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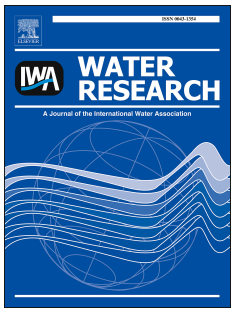

Removal of selected emerging PPCP compounds using greater duckweed (*Spirodela polyrhiza*) based lab-scale free water constructed wetland

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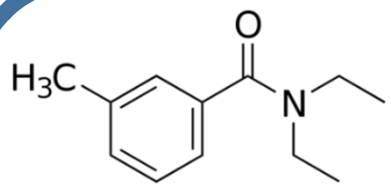

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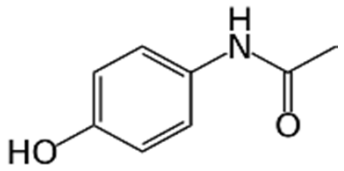

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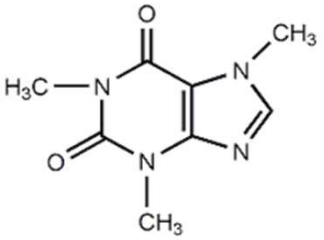

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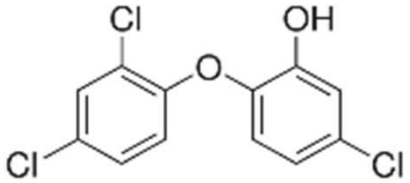
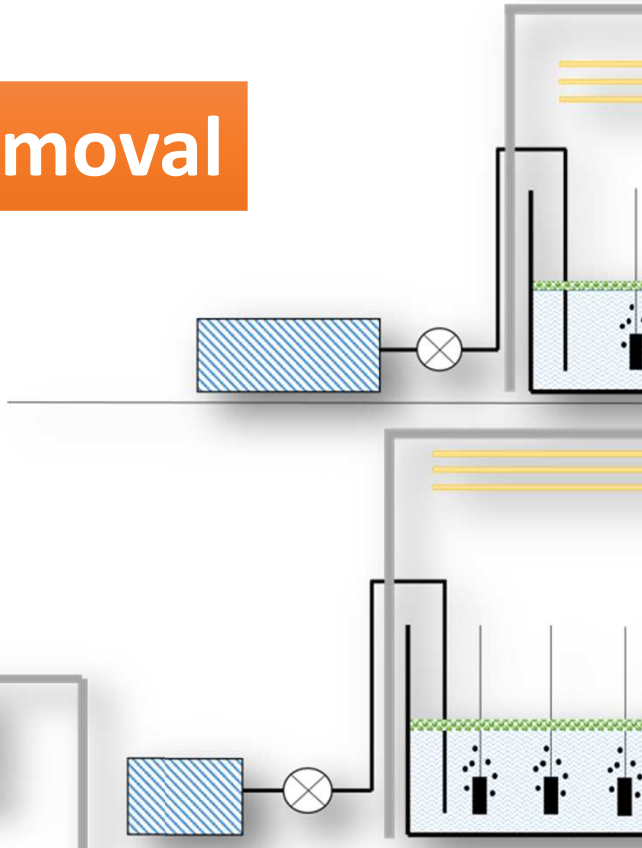


Triclosan

PPCPs' Removal



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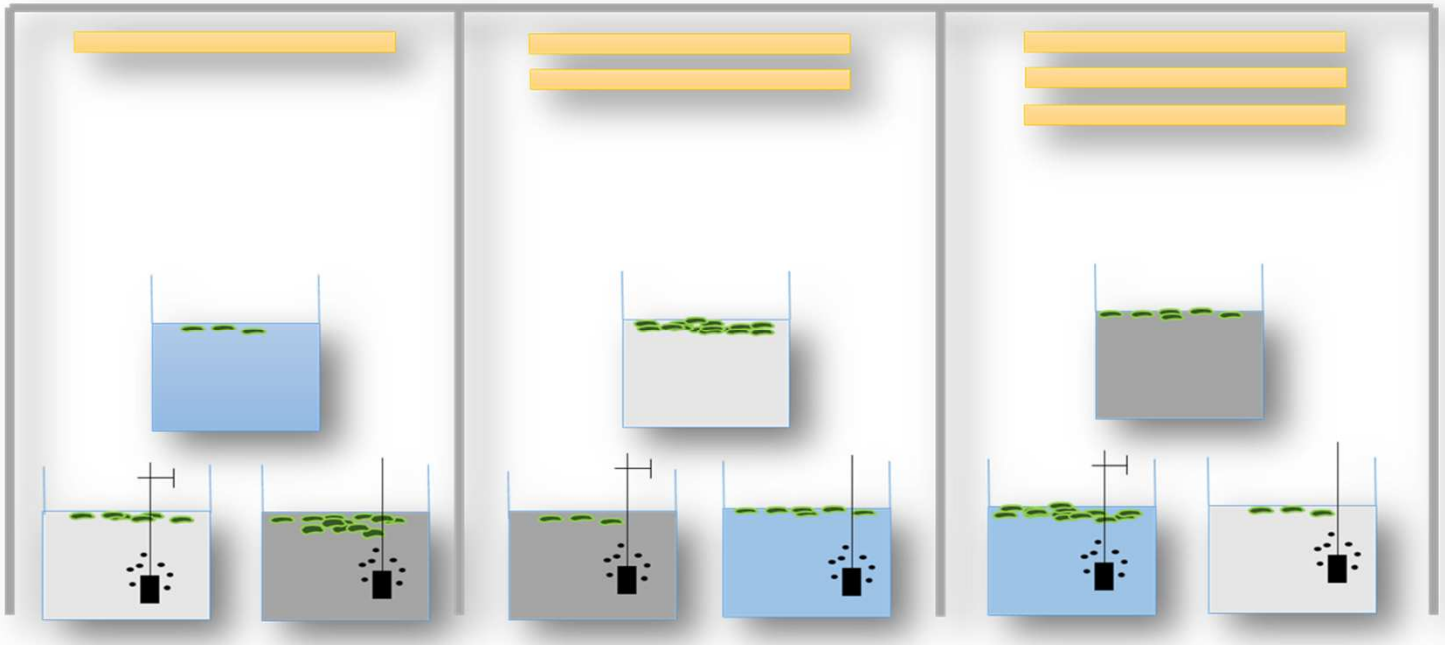

