 2014; Zhao et al., 2018a, 2018b). The RAB system consists of a liquid reservoir and a vertically oriented belt revolving between the nutrient-rich liquid and air phase. Algal cells grow on the surface of the belt and are harvested by scraping. The RAB reactors have demonstrated much higher biomass productivity than the algae suspension culture systems due to the enhanced gas and nutrients transfer (Gross et al., 2013; Lee et al., 2014; Zhao et al., 2018a).

From PPCPs removal respective, the RAB reactor can be an ideal system to remove those compounds. It has been reported that the algal biomass can be a biosorbent to remove PPCPs from waterbody (Wang et al., 2017b; Xie et al., 2020). Considering the large quantities of biomass it can produce, the RAB reactor is an ideal system for pollutant adsorption. The algal biofilm in the RAB reactor contains a high content of Extracellular Polymeric Substances (EPS), which are mainly polysaccharides and proteins (Adeleye and Keller, 2016). Those amphiphilic biopolymers with hydrophobic patches on the external surface provided binding sites for organic compounds (Zhao et al., 2017; Peng et al., 2019) and thus, may benefit the adsorption of PPCP compounds. In addition, algal biofilm contains a wider multivariate microbial community structure compared to the suspended algae culture (Niederdorfer et al., 2016). Such biodiversity has been reported to have a positive correlation with the removal of micro-pollutants (Torresi et al., 2016). Based on these advantages, the aim of this work was to evaluate the potential of using the RAB reactor as an effective system to remove PPCPs from the waterbody.

2. Materials and methods

2.1. PPCP compounds and PPCPs-containing medium

Ibuprofen, oxybenzone, triclosan, bisphenol A, and N, N-diethyl-3-methylbenzamide (DEET) were used as the model PPCP compounds, which represent a wide spectrum of substances such as analgesic substances, cosmetic products, antimicrobial agents, plasticizers, and insect repellents. Those compounds have been reported in the wastewater treatment plant or the waterbody (Snyder et al., 2007; Mohapatra et al., 2016) with the concentrations in line with those studied in this work (Matamoros et al., 2015; Kapelewska et al., 2016). Therefore, the results obtained in this work will provide practical references and guidance for wastewater treatment practitioners. The PPCPs-containing water was prepared by adding these compounds to Bold's Basal Medium (BBM) containing 2.5 g/L $NaNO_3$, 0.25 g/L $CaCl_20.2H_2O$, 0.75 g/L $MgSO_40.7H_2O$, 0.75 g/L K_2HPO_4, 1.75 g/L KH_2PO_4, 0.25 g/L NaCl, and trace elements. We added PPCP compounds in synthetic algal growth medium to mimic the real wastewater as this work focuses on the feasibility study of RAB as a potential PPCPs removal system.

2.2. Algae seed culture

Algae seed culture (0.5–1 g/L, dry basis) was maintained in a raceway pond with 1,000-L of working volume in a greenhouse at Iowa State University campus in Ames, IA, USA. The greenhouse uses natural sunlight with temperature ranging 15–30 °C throughout the year. The algae were grown in the BBM medium with 14-day hydraulic retention time (HRT). The algal seed culture was monitored through microscopic observation. Green algae, diatom and cyanobacteria assemblages were found as the major species in the microscopic images. Prior to inoculation, the algae suspensions harvested from the raceway pond were thickened through sedimentation.

2.3. RAB culture system

Lab-scale RAB reactors were used to treat PPCPs-containing influent in a semi-continues mode with a 5-day HRT. The details of the RAB reactor design were reported previously (Gross et al., 2015b; Gross and Wen, 2018; Zhou et al., 2018b). A schematic diagram is also available in the previous report (Gross et al., 2015b). In brief, the RAB reactor consisted of a liquid reservoir with 1.5 L working volume and a vertical revolving belt with a surface area of 0.13 m^2 for algae attachment. The belt rotated between the liquid and air phases with a linear velocity of 4 cm/s. The RAB reactors were illuminated with continuous fluorescent at 130 μmol m^{-2} s^{-1}. The temperature was at 25 °C. To initiate the culture in the RAB reactor, the algae seed was added to the liquid reservoir and progressively attached to the belt. The establishment of algal biofilm took approximately three weeks, during which 300 mL liquid in the reservoir was exchanged with 30 mL seed culture and 270 mL fresh BBM medium on a daily basis.

After a stable biofilm in the RAB belt was established, the RAB reactors were switched to PPCPs treatment mode. On a daily basis, the liquid reservoir was discharged 300 mL effluent and received the same volume of PPCPs-containing influent. Two levels of PPCP concentrations in the influent were used. The high concentration level included 100.4 μg/L ibuprofen, 14.1 μg/L oxybenzone, 3.4 μg/L triclosan, 3.9 μg/L bisphenol A, and 135.1 μg/L DEET. The low concentration level included 45.7 μg/L ibuprofen, 6.9 μg/L oxybenzone, 2.9 μg/L triclosan, 2.2 μg/L bisphenol A, and 63.9 μg/L DEET. Those targeted concentrations were based on those commonly reported in the effluent of municipal wastewater treatment plants (Snyder et al., 2007; Salgado et al., 2010; De Graaff et al., 2011; Hedgespeth et al., 2012; Mohapatra et al., 2016).

The biomass of the RAB reactor was harvested by scrapping the biofilm every 7 days. The operation was sustained for four consecutive harvest cycles. The removal of PPCPs, as well as nitrate, phosphorus and sulfate of the RAB reactors were evaluated by comparing the concentration of each pollutant in the influent and effluent. The biomass was characterized for EPS compositions, chlorophyll a (*Chl a*) content, and microbial community diversity.

2.4. Bubble column culture system

A suspended algal culture in bubble column reactors was also performed as a control. The bubble columns (1 L working volume) were made of glass tubes with 6.5 cm inter-diameter and 50 cm height. The reactor was continuously illuminated with fluorescent at 130 μmol m^{-2} s^{-1} and aerated with air at 500 mL min^{-1} (0.5 vvm). The culture was operated at 25 °C in a batch mode for 7 days, with the initial PPCPs concentrations set at the same levels as those used in the influent of the RAB culture. The cell growth was monitored with optical cell density at 680 nm (OD_{680}) (Ma et al., 2012).

2.5. Analyses

2.5.1. Determination of PPCPs in liquid and microalgal biomass

To determine PPCPs concentration in liquid, the sample (250 mL) was centrifuged at 7,435 g to remove solid residues. The supernatant was spiked with triclosan-d_3 (CDN isotopes, Canada) to 8 μg/L, and acidified to pH 2 with sulfuric acid (Yu and Chu, 2009). PPCPs in the supernatant were then concentrated by solid-phase extraction. In brief, a cartridge (Waters Oasis HLB cartridges, WAT 106202, Waters

Table 1
Monitored mass ions, limit of quantitation, sample concentration and recovery for target PPCPs (n = 3).

Compound	[a]MW	Retention time (min)	Qualifier ion	[b]LOD (ng L^{-1})	[c]LOQ (ng L^{-1})	Concentrations in low loading level (ng L^{-1})	[d]RSD (%)	Recovery (%)
Ibuprofen	206	26.1	161	1,282	4,000	45,700	7.3	94.2
Oxybenzone	228	33.7	227	326	400	6,900	3.4	91.4
Triclosan	290	34.4	290	351	400	2,900	4.1	75.7
Bisphenol A	228	35.1	213	360	400	2,200	7.9	72.5
DEET	191	25.0	190	3,344	4,000	63,900	5.2	99.5

[a] MW: Molecular Weight.
[b] LOD: Limit of Detection.
[c] LOQ: Limit of Quantitation.
[d] RSD: Relative Standard deviation.

Corporation) filled with the sorbent materials was pre-conditioned with 30 mL methanol/acetone (1:1, v/v) solution followed with 15 mL water. The supernatant flowed through the cartridge at 1 mL min^{-1} for PPCPs absorption. The PPCPs-loaded cartridge was then washed with 15 mL methanol/acetone (1:1, v/v). The PPCPs in the eluate were dried by evaporating the solvent through blowing nitrogen gas in a nitrogen blowdown station, and re-dissolved with 450 μL Dimethylformamide (DMF) for GC-MS analysis.

Agilent 5973 GC-MS system equipped with a DB-5 fused silica capillary column (Yu and Chu, 2009) was used to quantify the PPCPs compounds. Samples were injected in a splitless mode. Helium was used as carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature program started at 60 °C (held for 1 min) and ramped to 320 °C at 5 °C min^{-1}. The mass spectrometer was operated in the positive ion-electron impact (EI) mode with an ionization voltage of 70 eV and a source temperature of 200 °C using selected ion monitoring. The qualifier ions selected for quantification are summarized in Table 1. The recovery, precision (related standard deviation % RSD), limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the method described previously (Hu and Zhou, 2011) and the results are also summarized in Table 1.

To determine the PPCPs content in the algal biomass, the PPCP compounds were first extracted by sonication. One gram of lyophilized biomass was mixed with 10 mL methanol/acetone mixture (1:1, v/v) and sonicated in an ultrasonic water bath for 30 min followed with centrifugation at 7,435 g. The extraction was repeated twice. The PPCPs-containing supernatants from the extraction process were combined in a 50 mL glass tube and evaporated by passing nitrogen gas in a nitrogen blowdown station. The PPCPs were re-dissolved in 450 μL Dimethylformamide (DMF) for GC-MS analysis based on the procedure described above. The PPCPs content in the biomass was calculated based on the PPCPs concentration in the supernatant and the weight of the biomass.