Batch and Verification Tests

Removal of Selected Emerging PPCP Compounds using Greater Duckweed (*Spirodela polyrhiza*) Based Lab-scale Free Water Constructed Wetland

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Abstract

Greater duckweed (*Spirodela polyrhiza*) based lab-scale free water constructed wetland (CW) was employed for removing four emerging pharmaceuticals and personal care products (PPCPs) compounds (i.e. DEET, paracetamol, caffeine and triclosan). Orthogonal design was used to test the effect of light intensity, aeration, *E.coli* abundance and plant biomass on the target compounds. Synthetic wastewater contaminated with the target compounds at concentration of 25 μg/L was prepared, and both batch and continuous flow experiments were conducted. Up to 100% removals were achieved for paracetamol (PAR), caffeine (CAF) and tricolsan (TCS) while the highest removal for DEET was 32.2% in batch tests. Based on orthogonal Duncan analysis, high light intensity (240 $\mu molm^{-2}s^{-1}$), full aeration, high plant biomass (1.00 kg/m^2) and high *E.coli* abundance (1.0×10^6 CFU/100 mL) favoured elimination of the PPCPs. Batch verification test achieved removals of 98.8%, 96.4%, 95.4% and 17.1% for PAR, CAF, TCS and DEET, respectively. Continuous flow tests with CW only and CW followed by stabilization tank (CW-ST) were carried out. Final removals of the PPCP contaminants were 32.6%, 97.7%, 98.0% and 100% for DEET, PAR, CAF and TCS, respectively, by CW system alone, while 43.3%, 97.5%, 98.2% and 100%, respectively, were achieved by CW-ST system. By adding the ST tank, PPCP concentrations decreased significantly faster ($p<0.05$) compared with continuous flow CW alone. In addition, after removing aerators during continuous flow CW experiments, the treatment systems presented

good stability for the PPCP removals. CW-ST showed better chemical oxygen demand (COD) and total organic carbon (TOC) removals (89.3%, 91.2%, respectively) than CW only (79.4%, 85.2%, respectively). However, poor DEET removal (<50%) and high *E.coli* abundance (up to 1.7 log increase) in the final treated water indicated further treatment processes may be required. Correlation analysis showed significant correlations (p<0.05) between PPCPs and water quality parameters (e.g. COD, nitrate, phosphate), and between the four PPCP compounds for the continuous flow CW and CW-ST systems. Positive results encourage the test of Greater duckweed at pilot scale CW using real wastewater.

Keywords: Greater duckweed; PPCPs; Constructed wetland; Stabilization tank; Treatment; Orthogonal design

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1. Introduction

Pharmaceuticals and personal care products (PPCPs) as emerging environmental contaminants have increased concerns from researchers and public over the last two decades (Zhu and Chen, 2014). These contaminants have been widely detected in water environments (wastewater, drinking water, river water) in China, Romania and other countries (Chen et al., 2016; Li et al., 2015; Moldovan, 2006). Concentrations of these compounds vary with water quality and their fate usually depends on physicochemical properties, temperature, rainfall, sunlight and treatment techniques (Hijosa-Valsero et al., 2010; Li et al., 2016). Effluents from wastewater treatment plants (WWTPs) are considered as important sources of these emerging contaminants in the environment (Chen et al., 2012). Conventional WWTP techniques are generally designed to remove organic matter, nitrogen and phosphate, but not the emerging compounds (Zhu and Chen, 2014). Compared with conventional pollutants, emerging

contaminant concentrations are usually low (ng/L-μg/L). But their persistence, toxicity and associated problems (such as antibiotic resistance) may cause potential risks to human health and the environment (Li et al., 2015; Zhu et al., 2013).

DEET, paracetamol (PAR), caffeine (CAF) and triclosan (TCS) are among emerging PPCP contaminants. DEET is an insect repellent and widely detected in stream water, wastewater, drinking water and sludge (Stackelberg et al., 2004; Zhu and Chen, 2014). Paracetamol is heavily used and prescribed analgesic and antipyretic drug (Yang et al., 2008). Roberts and Thomas (2006) reported paracetamol in wastewater at the concentration of 69,570 ng/L, and more than 10,000 ng/L was detected in hospital and natural waters (Thomas et al., 2007;Gomez et al., 2006). Caffeine is a drug commonly used in stimulants and pharmaceuticals. High detection frequencies and different concentrations of caffeine were found in wastewater and river water (Moldovan, 2006; Zhu and Chen, 2014). Triclosan is antibacterial and antifungal compound, and is considered as a ubiquitous pollutant and can be detected in all types of aquatic environments, scaling from 1 ng/L to 10,000 ng/L (Kumar et al., 2010; Li et al., 2010).