2.5.2. Determination of Chl a and EPS content in biomass

Chl a content in algal biomass was measured based on the method reported previously (Lv et al., 2017). In brief, lyophilized biomass was suspended in 95% (v/v) ethanol at 4 °C in the dark for 24 h. The suspension was centrifuged at 7,435 g, and the supernatant was collected for measuring absorbance at 649 nm (A_{649}) and 665 nm (A_{665}). The Chl a concentration in the supernatant ($C_{Chl\ a}$, μg mL^{-1}) was calculated as follows,

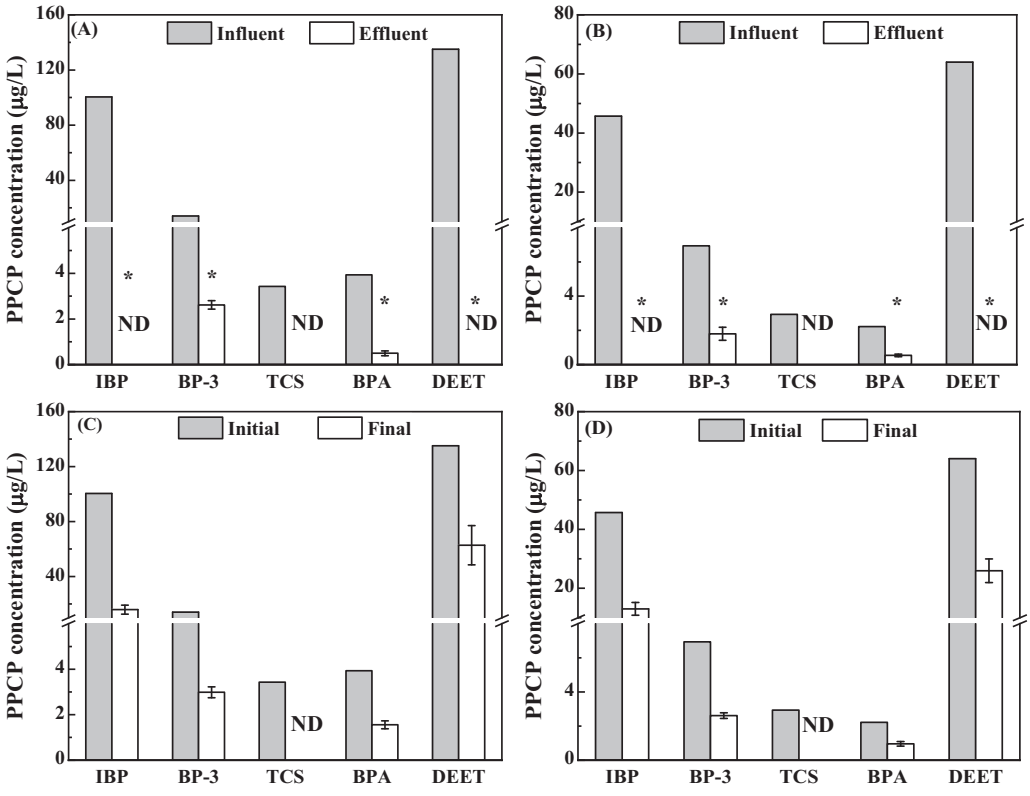

Fig. 1. Concentrations of individual PPCP compounds in influent and effluent of the RAB reactor during continuous operation, and in initial and final culture of the bubble column during the batch operation. (**A**) RAB reactor with high PPCP concentrations in influent, (**B**) RAB reactor with low PPCP concentrations in influent, (**C**) Bubble column with high initial PPCP concentrations, and (**D**) Bubble column with low initial PPCP concentrations. Abbreviation: IBP: ibuprofen, BP-3: oxybenzone, TCS: triclosan, BPA: bisphenol A, DEET: N, N-diethyl-3-methylbenzamide. ND: Not Detected. The data for each PPCP in the bubble columns (**C** & **D**) were set as the baseline, the corresponding PPCP data in RAB reactors with the asterisk (*) (**A** & **B**) indicate significant differences from the bubble column results ($p \leq 0.05$). The sample size of PPCPs related parameters in the RAB reactor is 8, including the steady-state liquid samples at four alternative days with duplicates each day. The sample size of the bubble column is 2, including duplicate samples at the end of batch culture.

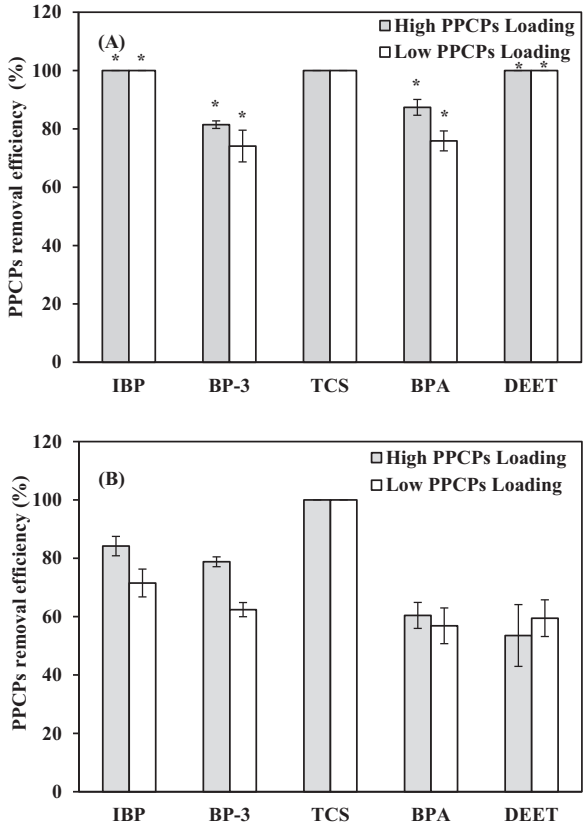

Fig. 2. Removal efficiencies of PPCP compounds in the RAB reactor (**A**) and the bubble column reactor (**B**). Abbreviation: IBP: ibuprofen, BP-3: oxybenzone, TCS: triclosan, BPA: bisphenol A, DEET: N, N-diethyl-3-methylbenzamide. The data for each PPCP in the bubble columns (**B**) were set as the baseline, the corresponding PPCP data in RAB reactors with the asterisk (*) (**A**) indicate significant differences from the bubble column results ($p \leq 0.05$). The sample size of PPCPs related parameters in the RAB reactor is 8, including the steady-state liquid samples at four alternative days with duplicates each day. The sample size of the bubble column is 2, including duplicate samples at the end of batch culture.

$$C_{Chla} = 13.95 \cdot A_{665} - 6.88 \cdot A_{649} \quad (1)$$

The Chl a concentration was then converted to the Chl a content in biomass based on the weight of biomass and the volume of the supernatant.

Extraction of the EPS from the biomass was based on the method reported previously (García-Meza et al., 2005). Briefly, the lyophilized biomass was suspended in 0.1 M H_2SO_4 solution at 95 °C for 30 min. The EPS extract was centrifuged at 7,435 g after 30 min incubation. The supernatant was collected for analysis of the polysaccharides and proteins concentrations. Polysaccharides concentration was measured by using the phenol-sulfuric acid method (Dubois et al., 1956). Proteins concentration was determined based on Coomassie brilliant blue G250 dye method (Bradford, 1976). The polysaccharides and proteins concentrations in the supernatant were then converted to their contents in biomass based on the weight of dry biomass and the volume of the supernatant.

2.5.3. Determination of nitrate, phosphate, and sulfate concentrations

The nitrate, phosphate and sulfate concentrations in the liquid were determined by Ion chromatography. The liquid sample was filtered through a 0.2 μm syringe filter to remove solid particles. The filtered liquid (10 μL) was injected into the Ion Chromatography system (ICS-900, Dionex) equipped with an IonPac AG12A guard column followed by an IonPac AS12A (AS12A) analytical column and a conductivity detector. Isocratic elution with 2.7 mM Na_2CO_3 and 0.3 mM $NaHCO_3$ was used as the mobile phase at a flow-rate of 1.5 mL min^{-1}. An Auto Supression Recycle mode was used with an Anion Electrolytically-Regenerating Suppressor (ERS-500, Dionex). The nitrate, phosphate

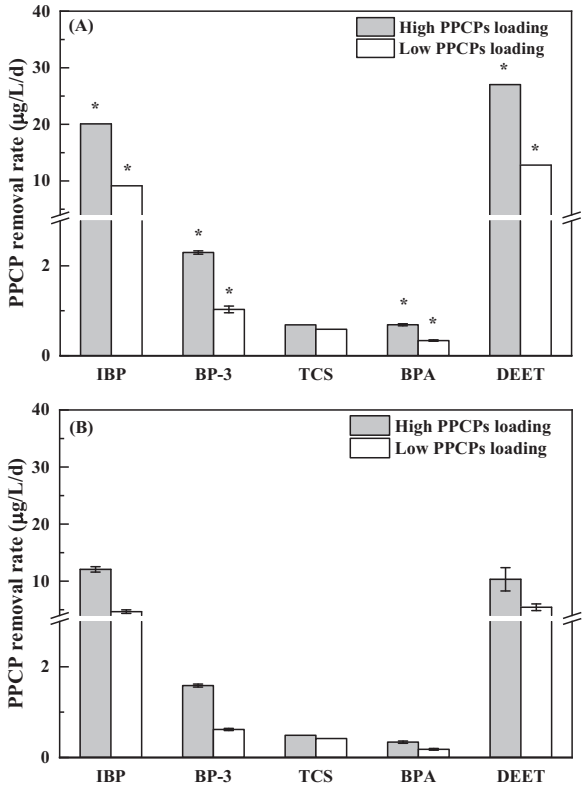

Fig. 3. Removal rates of PPCPs in RAB reactor (**A**) and bubble column (**B**). Abbreviation: IBP: ibuprofen, BP-3: oxybenzone, TCS: triclosan, BPA: bisphenol A, DEET: N, N-diethyl-3-methylbenzamide. The data for each PPCP in the bubble columns (**B**) were set as the baseline, the corresponding PPCP data in RAB reactors with the asterisk (*) (**A**) indicate significant differences from the bubble column results ($p \leq 0.05$). The sample size of PPCPs related parameters in the RAB reactor is 8, including the steady-state liquid samples at four alternative days with duplicates each day. The sample size of the bubble column is 2, including duplicate samples at the end of batch culture.

Table 2
Content of PPCP compounds in biomass of the RAB reactors (n = 2) and bubble columns (n = 2)[a].

Name	PPCPs content (μg/g)	
	High PPCPs Loading	Low PPCPs Loading
RAB reactor		
Ibuprofen	0.00 ± 0.00	0.00 ± 0.00
Oxybenzone	2.05 ± 0.26	0.86 ± 0.06
Triclosan	0.77 ± 0.02	1.54 ± 0.11
Bisphenol A	0.00 ± 0.00	0.00 ± 0.00
DEET	0.00 ± 0.00	0.00 ± 0.00
Bubble column		
Ibuprofen	0.00 ± 0.00	0.00 ± 0.00
Oxybenzone	1.17 ± 0.40	1.03 ± 0.10
Triclosan	1.07 ± 0.14	0.98 ± 0.23
Bisphenol A	1.20 ± 0.07	0.94 ± 0.13
DEET	2.54 ± 0.26	0.00 ± 0.00

[a] The sample size (n) of the biomass for PPCPs accumulation analysis from the RAB reactor is 2, including duplicate biomass samples at the steady state (week 4). The sample size of the biomass from bubble column is 2, including duplicate biomass samples at the end of batch culture. The results are presented as arithmetic means ± standard errors.

Table 3
Accumulation and degradation rates of each PPCP compound in RAB (n = 2) and bubble column (BC) reactors (n = 2), and their contribution to the overall removal rate from the liquid[a, b].

PPCP compound		Accumulation rate ($\mu g\ L^{-1}day^{-1}$)		Degradation rate ($\mu g\ L^{-1}day^{-1}$)	
		High loading	Low loading	High loading	Low loading
Ibuprofen	RAB	0.00 ± 0.00 (0%)	0.00 ± 0.00 (0%)	20.08 ± 0.00 (100%)	9.14 ± 0.00 (100%)
	BC	0.00 ± 0.00 (0%)	0.00 ± 0.00 (0%)	12.08 ± 0.47 (100%)	4.64 ± 0.31 (100%)
Oxybenzone	RAB	0.06 ± 0.01 (2%)	0.05 ± 0.01 (6%)	2.24 ± 0.10 (98%)	0.87 ± 0.05 (94%)
	BC	0.09 ± 0.03 (6%)	0.08 ± 0.01 (14%)	1.55 ± 0.01 (94%)	0.48 ± 0.04 (86%)
Triclosan	RAB	0.05 ± 0.00 (7%)	0.04 ± 0.00 (7%)	0.64 ± 0.00 (93%)	0.55 ± 0.00 (93%)
	BC	0.08 ± 0.01 (17%)	0.08 ± 0.02 (18%)	0.41 ± 0.01 (83%)	0.34 ± 0.02 (82%)
Bisphenol A	RAB	0.00 ± 0.00 (0%)	0.00 ± 0.00 (0%)	0.79 ± 0.00 (100%)	0.42 ± 0.04 (100%)
	BC	0.09 ± 0.01 (28%)	0.07 ± 0.01 (41%)	0.25 ± 0.02 (72%)	0.11 ± 0.03 (59%)
DEET	RAB	0.00 ± 0.00 (0%)	0.00 ± 0.00 (0%)	27.03 ± 0.00 (100%)	12.80 ± 0.00 (100%)
	BC	0.20 ± 0.02 (2%)	0.00 ± 0.00 (0%)	10.14 ± 4.11 (98%)	5.44 ± 1.15 (100%)

[a] The sample size (n) of the biomass for PPCPs accumulation analysis from the RAB reactor is 2, including duplicate biomass samples at steady state (week 4). The sample size of the biomass from bubble column is 2, including duplicate biomass samples at the end of batch culture. The results are presented as arithmetic means ± standard errors.
[b] The numbers in the parentheses represent the contribution percentage of accumulation and degradation for PPCPs removal from the liquid.

and sulfate were then converted to nitrogen, phosphorus and sulfur content, respectively.

2.6. Microbial community characterization

Algal biomass harvested from the RAB reactors was centrifuged at 7,435 g to remove free water. The algal pellet was collected and freeze-dried for microbial community analysis. The total genomic DNA of the biomass samples was extracted based on Cetyl Tri-methyl Ammonium Bromide (CTAB), and Sodium Dodecyl Sulfate-based (SDS) protocols (Barbier et al., 2019). The quality of extracted DNA was examined using electrophoresis with 1% agarose gel in which the Red Sage was added to the gel to stain nucleic acids.

The V4 region of eukaryotes 18S rRNA was amplified using the primers 528F (GCGGTAATTCCAGCTCCAA) and 706R (AATCCRAGAATTTCACCTCT). The V4 and V5 regions of prokaryotic 16S rRNA were amplified with the primers 515 F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′). The PCR reaction was performed using 15 μL 2 × Phusion Master Mix, 3 μL of each primer (6 μM), 10 μL template DNA and 2 μL ultrapure H_2O. Amplification was conducted under the following conditions, polymerase activating at 94 °C for 130 s, annealing at 56 °C for 45 s, and extension at 72 °C for 130 s. This process was repeated for 35 cycles. The last cycle ended at 72 °C with an additional 6 min and cooling down to 4 °C. The PCR products were purified with GeneJET™ Gel Extraction kit (Thermo Scientific, USA) and the fragment size was determined by agarose gel electrophoresis. The diversity of the eukaryotic communities and prokaryotic communities was evaluated using Illumine high- throughput sequencing with an Ion S5TM XL platform (Thermo Scientific, USA) at single-end reads of 250 bp for 18S rRNA and 450 bp for 16S rRNA, respectively. The sequences obtained were compared with the Silva Database.

2.7. Statistical analysis

Generalized linear models (GLMs) were used and Multivariate Analysis of Variance (MANOVA) was performed. A p-value equal to and less than 0.05 was considered statistical significance. Modeling was performed with the lme4 package in R software (V 3.5.0).

3. Results

3.1. PPCPs removal in RAB reactor and bubble column

The PPCPs concentrations in the influent and effluent of the RAB reactors are shown in Fig. 1. As a comparison, the initial and final PPCP concentrations in the bubble column culture were also presented. As shown in Fig. 1A and B, ibuprofen, triclosan, and DEET were completely removed in the RAB reactors as none of these compounds were detected in the effluent. Oxybenzone and bisphenol A concentrations in the RAB effluent were detected but significantly lower ($p < 0.05$) than those in the influent. For the bubble column culture, the concentrations of all the PPCP compounds were reduced after seven days of cultivation (Fig. 1C and D). Triclosan was completely removed in the bubble column. Contrary to the RAB reactor, ibuprofen and DEET were detected at the end of bubble column culture. Overall, Fig. 1 shows that the RAB reactors outperformed bubble columns for PPCPs removal, the ANOVA test shows the statistically significant difference ($p < 0.05$) between the two groups.

The removal efficiencies (%) of each PPCP compound in the RAB reactor (E_{RAB}) and bubble column (E_{BC}) were evaluated as follows,

$$E_{RAB} = \frac{C_{in} - C_{eff}}{C_{in}} \times 100\% \quad (2)$$

$$E_{BC} = \frac{C_{initial} - C_{final}}{C_{initial}} \times 100\% \quad (3)$$

where C_{in} and C_{eff} are the concentrations of each PPCP in influent and effluent of the RAB reactor at the steady state, respectively. The $C_{initial}$ and C_{final} are the concentrations of each PPCP compound in the initial and final culture in the bubble columns, respectively. As shown in Fig. 2A, the RAB reactor achieved 100% removal of ibuprofen, triclosan and DEET. In the bubble column, only triclosan was removed at 100% (Fig. 2B). Overall, the removal efficiencies of most PPCP compounds in RAB reactors were higher ($p < 0.05$) than those in the bubble columns (Fig. 2).

PPCPs removal rates (μg/L/d) of the RAB reactor (R_{RAB}) and bubble column (R_{BC}) were also evaluated using the following equations,

$$R_{RAB} = \frac{F(C_{in} - C_{eff})}{V_{RAB}} = \frac{C_{in} - C_{eff}}{HRT} \quad (4)$$

$$R_{BC} = \frac{C_{initial} - C_{final}}{t} \quad (5)$$

where F is the daily exchange rate of the RAB reactor (mL/day), V_{RAB} is the working volume of the liquid reservoir in the RAB reactor, HRT is the hydraulic retention time of the RAB reactor and t is the culture time of the bubble column. As shown in Fig. 3, the RAB reactors resulted in higher removal rates ($p < 0.05$) then the bubble columns for all the PPCP compounds.