In the last few decades, constructed wetlands (CWs) have become popular and have been regarded as promising tertiary treatment techniques in the wastewater treatment process (Zhang et al., 2014b). In comparison with conventional WWTP techniques, CWs are low-cost and eco-friendly (Zhu and Chen, 2014). In recent years, more studies have been focused on removal of PPCPs using CWs (Huang et al., 2015; Sharif et al., 2014). And various mechanisms (e.g. microbial biodegradation, photodegradation and plant effect) were regarded effective (Zhang et al., 2014a). From simple to complex, single to hybrid, microcosm to pilot-scale, CWs present a potential ability to treat emerging contaminants which are not removed thoroughly by conventional WWTP processes (Ávila et al., 2015 Hijosa-Valsero et al., 2011;Sehar et al., 2015).

Different CW aquatic plants have been tested, such as *Typha angustifolia*, *Hydrilla verticillata*, *Salvinia natans*, *Lemna minor* and *Phragmites australis* (Hijosa-Valsero et al., 2011; Reinhold et al., 2010; Weber et al., 2011; Zhao et al., 2015). But *Spirodela polyrhiza* (Greater duckweed) has not been tested yet for the removal of the aforementioned PPCP contaminants. As a member of the *Lemnaceae* family, it has advantages such as ability to survive in dry conditions, low temperature endurance, and ammonia preference uptake (Hillman and Culley, 1978; Porath and Agami, 1986; Rahman et al., 2007). But it does not propagate as quickly as other *Lemnaceae* species such as *Lemna minor*, making it easy to handle and a potential choice for CW vegetation (Landolt and Kandeler, 1987; Lemon et al., 2001).

CWs dealing with wastewater at high COD (>100 mg/L) are usually subsurface horizontal and vertical flow CWs, and hybrid CWs which have complex structures and need careful maintenance (Ávila et al., 2015; Huang et al., 2015; Zhang et al., 2012). Free water surface flow at high organic load has not been tested to remove DEET, PAR, CAF and TCS. Stabilization tank (ST) is another common wastewater treatment process (Verbyla and Mihelcic, 2015). A study on ST followed by CW was reported by Steinmann et al. (2003) and this combination was evaluated to remove 15 pharmaceutical compounds (Conkle et al., 2008). ST (as maturation pond) following CW was also investigated by Mburu et al. (2013) to degrade nutrients but not PPCPs.

In the present study, Greater duckweed (*Spirodela polyrhiza*) based lab-scale free water CW was employed to remove DEET, PAR, CAF and TCS from synthetic wastewater at high COD load (300mg/L). Batch tests were developed by the aid of orthogonal design to optimize factors (i.e. light intensity, aeration, plant biomass and *Escherichia coli* (*E.coli*) abundance) affecting PPCP removals (Lan et al., 1994). *E. coli* was used to represent bacteria abundance present in wastewater and to determine their effect on PPCP degradation. Batch verification

and continuous flow tests were experimented under the optimized factor levels. In addition, CW tank followed by one ST tank was tested under the optimized conditions. To our knowledge, it is the first report that Greater duckweed has been used in lab-scale CW to treat PPCP compounds.

2. Materials and methods

2.1 Chemicals and materials

Standards and chemicals of DEET, PAR, CAF and TCS were purchased from Sigma-Aldrich (UK). Methanol and acetonitrile (both HPLC grade) were purchased from Fisher Scientific (UK). Characteristics of the PPCP compounds are presented in Table S1. Stock solutions of individual compounds were prepared at 1mg/mL in acetonitrile and stored in the dark at -20℃. New stock solutions were made every 3 months and kept in the fridge. Standard solutions were prepared by diluting stock solutions with acetonitrile, and solutions of mixed PPCPs were prepared at 1mg/L in methanol every two weeks.

Greater duckweed (*Spirodela polyrhiza*) was purchased from Claremontaquatic Leyland Company (UK) and placed in hydrophyte nutrient solution. Since microbes are always associated with plants, Greater duckweed was washed 10 times to remove existing *E.coli* as much as possible (Compant et al., 2010). *E.coli* abundance attaching to cleaned Greater duckweed was left 24 hours in a sterile wastewater and, at the end was found to be 2-7 CFU/100mL. Plastic CW tanks (25×16×11 cm) and containers (44×32×21 cm, 32×22×17 cm) were used in this study (Figures1 and 2). Each aerator had an output of 3.2 L/min.

The total experimental period was about 4 months. For all tests, triplicates were conducted. Each CW was fed with synthetic wastewater, prepared with tap water using 300 mg/L COD (glucose), 80 mg/L NH_4Cl, 12.8 mg/L K_2HPO_4, 0.05 mg/L $FeCl_3$, 4.5 mg/L $MgSO_4 \cdot 7H_2O$ and 7.3 mg/L $CaCl_2 \cdot 2H_2O$ (Liu et al., 2013; Zhang et al., 2012). As a

commonly and widely found microbe in wastewater, *E.coli* has been applied in synthetic wastewater as surrogate organisms (Decamp and Warren, 2000; Antoniadis et al., 2007). Three *E.coli* (ATCC 11775, Sigma-Aldrich, UK) levels (none, 1×10^4 and 1×10^6 CFU/100 mL) were used to prepare the synthetic wastewater to be tested (Ávila et al., 2015; Boutilier et al., 2009). DEET, PAR, CAF and TCS solutions were mixed in synthetic wastewater to reach a final concentration of 25 μg/L.

2.2 Batch tests

Light intensity (80, 160, ad 240 $\mu molm^{-2}s^{-1}$), oxygen (no aeration, intermittent and full aeration), plant biomass (0.25, 0.50 and 1.00 kg/m^2) and *E.coli* abundance (none *E.coli*, 1.0×10^4 and 1.0×10^6 CFU/100mL) were chosen as factors impacting the PPCP removal (Wang et al., 2014; Zhang et al., 2014a). Orthogonal design (four factors with three levels) was conducted to reduce the number of experiments, resulting in nine runs (Table 1). Three litres of synthetic wastewater contaminated with PPCPs were placed in each CW tank and the experimental area was covered using reflective fabric, which made the light spread evenly upon the CW tanks (Figure 1). Cleaned Greater duckweed was put into each CW. Lights were placed on the top of the CW surface areas (50×40×70 cm) under the fabric and light intensity was monitored by a photon flux density meter (Rectifier SKKH 72/20E). Aerators were placed in the water to supply dissolved oxygen (DO). For intermittent aeration, aerators were switched on for 2 hours and then off for 2 hours. This cycle was repeated six times a day. The lighting was left on for a period of 14 hours and off for 10 hours (Clyde-Smith, 2016). Room temperature was around 23 ℃ constantly. Hydraulic retention time (HRT) in CW operations varies considerably from 1 to 12.9 days (Carranza-Diaz et al., 2014; Chen et al., 2016; Verlicchi et al., 2013). For practical sampling, the period (i.e. HRT) for the batch experiment and subsequent tests were set at seven days. During this period, pH, conductivity and redox potential of each CW were measured at the same time each day (excluding

weekend). DO concentrations were measured in non-aerated CWs only. After seven days, PPCP compounds, NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}, COD and *E.coli* abundance of the treated synthetic wastewater in each CW were determined.

Additional tests (ATs) were conducted to investigate the effect of light (i.e. photodegradation) and *E.coli* (i.e. biodegradation) on removing PPCP compounds (Table 1). To identify Greater duckweed's role, aseptic Greater duckweed plants were tested (AT7) under the same condition of CW9 as well, using 0.1% bleach adapted from the method of Oyebanji et al, (2009). Sterilization process is shown in Text S1 (see Supplementary Data). PPCP concentrations in the treated synthetic wastewater were quantified at the end.

2.3 Batch verification

A batch verification test was conducted to verify the effect of the combined optimized factors on PPCP removal under the same conditions given by the orthogonal Duncan analysis. Experimental apparatus and lighting were the same as in the batch test. High light intensity (240 $\mu molm^{-2}s^{-1}$), full aeration, high plant biomass (1.00 kg/m^2) and high *E.coli* abundance (1.0×10^6 CFU/100mL) were chosen as optimum parameters. The target PPCP concentrations and relevant quality parameters were determined at the end of the test (day 7). One control test using optimum factor level conditions without Greater duckweed was also conducted to verify the role of Greater duckweed.

2.4 Continuous flow test

The experimental conditions followed the optimum factor levels. The continuous flow CW (Figure 2a) consisted of one inflow tank, one CW tank (44×32×21 cm) and one outflow tank. Fresh and cleaned Greater duckweed (1.00kg/m^2, 140 g) was put in the CW tank. The area (100×40 cm) above the CW tank was covered by a reflective fabric while inflow and outflow tanks were covered by black paper to prevent PPCP photodegradation (Aranami and

Readman, 2007). Lights were placed over the CW surface area under the fabric. Fourteen litres of synthetic wastewater contaminated with the PPCP compounds and 1.0×10^6 CFU/ 100mL *E.coli* were added into the CW tank. The HRT was set at 7 days (two litres in and out every day, actual 6.7 days) as the batch experiment and the peristaltic pump ensured the inflow and outflow of water was consistently kept at 1.38 mL/min. The system was operated for 4 weeks and was left under lighting for a period of 14 hours and 10 hours in darkness. The room temperature was constantly around 23 ℃. Aerators were placed evenly at the bottom of the tank to make sure DO was saturated in the CW tank. In order to explore the CW performance without aeration, at day 17 all aerators were removed after sampling. The inflow synthetic wastewater was freshly made every day. Both inflow and outflow tanks were sterilized by 70 % alcohol and antimicrobial before refilling. The pH, conductivity and redox potential of the treated synthetic wastewater and DO in the CW tank were measured every working day. For quantification of the PPCPs, NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, COD, TOC and *E.coli* abundance, samples were collected three times a week on Mondays, Wednesdays and Fridays.

2.5 Continuous flow with CW-ST test

The continuous flow CW-ST (Figure 2b) consisted of one inflow tank, one CW tank (32×22×17 cm), one ST tank (32×22×17 cm) and one outflow tank, successively connected by peristaltic pump (speed at 1.38 mL/min). Fresh and clean Greater duckweed (1.00kg/m^2, 70 g) was put in the CW tank. The area (100×40 cm) above the CW and ST tanks was covered by reflective fabric and inflow and outflow tanks were covered by black paper. Lights were put over the CW-ST area, and room temperature was constantly 23 ℃. For comparing this system with the continuous flow CW only, seven litres of the synthetic wastewater were initially added in CW and ST tanks separately. Aerators were evenly placed at the CW tank bottom. The total HRT of the system was set at 7 days (2 litres in and out

every day, 3.5 days in CW tank and 3.5 days in ST tank, actual 6.9 days). The duration of this experiment (4 weeks) and aerator removal strategy (at day 17) were the same as for the continuous flow CW test (Section 2.4). Every day, inflow synthetic wastewater was freshly prepared. Before reloading, inflow and outflow tanks were cleaned and sterilized to avoid contamination. Sampling strategy and parameter monitoring were the same as for the continuous flow CW test.

2.6 Analytical procedures for PPCP determination

Purification and analytical procedure methods of the target PPCP compounds are described in Text S2 (see Supplementary Data). For each target compound, three diagnostic (m/z) ions were selected (Table S2).