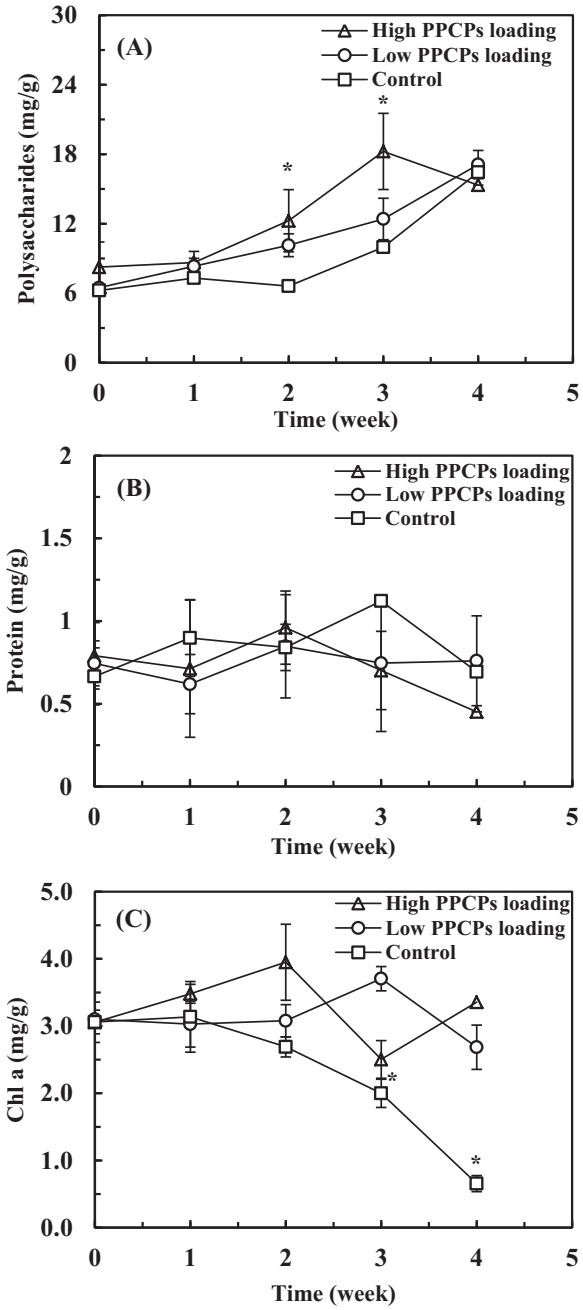

Fig. 4. The EPS polysaccharide content (**A**), EPS protein content (**B**), and Chl a content (**C**) in biomass of the RAB reactor fed with different concentrations of PPCP. The control group was fed with PPCPs-free influent. The data point with the asterisk symbol (*) indicates a significant difference from other groups ($p \leq 0.05$). The sample size at each level of PPCPs loading is 10, including duplicate samples per week at five consecutive weeks.

3.2. Fate of PPCPs in RAB reactors and bubble columns

To study the fate of PPCPs during the treatment, the PPCP contents in algal biomass were determined. As shown in Table 2, ibuprofen was not detected in the biomass of the two reactors, while the accumulation of oxybenzone and triclosan in biomass were found in both of the two reactors. The accumulation of bisphenol A and DEET were not found in the RAB reactor, whereas these two compounds were detected in the biomass of the bubble column.

The accumulation rate (μg/L/d) of the PPCPs in the biomass of the RAB reactor (A_{RAB}) and the bubble column (A_{BC}) was further determined as follows.

$$A_{RAB} = \frac{M \cdot C_{PPCP}}{\mathrm{SRT} \cdot V_{RAB}} \quad (6)$$

$$A_{BC} = \frac{M \cdot C_{PPCP}}{t \cdot V_{BC}} \quad (7)$$

where M is mass of biomass, C_{PPCP} is the content of each PPCP compound in the biomass, SRT is the solid retention time (i.e. biomass harvesting frequency) in the RAB reactor. V_{RAB} and V_{BC} are the working volume of the RAB liquid reservoir and the bubble column, respectively.

Based the rates of PPCP removal from liquid and accumulation in the biomass, the degradation rate (μg/L/d) of the PPCPs in the RAB reactor (D_{RAB}) and bubble column (D_{BC}) can be determined, i.e.,

$$D_{RAB} = R_{RAB} - A_{RAB} \quad (8)$$

$$D_{BC} = R_{BC} - A_{BC} \quad (9)$$

Table 3 shows the accumulation and degradation rates of each PPCP compound and their contributions to the rate of PPCPs removal from the liquid. As shown in the table, the degradation contributed to the majority of the PPCPs removal. Degradation contributed 100% to ibuprofen removal for both the RAB reactor and bubble column. Degradation also contributed the majority of oxybenzone and DEET removed from the liquid. Triclosan and bisphenol A had higher biomass accumulation percentage ($p < 0.05$) in bubble column culture compared to the RAB.

Table 4
The nitrogen, phosphorus and sulfur removal performances (n = 14) by the RAB reactors fed with different levels of PPCPs[a,b].

Parameters		Influent		
		High PPCP concentrations	Low PPCP concentrations	Control (PPCP-free)
Nitrate-N	Removal efficiency (%)	98 ± 1[c]	97 ± 1[c]	98 ± 1[c]
	Removal rate (mg-N/L/d)	47 ± 1[c]	47 ± 1[c]	48 ± 1[c]
Phosphate-P	Removal efficiency (%)	45 ± 5[c]	53 ± 6[c]	53 ± 4[c]
	Removal rate (mg-P/L/d)	24 ± 3[c]	28 ± 3[c]	28 ± 2[c]
Sulfate-S	Removal efficiency (%)	53 ± 8[c]	65 ± 8[c]	60 ± 8[c]
	Removal rate (mg-S/L/d)	4 ± 1[c]	5 ± 1[c]	5 ± 1[c]

[a] The sample size (n) of nutrients removal in the RAB reactor is 14, including steady-state (week 4) liquid samples at several consecutive days with duplicates each day.
[b] The results are presented as arithmetic means ± standard deviations.
[c] No significant difference.

3.3. The biofilm compositions and nutrients removal capacity of PPCPs-loaded RAB reactors

The EPS and Chl a contents in biomass of the RAB reactor as a response to exposure to the PPCP compounds were also evaluated. As shown in Fig. 4A, PPCPs treatment significantly ($p < 0.05$) increased polysaccharide content during the first three weeks. For the extracellular protein content, however, there was no significant difference ($p > 0.05$) between PPCPs treatment and control (Fig. 4B). Fig. 4C shows that the Chl a content of the PPCPs-treated biomass maintained at a relative

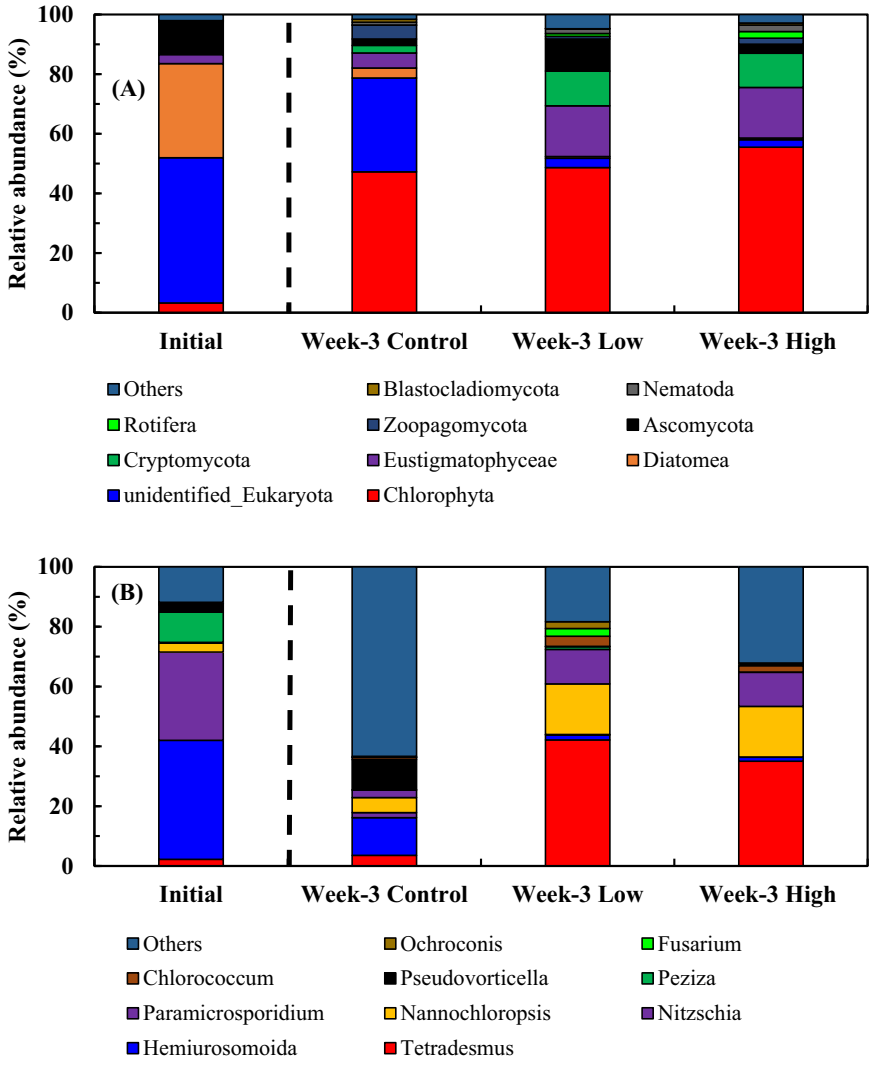

Fig. 5. Taxonomic assignment of Eukaryotic sequences of biomass at **(A)** phylum and **(B)** genus levels in biomass samples from the RAB reactor. Data are presented in relative abundance. "Initial" represents the biomass samples at the onset of feeding PPCPs-containing influent. "High, low and control" refers to the biomass samples that were fed with influent of high concentrations of PPCPs, low concentrations of PPCPs, and PPCPs-free for three weeks, respectively.

constant level (~3 mg/g) throughout the 4-week culture period. However, the biomass in the control group, which was fed with PPCPs-free medium recorded a decreased Chl a content with the culture progression.

The removal of major nutrients (nitrate-N, phosphate-P, and sulfate-S) in the RAB reactor were also evaluated. As shown in Table 4, the removal efficiencies and rates of these three nutrients were similar, with no significant difference ($p > 0.05$) among different PPCPs loadings and the control group.

3.4. Characterization of microbial communities of the RAB biofilm

The microbial community populations in the RAB reactors were also characterized. Fig. 5 shows the taxonomic assignment of eukaryotic sequences at the phylum and genus levels. As shown in Fig. 5A, the phyla of eukaryote at the initial stage was dominated by *Diatomea* with a limited amount of *Chlorophyta*. After fed with PPCPs-containing influent for three weeks, *Chlorophyta* dominated the eukaryote community. The biofilm also consisted of *Diatomea, Eustigmatophyceae, Cryptomycota* and *Ascomycota* as the major phyla. Fig. 5B shows that at genus level, the relative abundance of *Tetradesmus, Nitzschia* and *Nannochloropsis* increased after 3 weeks cultivation.

The prokaryotic community characteristics are shown in Fig. 6. Fig. 6A shows that *Oxyphotobacteria*, *Proteobacteria,* and *Bacteroidetes* were the dominant phyla in the consortia. At genus level, several unidentified genera in the family of *Pseudanabaenacea* and phyla of *Oxyphotobacteria* were widely presented in RAB reactors. *Sphingopyxis, Cytophaga,* and *Pseudofulvimonas* were observed in all PPCPs loading conditions.

4. Discussion

This work demonstrated that the RAB reactor is an efficient system to remove PPCP compounds from waterbody. The removal efficiency and the removal rate of most PPCPs in the RAB reactor exceeded the bubble column. Biomass accumulation and degradation were two mechanisms for PPCPs removal by microalgae. It was found that the majority of the PPCP removal was through degradation, which may be due to the enzymes produced by the algae to metabolize the PPCP compounds (Wang et al., 2017b). The metabolism of PPCPs by microalgae has been reported previously (Zamek-Gliszczynski et al., 2006; Nakajima et al., 2007; Calza et al., 2011; Im and Löffler, 2016; Chen et al., 2017; Ding et al., 2017; Wang et al., 2018). PPCP degradation by algae commonly initiates with hydroxylation (Ding et al., 2017), demethylation (Calza et al., 2011; Chen et al., 2017) and dichlorination (Wang et al., 2018), which transform PPCPs into more hydrophilic compounds to increase

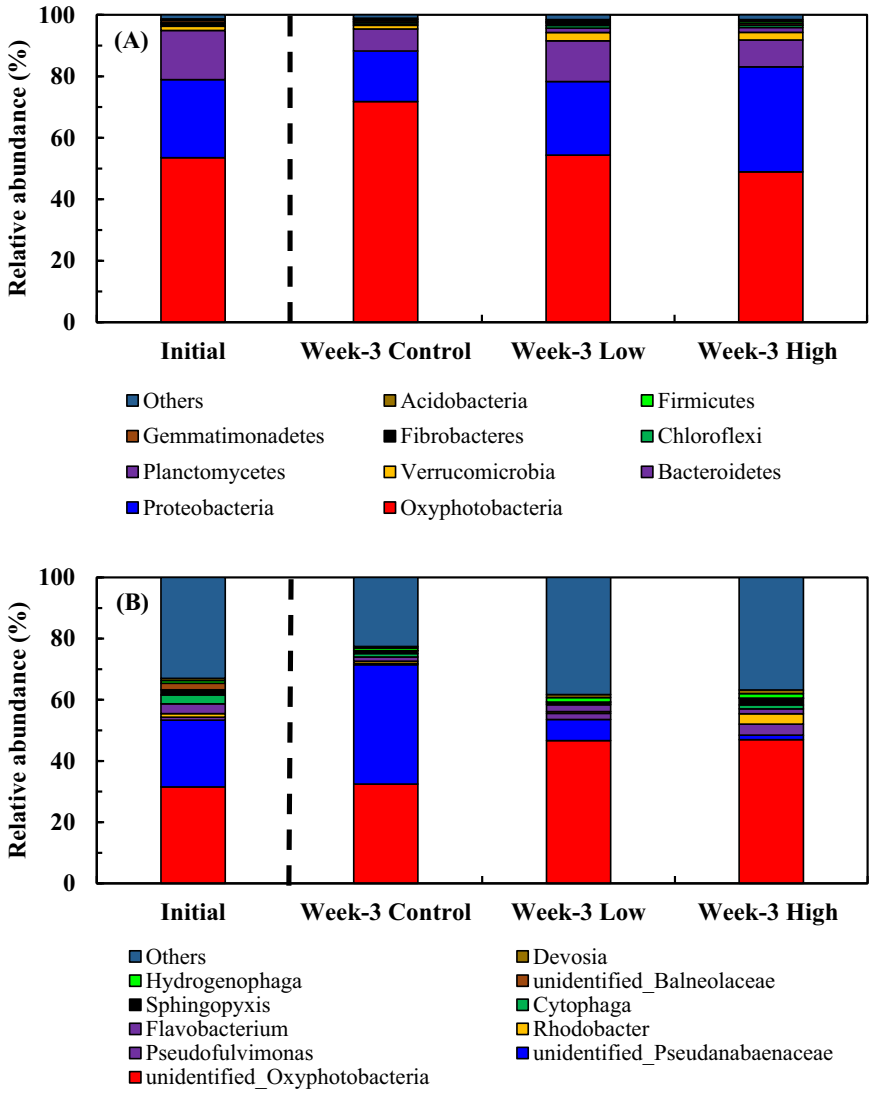

Fig. 6. Taxonomic assignment of Prokaryote sequences of biomass at **(A)** phylum and **(B)** genus levels in biomass samples from the RAB reactor. Data are presented in relative abundance. "Initial" represents the biomass samples at the onset of feeding PPCPs-containing influent. "High, low and control" refers to the biomass samples that were fed with influent of high concentrations of PPCPs, low concentrations of PPCPs, and PPCPs-free for three weeks, respectively.

their bioavailability. After these initial steps, the PPCPs can conjugate with glucoside, malonyl-glucoside and sulfate to form conjugated complexes for further degradation (Wang et al., 2017b).

For the PPCP compounds that were partially accumulated in the biomass (Table 3), such an accumulation can be explained by their octanol/water partition coefficient (K_{ow}) and pH-dependent partition coefficient (D_{ow}), which describe the partitioning of the ionizable PPCP compounds between lipophilic and hydrophilic phases (Swackhamer and Skoglund, 1993; Lindholm-Lehto et al., 2017). In general, the higher the K_{ow} and D_{ow} values, the more tendency for the PPCPs to accumulate in the cells. In this work, the values of log K_{ow} and log D_{ow} (at two pH levels) for each PPCP compound and their accumulations followed this trend. For example, the log K_{ow} and log D_{ow} values for oxybenzone, triclosan and bisphenol were higher than ibuprofen and DEET (Table 5), which explains the certain degree of accumulation of these three compounds in the biomass (Table 3). However, it should be noted that the accumulation and degradation of PPCP compounds are also affected by other factors in addition to K_{ow} and Dow. For example, Arnot and Gobas (2006) reported that the metabolic biotransformation rate and the concentrations of organic carbon can affect bioaccumulation endpoints of the PPCP compounds. This work also demonstrated that different reactors (RAB vs bubble column) (Table 3) and the microbial community structures can cause the different scenarios of the bioaccumulation and degradation. These variabilities may explain the slight accumulation (2%) of DEET in the bubble column reactor (Table 3).

Polysaccharides and proteins are the major compositions of EPS. The

Table 5
Octanol/water partition coefficients (K_{ow} and D_{ow}) of the target PPCP compounds[a].

PPCP compound	log K_{ow}	pKa	log D_{ow} (pH = 6.6)	log D_{ow} (pH = 10)	References
Ibuprofen	2.48	4.52	0.40	− 3.00	Scheytt et al., 2005
Oxybenzone	4	7.56	2.99	1.56	Kim and Choi, 2014
Triclosan	4.76	8.14	3.20	2.89	Wang et al., 2017a
Bisphenol A	3.32	9.50	0.42	2.70	Wang et al., 2017a
DEET	2.18	0.67	− 3.75	− 7.15	McEachran et al., 2017

[a] The values of log D_{ow} of the PPCP compounds were calculated based on log K_{ow} and acid dissociation constant (pKa) as described in the previous research (Yang et al., 2020).

Table 6
Comparison of PPCPs removal by different technologies.