2.7 Quality control of PPCP determination

Calibration curves, limits of detection (LODs), limits of quantification (LOQs) equipment relative standard deviations (RSDs) of the target compounds are shown in Table S3. The recoveries and RSDs of the quantification method are shown in Table S4.

2.8 Analysis of monitored parameters

COD of samples were determined by using HACH COD TNT digestion solution (0-1500 mg/L, HACH Company, UK). TOC were determined by Shimadzu TOC-L machine (UK) while ion chromatography (IC, Dionex ICS 1100, US) was used to detect and measure the concentrations of NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}. Conductivity, pH and redox potential were measured using a Mettler Toledo meter. DO was determined using Jenway 9200 meter. Selective plate counting (eosin methylene blue agar, EMB) was used to quantify the abundance of *E.coli*.

2.9 Statistical Analysis

Orthogonal design was performed by using IBM SPSS Statistics 22 to plan the experiments. Duncan analysis was used for the orthogonal result evaluation (Lan et al., 1994). ANOVA and correlation tests were conducted by using IBM SPSS Statistics 22, and *p*-value<0.05 was considered statistically significant.

3. Results and discussion

3.1 Batch experiment

3.1.1 Target compounds removal

Figure 3 and Table S5PAR, CAF and TCS achieved good removals in the batch tests. For PAR, CWs 5, 6 and 8 showed no detectable PAR and the other CWs' removals ranged from 94.0-99.0%, indicating excellent PAR (p<0.01) removal by the CW system. All CAF concentrations were below 10 μg/L (not detected in CWs 2 and 4) except for CW 9 (18.36 μg/L). Very good TCS removals were also achieved and final TCS concentrations varied from 0-4.57 μg/L. However, it can be seen from Figure 3 DEET concentrations in the treated wastewater were still high. The lowest concentration of DEET was 16.94 μg/L in CW3 (32.2% removal) and the highest was 26.88 μg/L in CW8. The negative removal of DEET in CWs 5 and 8 might be due to water evaporation which led increasing remaining concentrations (Hijosa-Valsero et al., 2010). DEET removal by wetlands and other *Lemnaceae* species were found to be none or very poor (Reinhold et al., 2010; Zhu and Chen, 2014). Interestingly, Greater duckweed in the present study showed to improve its removal (up to 32.2%).

Light effect on PPCP degradation was investigated in AT1, AT2 and AT3 (Table 1). PAR concentration decreased from 15.28 μg/L to 13.45 μg/L when light intensity increased from 80 to 240 $\mu molm^{-2}s^{-1}$, indicating photodegradation was one of the mechanisms responsible for PAR elimination (Figure 3). This is in agreement with the findings of Yamamoto et al (2009). TCS removal also increased from 8.8% to 57.6% with increased light

intensity, and TCS photodegradation agrees well with the findings of Aranami and Readman (2007). In contrast, DEET demonstrated not to be light sensitive (1.2% removal at highest), supporting its poor degradation from CW1-CW9. Also, CAF (7.0% at highest) behaved recalcitrant under visible light, confirming the findings by Arfanis et al. (2017) and Trovó et al. (2013) who found CAF degradation by photocatalysis or photo-Fenton processes.

E.coli's effect on target compounds removal was studied in AT4, AT5 and AT6 (Table1). *E.coli* biodegradation of PAR was moderately effective (compared with 19.0% removal without *E.coli* addition) but no significant difference (49.5% in AT5 and 48.8% in AT6) was found between the two abundance levels. CAF behaved recalcitrant under visible light but showed more degradation by using *E.coli* (46.5% under high *E.coli* abundance in AT6). *E.coli* also favoured TCS elimination. Although 83.8% removal achieved by *E.coli* (AT6) was higher than the 57.6% by light (AT3), there was no significant difference between light and *E.coli* effect ($p>0.05$). For DEET, the highest removal of 4.5% was observed in AT6. Biodegradation of PAR, CAF and TCS was suggested as one degradation mechanism by other researchers (Roberts and Thomas, 2006; Lin et al., 2010; Zhang et al., 2015). The present results show that pure cultures of *E. coli* was capable of degrading the concentration of 25 μg/L PPCPs. Degradation of organics (e.g. phenol) by *E.coli* (e.g. ATCC 33456) and other pure bacterial strains (e.g. ATCC 11172) have been observed (Molin and Nilsson, 1985; Shen and Wang, 1995). Similarly, in the present study *E.coli* (ATCC 11775) was found capable of degrading PPCPs. This suggests to further investigate the biodegradation mechanisms of PPCPs.

AT7 (light and aseptic plant) showed removals of 2.8%, 91.8%, 2.9% and 38.7% for DEET, PAR, CAF and TCS respectively, showing that Greater duckweed contributed to the removal of the target PPCPs, especially PAR and TCS. From CW9 (light and non-aseptic plant) and AT1 (only light), it can be seen that Greater duckweed significantly enhanced

removal (p<0.05). CW9 achieved 7.0%, 98.3%, 26.6% and 81.7% removal of DEET, PAR, CAF and TCS respectively, compared with 0.6%, 38.9%, -0.6% and 8.8% removal in AT1. AT7 removal lay within the range of removals of CW9 and AT1, indicating that both Greater duckweed and associated microbes attaching to plants contributed to the PPCP degradation. Roles of plants in CWs include direct uptake of organic contaminants and creation of favourable conditions (e.g. biofilm anchorage) for their removal (Li et al., 2014; Verlicchi and Zambello, 2014). Studies of planted CWs showing significant better performance than unplanted beds were also reported (Sehar et al., 2015; Carranza-Diaz et al., 2014). Thus, the fate of PPCP compounds by Greater duckweed and identification of microbial type should be further investigated.