Compound	Technology	Influent (μg/L)	Effluent (μg/L)	Removal efficiency (%)	HRT (d)	Type of wastewater	Reference
Ibuprofen	Activated sludge (WWTP 4)	0.711–17.94	0.313–3.77	56	12	Industrial, hospital and livestock wastewater	Lin et al., 2009
	Tubular photo-bioreactor	39–52.8	< 1	> 98	8	Toilet wastewater	Hom-Diaz et al., 2017
	High-rate algal ponds	~9	N/A	99 ± 1 (warm season) 86 ± 4 (cold season)	4	Primary effluent from the settlers	Matamoros et al., 2015
	RAB	100.4 (HL[a]); 45.7 (LL[a])	0 (HL); 0 (LL)	100 (HL); 100 (LL)	5	Culture medium	This work
Oxybenzone	Activated sludge	0–1.26	N/A	70.8	2.4	Municipal wastewater	Kapelewska et al., 2018
	High-rate algal ponds	1–12	N/A	97 ± 1(warm season) 75 ± 10 (cold season)	4	Primary effluent from the settlers	Matamoros et al., 2015
	RAB	14.1 (HL); 6.9 (LL)	2.62 ± 0.18 (HL) 1.80 ± 0.38 (LL)	81.4 ± 1.3 (HL) 74.1 ± 5.5 (LL)	5	Culture medium	This work
Triclosan	Activated sludge	0–0.67	N/A	90.1	2.4	Municipal wastewater	Kapelewska et al., 2018
	High-rate algal ponds	< 1	N/A	93 ± 1(warm season) 49 ± 5 (cold season)	4	Primary effluent from the settlers	Matamoros et al., 2015
	RAB	3.4 (HL); 2.9 (LL)	0 (HL); 0 (LL)	100 (HL); 100 (LL)	5	Culture medium	This work
Bisphenol A	Activated sludge	0.01–12.06	N/A	87.2	2.4	Municipal wastewater	Kapelewska et al., 2018
	High-rate algal ponds	< 0.10	N/A	72 ± 14 (warm season) 66 ± 16 (cold season)	4	Primary effluent from the settlers	Matamoros et al., 2015
	RAB	3.9 (HL); 2.2 (LL)	0.49 ± 0.11 (HL) 0.54 ± 0.75 (LL)	87.4 ± 2.7 (HL) 75.9 ± 3.4 (LL)	5	Culture medium	This work
DEET	Activated sludge	0.15–1	N/A	69 ± 21	0.6	Domestic wastewater	Sui et al., 2010
	Activated sludge	0–2.62	N/A	38.9	2.4	Municipal wastewater	Kapelewska et al., 2018
	RAB	135 (HL); 63.9 (LL)	0 (HL); 0 (LL)	100 (HL); 100 (LL)	5	Culture medium	This work

[a] HL: High loading, LL: low loading

participation of polysaccharides, such as stretching vibration of C–O–C have been observed after PPCPs adsorption (Xie et al., 2020). The extracellular enzymes that are secreted by the cells to serve as an external digestion system also interact with polysaccharide and accumulate in the biofilm (Flemming et al., 2016). Protein-like substances can provide active sites, such as carboxyl, amine, hydroxyl groups, and hydrophobic regions for organic micropollutants absorption (Zhang et al., 2018). A recent study reported that PPCPs can be trapped in the EPS and then transported inside cells for complete degradation (Xie et al., 2020).

In this work, the polysaccharide contents in RAB biomass increased with PPCPs loading and culture time (Fig. 4A), probably due to the succession of biofilm under stressful environment (Miao et al., 2009; Iswarya et al., 2017) as polysaccharides functions as a diffusion barrier for antimicrobials by chelation reaction, enzyme degradation, and sacrificial reaction (Flemming et al., 2016).

The photosynthesis pigment chlorophyll-a reflects the efficiency of light absorption by algae cells (Sutherland et al., 2015). The *Chl-a* content in biomass of PPCPs-free culture decreased but maintained constant in biomass fed with PPCPs (Fig. 4C). This phenomenon was possibly due to the degradation of chlorophyll-a as a nitrogen source by the biomass in PPCPs-free culture (Li et al., 2008), while the biomass fed with PPCP compounds can use nitrogen-containing DEET as a nitrogen source (Antonopoulou et al., 2013) which protected chlorophyll-a from degradation. Another possible reason may be the hormesis effect, as a small dose of PPCPs has been reported to stimulate the Chl-a content (Pinckney et al., 2013; Ding et al., 2017; Zhang et al., 2020).

Several eukaryotic microorganisms capable of degrading targeted PPCP compounds were found enriched in the RAB reactors. For example, at the genus level, the dominant eukaryote *Tetradesmus* (Fig. 5) is a green alga for the degradation of ibuprofen and phenolic compounds (Papazi and Kotzabasis, 2007; Matamoros et al., 2016) while *Nannochloropsis* was capable of degrading bisphenol A (Ishihara and Nakajima, 2003). Some prokaryotic microorganisms capable of degrading PPCPs were also observed in the RAB reactor (Fig. 6). For example, *Cytophaga* was reported responsible for ibuprofen degradation (Prasertkulsak et al., 2016). *Sphingopyxis* was able to degrade triclosan and bisphenol A (Zhou et al., 2013). *Pseudofulvimonas* observed in the RAB reactor is classified as gammaproteobacteria, which was reported to degrade DEET (Rivera-Cancel et al., 2007). Such a diversity of the microbial community enhanced the PPCPs removal performance by the RAB reactor.

A comprehensive comparison of different technologies for treating PPCP compounds is shown in Table 6. Overall, the RAB reactors achieved good performance in PPCPs removal compared with other technologies. For example, the RAB reactors achieved complete removal of ibuprofen, triclosan and the DEET, which was relatively recalcitrant in the activated sludge process. The removal efficiencies of oxybenzone and bisphenol A were equal to the other technologies even though the PPCPs loading levels of the RAB reactors were high. For the comparison of removal between different PPCPs, the results were consisted with other algae-based technologies such as tubular photo-bioreactor and high rate algal ponds, in which the ibuprofen and triclosan reached more than 90% removal in the warm season, compared with the removal of bisphenol A was lower than 72% (Matamoros et al., 2015; Hom-Diaz

et al., 2017). In the activated sludge processes, the oxybenzone, triclosan and bisphenol A were easier to degrade compared with the DEET resisted in water (Kapelewska et al., 2018).

5. Conclusion

This work demonstrates that the RAB reactors achieved 70%− 100% removal of PPCP compounds. The PPCPs removal by algal biomass was primarily due to degradation, with small portion (< 10%) attributed to biomass accumulation. Treating the RAB reactor with PPCPs did not reduce its nitrate, phosphorus, and sulfate removal performance. The PPCP treatment also increased the extracellular polysaccharides content of the biomass in the RAB, and keep Chl a content from degradation. The algal species capable of PPCPs degradation in the microalgal community of the RAB biofilm were enriched with the PPCPs treatment, demonstrating a robust tolerance to PPCPs compounds by the RAB reactor. Finally, it should be noted that the PPCPs removal reported here was based on lab scale RAB reactors. Future work is needed for scaling up of the RAB reactor to meet the requirements of high wastewater volume of wastewater treatment plants. the performance by the RAB system to treat real wastewater stream need to be studied to validate the results obtained in this work. In addition, more PPCP compounds with different characteristics and their transformation products should be explored.

CRediT authorship contribution statement

Si Chen: Conceptualization, Methodology, Investigation, Visualization, Data curation, Writing - original draft. **Jiahui Xie:** Resources. **Zhiyou Wen:** Conceptualization, Supervision, Project administration, Writing - review & editing.

Declaration of Competing Interest

Z. Wen has interests in Gross-Wen Technologies, Inc., a company that may potentially benefit from the research results, and also serve on the company's Scientific Advisory Board. The terms of this arrangement have been reviewed and approved by Iowa State University in accordance with its conflict of interest policies.

Acknowledgments

The authors would like to acknowledge Show-Ling Lee, Haoyuan Zhou and Xuefei Zhao (all from Iowa State University) for their contributions to the experiment setup. This work has been supported by the Iowa State University President Graduate Scholarship (to Si Chen).

References

Adeleye, A.S., Keller, A.A., 2016. Interactions between algal extracellular polymeric substances and commercial TiO_2 nanoparticles in aqueous media. Environ. Sci. Technol. 50, 12258–12265. https://doi.org/10.1021/acs.est.6b03684.

Antonopoulou, M., Giannakas, A., Deligiannakis, Y., Konstantinou, I., 2013. Kinetic and mechanistic investigation of photocatalytic degradation of the N,N-diethyl-m-toluamide. Chem. Eng. J. 231, 314–325. https://doi.org/10.1016/j.cej.2013.06.123.

Arnold, K.E., Boxall, A.B., Brown, A.R., Cuthbert, R.J., Gaw, S., Hutchinson, T.H., Jobling, S., Madden, J.C., Metcalfe, C.D., Naidoo, V., 2013. Assessing the exposure risk and impacts of pharmaceuticals in the environment on individuals and ecosystems. Biol. Lett. 9, 20130492 https://doi.org/10.1098/rsbl.2013.0492.

Arnot, J.A., Gobas, F.A., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14, 257–297. https://doi.org/10.1139/A06-005.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Calza, P., Medana, C., Raso, E., Giancotti, V., Minero, C., 2011. N,N-diethyl-m-toluamide transformation in river water. Sci. Total. Environ. 409, 3894–3901. https://doi.org/10.1016/j.scitotenv.2011.06.006.

Chen, F., Huber, C., Schröder, P., 2017. Fate of the sunscreen compound oxybenzone in Cyperus alternifolius based hydroponic culture: uptake, biotransformation and phytotoxicity. Chemosphere 182, 638–646. https://doi.org/10.1016/j.chemosphere.2017.05.072.