3.1.2 Orthogonal Duncan analysis

Table 2 shows the analysis results for individual target compounds and Table 3 presents the results based on average removals of the four PPCP compounds in each CW experiment.

For individual PPCP compounds, high light intensity favoured DEET and TCS degradations, while medium light intensity significantly decreased (p<0.01) PAR and CAF concentrations. CAF also achieved the highest removal (7.0%) under medium light intensity in the AT sets (Table S5). Except PAR, the other three compounds were removed mostly under full aeration. Most efficient removal of PAR was without aeration (p<0.01). As for *E.coli* biodegradation, abundance of 1.0×10^6 CFU/100 mL considerably helped to reduce DEET, CAF and TCS concentrations. However, 1.0×10^4 CFU/100 mL *E.coli* favoured PAR reduction, confirming the AT set results (see AT4, AT5 and AT6 in Table S5). Greater duckweed is a floating plant which leaves are spread on the water surface. More plants on the water surface can cause less light penetration, reducing photodegradation. Therefore, it may be assumed that results that showed higher removal of DEET and PAR under low plant

biomass may be due to higher photodegradation effect. However, CAF and TCS concentrations decreased mostly with high plant biomass, and this may be attributed to plant uptake and/or plant roots which provide adherent substrate and habitat for microbes to biodegrade organics (Wang et al., 2014).

Results of the orthogonal Duncan analysis for the batch test (Table 3) showed that under the combination of high light intensity, full aeration, high abundance of *E.coli* and high plant biomass, average PPCP removal could significantly increase (p<0.01). Because the removals of the four PPCP compounds by CW varied (Table S5) and the analysis (Table 2) showed different optimum factor combinations for each compound to balance all PPCP removals and get the best optimum average removal, combined factor levels (240 $\mu molm^{-2}s^{-1}$ light intensity, full aeration, 1.00 kg/m^2 plant biomass and 1.0×10^6 CFU/100 mL *E.coli* abundance) were used in following tests as the optimum conditions.

3.1.3 General water quality parameters

COD removal achieved around 90% in most batch tests except for CW 7 (Table S6), indicating very good COD removal by the CW system. Ammonium removal varied from 10.6-83.3% but increased by 55.3% in CW 6. In CW5 and CW9, nitrate was not detected while the other CWs removed 30.0-93.1%.In addition, results showed that 40.6-80.8% of phosphate was removed, however, nitrite was found in eight CWs (0.9-16.6 mg/L) and it was not detected in the synthetic water. An increase of nitrite concentration in CW was also observed by Schaafsma et al. (1999). Although DO concentration indicates aerobic conditions in the water, presence of nitrite suggests inadequate nitrification, which might have been caused by insufficient nitrobacteria (such as *Nitrobacter*), or due to more intense denitrification (Vermmat and Hanif, 1998). *E.coli* abundance in the final treated wastewater of all CWs increased by 0.9-2.0 orders of magnitude. This is not in agreement with published

work (Mantovi et al., 2003).This might be due to the fact, a single microbe (*E.coli*) was inoculated into the synthetic wastewater, potentially generating a dominant microbial community. Also the lack of predators such as protozoa and high COD concentration may have favoured *E.coli* proliferation, causing an increase of *E.coli* abundance.

All DO concentrations in CWs 5, 8 and 9 without aeration decreased in the first few days and then increased again to around 6 mg/L (Table S7). Oxygen consumption could increase under high organic load (Caffrey et al., 1993). Apart from oxygen natural diffusion from air to water, Greater duckweed may also transport oxygen from leaves to roots, increasing DO level. Reddy et al. (1990) found that two floating plants (i.e. *Hydrocotyle umbellata L.* and *Eichhornia crassipes*) increased DO concentration up to 6.1 mg/L. Patel and Kanungo (2010) also found that *Lemna minor* increased DO concentration during phytoremediation. Greater duckweed as a floating plant may have potentially this ability but this requires further investigation.

3.2 Batch test verification

Except for DEET, all the other three PPCP compounds achieved more than 90% removals in the batch test verification (Table S8). Results showed Greater duckweed based CW was effective to eliminate 98.8%, 96.4% and 95.4% of PAR, CAF and TCS, respectively at the batch scale, while it was less able to remove DEET (17.1%) and *E.coli* (increased by a 0.60 order of magnitude). Besides, 86.0% and 84.9% of COD and TOC, respectively, were removed. In the none-plant control test, removals of DEET, PAR, CAF and TCS were 7.9%, 84.4%, 82.4% and 84.2%, respectively (Table S8). The lower removals from the control test indicate that Greater duckweed played a role in enhancing the removal of the PPCPs by potentially direct uptaking the PPCPs and/or by creating favourable conditions (e.g. biofilm anchorage) for their removal within the system (Li et al., 2014; Verlicchi and Zambello,

2014). Ammonium was not detected in the final treated water, and this may be attributed to the Greater duckweed ammonia preference uptake (Hillman and Culley, 1978; Porath and Agami, 1986). Nitrate (30.0%) and phosphate (62.0%) were removed and this agrees well with other researchers (Sehar et al., 2015; Zhang et al., 2012; Zhang et al., 2014b).