Comninellis, C., Kapalka, A., Malato, S., Parsons, S.A., Poulios, I., Mantzavinos, D., 2008. Advanced oxidation processes for water treatment: advances and trends for R&D. J. Chem. Technol. Biotechnol. 83, 769–776. https://doi.org/10.1002/jctb.1873.

Ding, T., Yang, M., Zhang, J., Yang, B., Lin, K., Li, J., Gan, J., 2017. Toxicity, degradation and metabolic fate of ibuprofen on freshwater diatom Navicula sp. J. Hazard. Mater. 330, 127–134. https://doi.org/10.1016/j.jhazmat.2017.02.004.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, Pt, Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: an emergent form of bacterial life. Nat. Rev. Microbiol. 14, 563–575. https://doi.org/10.1038/nrmicro.2016.94.

Ganiyu, S.O., van Hullebusch, E.D., Cretin, M., Esposito, G., Oturan, M.A., 2015. Coupling of membrane filtration and advanced oxidation processes for removal of pharmaceutical residues: a critical review. Sep. Purif. Technol. 156, 891–914. https://doi.org/10.1016/j.seppur.2015.09.059.

García-Meza, J.V., Barrangue, C., Admiraal, W., 2005. Biofilm formation by algae as a mechanism for surviving on mine tailings. Environ. Toxicol. Chem. 24, 573–581. https://doi.org/10.1897/04-064R.1.

De Graaff, M., Vieno, N., Kujawa-Roeleveld, K., Zeeman, G., Temmink, H., Buisman, C., 2011. Fate of hormones and pharmaceuticals during combined anaerobic treatment and nitrogen removal by partial nitritation-anammox in vacuum collected black water. Water Res. 45, 375–383. https://doi.org/10.1016/j.watres.2010.08.023.

Gross, M., Henry, W., Michael, C., Wen, Z., 2013. Development of a rotating algal biofilm growth system for attached microalgae growth with in situ biomass harvest. Bioresour. Technol. 150, 195–201. https://doi.org/10.1016/j.biortech.2013.10.016.

Gross, M., Jarboe, D., Wen, Z., 2015a. Biofilm-based algal cultivation systems. Appl. Microbiol. Biotechnol. 99, 5781–5789. https://doi.org/10.1007/s00253-015-6736-5.

Gross, M., Mascarenhas, V., Wen, Z., 2015b. Evaluating algal growth performance and water use efficiency of pilot-scale revolving algal biofilm (RAB) culture systems. Biotechnol. Bioeng. 112, 2040–2050. https://doi.org/10.1002/bit.25618.

Gross, M., Wen, Z., 2014. Yearlong evaluation of performance and durability of a pilot-scale revolving algal biofilm (RAB) cultivation system. Bioresour. Technol. 171, 50–58. https://doi.org/10.1016/j.biortech.2014.08.052.

Gross, M.A., Wen, Z., 2018. Photobioreactor systems and methods. Google Patents.

Hedgespeth, M.L., Sapozhnikova, Y., Pennington, P., Clum, A., Fairey, A., Wirth, E., 2012. Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina. Sci. Total Environ. 437, 1–9. https://doi.org/10.1016/j.scitotenv.2012.07.076.

Hom-Diaz, A., Jaén-Gil, A., Bello-Laserna, I., Rodríguez-Mozaz, S., Vicent, T., Barceló, D., Blánquez, P., 2017. Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting. Sci. Total Environ. 592, 1–11. https://doi.org/10.1016/j.scitotenv.2017.02.224.

Hu, X., Zhou, Q., 2011. Comparisons of microwave-assisted extraction, simultaneous distillation-solvent extraction, Soxhlet extraction and ultrasound probe for polycyclic musks in sediments: recovery, repeatability, matrix effects and bioavailability. Chromatographia 74, 489–495. https://doi.org/10.1007/s10337-011-2084-5.

Im, J., Löffler, F.E., 2016. Fate of bisphenol A in terrestrial and aquatic environments. Environ. Sci. Technol. 50, 8403–8416. https://doi.org/10.1021/acs.est.6b00877.

Ishihara, K., Nakajima, N., 2003. Improvement of marine environmental pollution using eco-system: decomposition and recovery of endocrine disrupting chemicals by marine phyto-and zooplanktons. J. Mol. Catal. B Enzym. 23, 419–424. https://doi.org/10.1016/S1381-1177(03)00107-3.

Iswarya, V., Sharma, V., Chandrasekaran, N., Mukherjee, A., 2017. Impact of tetracycline on the toxic effects of titanium dioxide (TiO_2) nanoparticles towards the freshwater algal species, Scenedesmus obliquus. Aquat. Toxicol. 193, 168–177. https://doi.org/10.1016/j.aquatox.2017.10.023.

Kapelewska, J., Kotowska, U., Karpińska, J., Kowalczuk, D., Arciszewska, A., Świrydo, A., 2018. Occurrence, removal, mass loading and environmental risk assessment of emerging organic contaminants in leachates, groundwaters and wastewaters. Microchem. J. 137, 292–301. https://doi.org/10.1016/j.microc.2017.11.008.

Kapelewska, J., Kotowska, U., Wiśniewska, K., 2016. Determination of personal care products and hormones in leachate and groundwater from Polish MSW landfills by ultrasound-assisted emulsification microextraction and GC-MS. Environ. Sci. Pollut. Res. 23, 1642–1652. https://doi.org/10.1007/s11356-015-5359-9.

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., Flick, R.W., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. 104, 8897–8901. https://doi.org/10.1073/pnas.0609568104.

Kim, S., Choi, K., 2014. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. Environ. Int. 70, 143–157. https://doi.org/10.1016/j.envint.2014.05.015.

Lee, S.H., Oh, H.-M., Jo, B.H., Lee, S.-A., Shin, S.Y., Kim, H.-S., Lee, S.-H., Ahn, C.-Y., 2014. Higher biomass productivity of microalgae in an attached growth system, using wastewater. J. Microbiol. Biotechnol. 24, 1566–1573. https://doi.org/10.4014/jmb.1406.06057.

Liang, R., Van Leuwen, J.C., Bragg, L.M., Arlos, M.J., Li Chun Fong, L.C.M., Schneider, O. M., Jaciw-Zurakowsky, I., Fattahi, A., Rathod, S., Peng, P., Servos, M.R., Zhou, Y.N., 2019. Utilizing UV-LED pulse width modulation on TiO2 advanced oxidation processes to enhance the decomposition efficiency of pharmaceutical micropollutants. Chem. Eng. J. 361, 439–449. https://doi.org/10.1016/j.cej.2018.12.065.

Lindholm-Lehto, P.C., Ahkola, H.S.J., Knuutinen, J.S., 2017. Procedures of determining organic trace compounds in municipal sewage sludge—a review. Environ. Sci. Pollut. Res. 24, 4383–4412. https://doi.org/10.1007/s11356-016-8202-z.

Lin, T., Yu, S., Chen, W., 2016. Occurrence, removal and risk assessment of pharmaceutical and personal care products (PPCPs) in an advanced drinking water treatment plant (ADWTP) around Taihu Lake in China. Chemosphere 152, 1–9. https://doi.org/10.1016/j.chemosphere.2016.02.109.

Lin, A.Y.-C., Yu, T.-H., Lateef, S.K., 2009. Removal of pharmaceuticals in secondary wastewater treatment processes in Taiwan. J. Hazard. Mater. 167, 1163–1169. https://doi.org/10.1016/j.jhazmat.2009.01.108.

Li, Y., Horsman, M., Wang, B., Wu, N., Lan, C.Q., 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleoabundans. Appl. Microbiol. Biotechnol. 81, 629–636. https://doi.org/10.1007/s00253-008-1681-1.

Lv, J., Guo, J., Feng, J., Liu, Q., Xie, S., 2017. Effect of sulfate ions on growth and pollutants removal of self-flocculating microalga Chlorococcum sp. GD in synthetic municipal wastewater. Bioresour. Technol. 234, 289–296. https://doi.org/10.1016/j.biortech.2017.03.061.

Maruya, K.A., Dodder, N.G., Sengupta, A., Smith, D.J., Lyons, J.M., Heil, A.T., Drewes, J. E., 2016. Multimedia screening of contaminants of emerging concern (CECS) in coastal urban watersheds in southern California (USA). Environ. Toxicol. Chem. 35, 1986–1994. https://doi.org/10.1002/etc.3348.

Matamoros, V., Gutiérrez, R., Ferrer, I., García, J., Bayona, J.M., 2015. Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilot-scale study. J. Hazard. Mater. 288, 34–42. https://doi.org/10.1016/j.jhazmat.2015.02.002.

Matamoros, V., Uggetti, E., García, J., Bayona, J.M., 2016. Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study. J. Hazard. Mater. 301, 197–205. https://doi.org/10.1016/j.jhazmat.2015.08.050.

Ma, M., Liu, R., Liu, H., Qu, J., Jefferson, W., 2012. Effects and mechanisms of pre-chlorination on Microcystis aeruginosa removal by alum coagulation: significance of the released intracellular organic matter. Sep. Purif. Technol. 86, 19–25. https://doi.org/10.1016/j.seppur.2011.10.015.

McEachran, A.D., Shea, D., Nichols, E.G., 2017. Pharmaceuticals in a temperate forest-water reuse system. Sci. Total Environ. 581–582, 705–714. https://doi.org/10.1016/j.scitotenv.2016.12.185.

Miao, A.-J., Schwehr, K.A., Xu, C., Zhang, S.-J., Luo, Z., Quigg, A., Santschi, P.H., 2009. The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. Environ. Pollut. 157, 3034–3041. https://doi.org/10.1016/j.envpol.2009.05.047.

Milledge, J.J., Heaven, S., 2013. A review of the harvesting of micro-algae for biofuel production. Rev. Environ. Sci. Biotechnol. 12, 165–178. https://doi.org/10.1016/j.biortech.2011.08.010.

Mohapatra, S., Huang, C.-H., Mukherji, S., Padhye, L.P., 2016. Occurrence and fate of pharmaceuticals in WWTPs in India and comparison with a similar study in the United States. Chemosphere 159, 526–535. https://doi.org/10.1016/j.chemosphere.2016.06.047.

Nakajima, N., Teramoto, T., Kasai, F., Sano, T., Tamaoki, M., Aono, M., Kubo, A., Kamada, H., Azumi, Y., Saji, H., 2007. Glycosylation of bisphenol A by freshwater microalgae. Chemosphere 69, 934–941. https://doi.org/10.1016/j.chemosphere.2007.05.088.

Niederdorfer, R., Peter, H., Battin, T.J., 2016. Attached biofilms and suspended aggregates are distinct microbial lifestyles emanating from differing hydraulics. Nat. Microbiol. 1, 16178. https://doi.org/10.1038/nmicrobiol.2016.178.

Papazi, A., Kotzabasis, K., 2007. Bioenergetic strategy of microalgae for the biodegradation of phenolic compounds—exogenously supplied energy and carbon sources adjust the level of biodegradation. J. Biotechnol. 129, 706–716. https://doi.org/10.1016/j.jbiotec.2007.02.021.

Peng, J., Kumar, K., Gross, M., Kunetz, T., Wen, Z., 2019. Removal of total dissolved solids (TDS) from wastewater using a revolving algal biofilm reactor. Water Environ. Res. https://doi.org/10.1002/wer.1273.

Pinckney, J.L., Hagenbuch, I.M., Long, R.A., Lovell, C.R., 2013. Sublethal effects of the antibiotic tylosin on estuarine benthic microalgal communities. Mar. Pollut. Bull. 68, 8–12. https://doi.org/10.1016/j.marpolbul.2013.01.006.

Prasertkulsak, S., Chiemchaisri, C., Chiemchaisri, W., Itonaga, T., Yamamoto, K., 2016. Removals of pharmaceutical compounds from hospital wastewater in membrane bioreactor operated under short hydraulic retention time. Chemosphere 150, 624–631. https://doi.org/10.1016/j.chemosphere.2016.01.031.

Rivera-Cancel, G., Bocioaga, D., Hay, A.G., 2007. Bacterial degradation of N, N-diethyl-m-toluamide (DEET): cloning and heterologous expression of DEET hydrolase. Appl. Environ. 73, 3105–3108. https://doi.org/10.1128/AEM.02765-06.

Salgado, R., Noronha, J., Oehmen, A., Carvalho, G., Reis, M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Sci. Technol. 62, 2862–2871. https://doi.org/10.2166/wst.2010.985.

Scheytt, T., Mersmann, P., Lindstädt, R., Heberer, T., 2005. 1-octanol/water partition coefficients of 5 pharmaceuticals from human medical care: carbamazepine, clofibric acid, diclofenac, ibuprofen, and propyphenazone. Water Air Soil Pollut. 165, 3–11. https://doi.org/10.1007/s11270-005-3539-9.

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Wert, E.C., Yoon, Y., 2007. Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination 202, 156–181. https://doi.org/10.1016/j.desal.2005.12.052.

Sui, Q., Huang, J., Deng, S., Yu, G., Fan, Q., 2010. Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Res. 44, 417–426. https://doi.org/10.1016/j.watres.2009.07.010.

Sutherland, D.L., Howard-Williams, C., Turnbull, M.H., Broady, P.A., Craggs, R.J., 2015. Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. Bioresour. Technol. 184, 222–229. https://doi.org/10.1016/j.biortech.2014.10.074.

Swackhamer, D.L., Skoglund, R., 1993. Bioaccumulation of PCBs by algae: kinetics versus equilibrium. Environ. Toxicol. Chem. 12, 831–838. https://doi.org/10.1002/etc.5620120506.

Torresi, E., Fowler, S.J., Polesel, F., Bester, K., Andersen, H.R., Smets, B.F., Plosz, B.G., Christensson, M., 2016. Biofilm thickness influences biodiversity in nitrifying MBBRs: implications on micropollutant removal. Environ. Sci. Technol. 50, 9279–9288. https://doi.org/10.1021/acs.est.6b02007.

Wang, Y., Liu, J., Kang, D., Wu, C., Wu, Y., 2017b. Removal of pharmaceuticals and personal care products from wastewater using algae-based technologies: a review. Rev. Environ. Sci. Biotechnol. 16, 717–735. https://doi.org/10.1007/s11157-017-9446-x.

Wang, F., Lu, X., Peng, W., Deng, Y., Zhang, T., Hu, Y., Li, X.-y, 2017a. Sorption behavior of bisphenol A and triclosan by graphene: comparison with activated carbon. ACS Omega 2, 5378–5384. https://doi.org/10.1021/acsomega.7b00616.

Wang, S., Poon, K., Cai, Z., 2018. Removal and metabolism of triclosan by three different microalgal species in aquatic environment. J. Hazard. Mater. 342, 643–650. https://doi.org/10.1016/j.jhazmat.2017.09.004.

Xie, P., Chen, C., Zhang, C., Su, G., Ren, N., Ho, S.-H., 2020. Revealing the role of adsorption in ciprofloxacin and sulfadiazine elimination routes in microalgae. Water Res. 172, 115475 https://doi.org/10.1016/j.watres.2020.115475.

Xu, Y., Liu, T., Zhang, Y., Ge, F., Steel, R.M., Sun, L., 2017. Advances in technologies for pharmaceuticals and personal care products removal. J. Mater. Chem. 5, 12001–12014. https://doi.org/10.1039/C7TA03698A.

Yang, H., Lu, G., Yan, Z., Liu, J., Dong, H., Bao, X., Zhang, X., Sun, Y., 2020. Residues, bioaccumulation, and trophic transfer of pharmaceuticals and personal care products in highly urbanized rivers affected by water diversion. J. Hazard. Mater. 391, 122245 https://doi.org/10.1016/j.jhazmat.2020.122245.

Yoo, D.K., An, H.J., Khan, N.A., Hwang, G.T., Jhung, S.H., 2018. Record-high adsorption capacities of polyaniline-derived porous carbons for the removal of personal care products from water. Chem. Eng. J. 352, 71–78. https://doi.org/10.1016/j.cej.2018.06.144.

Yu, C.-P., Chu, K.-H., 2009. Occurrence of pharmaceuticals and personal care products along the West Prong Little Pigeon River in east Tennessee, USA. Chemosphere 75, 1281–1286. https://doi.org/10.1016/j.chemosphere.2009.03.043.

Zamek-Gliszczynski, M.J., Hoffmaster, K.A., Nezasa, K.-i, Tallman, M.N., Brouwer, K.L. R., 2006. Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. Eur. J. Pharm. Sci. 27, 447–486. https://doi.org/10.1016/j.ejps.2005.12.007.

Zhang, H., Jia, Y., Khanal, S.K., Lu, H., Fang, H., Zhao, Q., 2018. Understanding the role of extracellular polymeric substances on ciprofloxacin adsorption in aerobic sludge, anaerobic sludge, and sulfate-reducing bacteria sludge systems. Environ. Sci. Technol. 52, 6476–6486. https://doi.org/10.1021/acs.est.8b00568.

Zhang, M., Steinman, A.D., Xue, Q., Zhao, Y., Xu, Y., Xie, L., 2020. Effects of erythromycin and sulfamethoxazole on Microcystis aeruginosa: cytotoxic endpoints, production and release of microcystin-LR. J. Hazard. Mater. 399, 123021 https://doi.org/10.1016/j.jhazmat.2020.123021.

Zhao, J., Cao, X., Wang, Z., Dai, Y., Xing, B., 2017. Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae. Water Res. 111, 18–27. https://doi.org/10.1016/j.watres.2016.12.037.

Zhao, X., Kumar, K., Gross, M.A., Kunetz, T.E., Wen, Z., 2018a. Evaluation of revolving algae biofilm reactors for nutrients and metals removal from sludge thickening supernatant in a municipal wastewater treatment facility. Water Res. 143, 467–478. https://doi.org/10.1016/j.watres.2018.07.001.

Zhou, N.A., Lutovsky, A.C., Andaker, G.L., Gough, H.L., Ferguson, J.F., 2013. Cultivation and characterization of bacterial isolates capable of degrading pharmaceutical and personal care products for improved removal in activated sludge wastewater treatment. Biodegradation 24, 813–827. https://doi.org/10.1007/s10532-013-9630-9.

Zhou, H., Sheng, Y., Zhao, X., Gross, M., Wen, Z., 2018b. Treatment of acidic sulfate-containing wastewater using revolving algae biofilm reactors: sulfur removal performance and microbial community characterization. Bioresour. Technol. 264, 24–34. https://doi.org/10.1016/j.biortech.2018.05.051.

Barbier, F.F., Chabikwa, T.G., Ahsan, M.U., Cook, S.E., Powell, R., Tanurdzic, M., Beveridge, C.A., 2019. A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. Plant Methods 15, 62. https://doi.org/10.1186/s13007-019-0447-3.