3.3 PPCP removal in continuous flow systems

Target PPCP concentrations of continuous CW and continuous CW-ST tests are shown in Figure 4 and Table S9. The final target compound removals by continuous CW only were 32.6%, 97.7%, 98.0% and 100%, respectively, for DEET, PAR, CAF and TCS. For the continuous flow CW-ST test, final removals of DEET, PAR, CAF and TCS were 43.3%, 97.5%, 98.2% and 100%, respectively. As it can be seen in Figure 4, in both systems, the removal of PPCP compounds occurred as soon as the tests started. While DEET was present at the highest concentration in all samples, PAR and TCS concentrations decreased quicker than DEET and CAF, demonstrating PAR and TCS were easier to be eliminated by both continuous flow CW and CW-ST systems than the other PPCPs. Although DEET concentrations decreased slowly with time, maximum removal was below 45%, confirming the results found in the batch experiments that DEET was recalcitrant (Sections 3.1 and 3.2). The lowest DEET concentrations were 16.85 μg/L in the continuous flow CW system and 14.17 μg/L in the continuous flow CW-ST. When aeration condition changed at day 17, DEET concentrations increased from 21.82 μg/L to 23.65 μg/L in the continuous flow CW and from 16.37 μg/L to 18.17 μg/L in the continuous flow CW-ST, then declined again in both systems. PAR and TCS removals did not show significant changes, indicating DEET removal was more oxygen sensitive than PAR and TCS. CAF concentration in the continuous flow CW test fluctuated between 9.19 and 12.89 μg/L (day 17 to 22) then decreased quickly to 1.11 μg/L. However, no decline of CAF removal occurred in the CW-ST, and this may be attributed to stable biodegradation in the ST tank as oxygen in the air may have diffused into

366 ST tank water continuously from day 17 when aerators were turned off and removed. The
367 sudden lack of oxygen could change the biotope of CW system, thus influencing the PPCP
368 removals. However, the PPCP removals in CW and CW-ST systems with and without
369 aeration showed no significant differences (p>0.05), indicating the CW and CW-ST tanks
370 were robust enough to degrade 25 μg/L of PPCP compounds when the operational conditions
371 changed.

372 The comparison between each individual PPCP removal for both continuous flow
373 systems (CW and CW-ST) can be seen in Figure S1. DEET concentrations in both systems
374 did not reduce as quickly as the other three compounds (p<0.05). From day 12, DEET
375 removal was higher in the continuous flow CW-ST system than those in the continuous flow
376 CW and this continued until the completion of the test. On the other hand, PAR
377 concentrations in CW-ST system decreased quickly from 25 μg/L (day 1) to 0.90 μg/L (day
378 10) then fluctuated until the end of test. In contrast, PAR concentration in the continuous CW
379 system did not decrease rapidly (p<0.05), but results from both systems showed no
380 significant difference from day 12 to the end of the test (p>0.05). Compared with the other
381 three compounds, CAF concentrations in both systems decreased more linearly with time but
382 were higher in the CW than in the CW-ST, except for day 8. At day 26, CAF concentrations
383 in both systems were below 0.5 μg/L. TCS concentration in CW-ST system decreased from
384 25 μg/L (day 1) to 0 (day 5) and then no TCS was detected in the subsequent samples,
385 probably due to the existence of ST tank that allowed more light penetration into the water
386 causing further TCS photodegradation (Aranami and Readman, 2007). For the CW system,
387 TCS concentrations decreased to 7.81 μg/L (day 5), and were eliminated gradually until day
388 26 when no detectable amount was found. By using ANOVA test, the four PPCP target
389 compounds were removed significantly faster using the CW-ST system (p<0.05) than using
390 the CW only.

The total water volume (14 litres) and the HRT (7 days) were the same in both continuous flow systems. However, the best removal of the target PPCP compounds occurred in the system with the adjunction of ST tank. This suggests that it not only ensured more direct light penetration for photodegradation but also compensated the small removals in the CW tank potentially caused by halving the HRT, and allowed more oxygen diffusion from air into water for biodegradation.

3.4 General water quality parameters

ANOVA-test showed both COD and TOC degraded significantly faster in the CW-ST system ($p<0.05$) than in the CW system (Table S10 and Figure S2). The final concentrations of COD and TOC in the CW-ST treated water were 32 mg/L and 13 mg/L (at removals of 89.3% and 91.3%, respectively), compared to 62 mg/L and 22 mg/L (at removals of 79.3% and 85.3%, respectively) using CW only.

E.coli abundance in the final treated wastewater increased 0.5 order of magnitude in both continuous systems (Table S11), due to the similar reason under batch tests (Section 3.1.3). Continuous flow CW presented a higher ammonium removal than CW-ST and ammonium removal increased from day 10 to the last day (51.6% to 100%), probably because of the longer contact time and Greater duckweed's ammonia preference uptake. As an intermediate product of nitrification and denitrification, nitrite in the continuous CW system varied greatly during the test period, being the final nitrite concentration was 6.4 mg/L. Nitrite was also present in the CW-ST since day 7 and from day 10, nitrite concentration went down gradually with time until 100% removal was achieved. Moreover, nitrates concentration in both systems initially decreased and then increased, but declined sharply after switching off the aerators. This may be explained by the fact under anoxic condition, denitrification can be active (Robertson and Kuenen, 1984). Both CW and CW-ST

systems showed removals of phosphate between 33% and 70%, respectively. Phosphate concentrations declined in the first few days and then varied between 3 to 6 mg/L. This result agrees well with Lin et al. (2002) who found phosphate removals of 32% to 71% in CW system only.

With aeration, DO concentration in the ST tank was lower than in the CW tanks for the first 17 days (Table S12). When aeration was switched off, DO concentration in the CW systems dropped to below 1 mg/L (anaerobic/anoxic condition), while DO in the ST tank remained above 2 mg/L and reached a stable value around 6 mg/l when exchange equilibrium of oxygen between air and water achieved. DO concentrations in all tanks (CWs and ST) increased after day 22, and the ST tank presented the highest DO concentration (> 6mg/L), suggesting DO was being consumed more in the CW system than in the ST tank. As DO is essential for biodegradation, the adjunction of a ST tank to the CW can potentially compensate the lack of DO in the CW.

3.5 Correlation analysis

COD and TOC concentrations both showed a significant relationship (p<0.05) with all four PPCPs (Table 4). COD correlated highly significantly (p<0.01) to PAR and TCS in the continuous flow CW system (r=0770, 0.767; p=0.003, 0.004 for PAR and TCS, respectively), and in the continuous flow CW-ST system. Also, COD showed a significant relationship with DEET, PAR and CAF (r=0.820, 0.821, 0.746; p=0.001, 0.001, 0.005, respectively). Similar results were also found for TOC which showed a significant relationship with PAR and TCS in the continuous flow CW system (r=0.794, 0.818; p=0.002, 0.001, respectively), and DEET, PAR and TCS in the continuous flow CW-ST system (r=0.739, 0.875, 0.776; p=0.006, 1.9E-04, 0.003, respectively). Significant correlations were also found between PPCPs and COD/TOC by Yoon et al. (2010) and Wang et al. (2012). Compared with COD and TOC,

nitrogen compounds had weak correlation with the PPCPs. Ammonium concentrations only correlated to PAR and TCS in the continuous flow CW system while it had correlations with all four targets in the CW-ST system, having strongest correlations with DEET, PAR and TCS (p<0.01). Matamoros et al. (2007) also observed significant positive correlations between ammonium and PPCPs in a vertical flow CW at pilot scale. Nitrate only correlated with CAF in the continuous flow CW (r=0.679; p=0.015), but PAR and TCS correlated more significantly with nitrate in the continuous flow CW-ST system (r=0.819, 0.853; p=0.001, 4.2E-04). However, nitrite concentrations fluctuated in both systems and no significant correlations were found between the four target compounds and nitrite (p>0.05). Wang et al. (2015) evaluated 28 PPCPs in urban river water samples and found most of them had positive correlations (p<0.05) with total nitrogen and total phosphorus concentrations. Chen et al. (2016) also found positive correlations (p<0.05) between PPCPs with ammonium and phosphate in rural wastewater treatment wetlands. In this study, phosphate concentrations also showed a positive and significant correlation with the PPCPs, except for with CAF in the continuous CW system (r=0.001; p=0.068).

Results showed (Table 5) all four PPCPs had statistically significant correlations with each other (p<0.05), having PAR the strongest correlation(r=0.979; p=3.0E-08) with TCS in the continuous flow CW system, and DEET with CAF (r=0.953; p=2.0E-06) in the continuous flow CW-ST system. Padhye et al. (2014) conducted a study in an urban drinking water treatment plant and found a strong correlation (r=0.97) between PPCPs and endocrine disrupting chemicals, which demonstrated potential relations between micropollutant concentrations. Correlations between pharmaceuticals in drinking water sources were also reported by Guo and Krasner (2009). As removal of contaminants is associated with chemical property, treatment conditions and removal preference (e.g. ammonia for duckweed),

463 statistical correlation does not always indicate "causal relationship" and mechanisms behind
464 the correlations need further investigation (Chen et al., 2016).

465 **4. Conclusion**

466 In this study, Greater duckweed based lab-scale free water CW was used for degrading
467 DEET, PAR, CAF and TCS at 25 μg/L in synthetic wastewater. Orthogonal design was used
468 for the batch experiment planning. The positive results encourage future work to be
469 conducted at medium and large scales with the use of real wastewater to examine the
470 performance of the proposed systems.

471 • Based on the orthogonal Duncan analysis, 240 $\mu molm^{-2}s^{-1}$ light intensity, full aeration,
472 1.00 kg/m^2 plant biomass and 1.0×10^6 CFU/100 mL *E.coli* abundance favoured the
473 degradation of the PPCP compounds (on average removal) in batch systems. Further
474 batch verification test achieved 98.8%, 96.4%, 95.4% and 17.1% removals for PAR,
475 CAF, TCS and DEET, respectively.
476 • For continuous systems, final PPCP removals achieved by the CW-ST system were
477 43.3%, 97.5%, 98.2% and 100% for DEET, PAR, CAF and TCS, respectively,
478 compared to 32.6%, 97.7%, 98.0% and 100%, respectively, by the CW system, .
479 PPCP removals by the CW-ST system were significantly faster (p<0.05) than those by
480 the CW alone. Both continuous flow systems (CW and CW-ST) demonstrated
481 treatment stability after aerators were switched off. Oxygen was considered an
482 important factor in the CW system and the lack of oxygen could be overcome by the
483 inclusion of a ST tank downstream the CW tank.
484 • Correlation analysis showed a number of significant correlations (p<0.05) between
485 PPCP compounds and water parameters removals (e.g. COD, nitrate, phosphate), as

well as between the four target compounds, in both continuous flow CW and CW-ST systems.

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675

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Table captions

Table 1 Orthogonal design of batch experiment and additional test (AT) sets

		Light ($\mu molm^{-2}s^{-1}$)	Aeration	*E.coli* abundance (CFU/100mL)	Plant biomass (kg/m^2) (g)
CW	CW 1	160	Full	None	0.50, 20
	CW 2	240	Intermittent	None	1.00, 40
	CW 3	240	Full	1.0×10^4	0.25, 10
	CW 4	80	Full	1.0×10^6	1.00, 40
	CW 5	240	None	1.0×10^6	0.50, 20
	CW 6	160	Intermittent	1.0×10^6	0.25, 10
	CW 7	80	Intermittent	1.0×10^4	0.50, 20
	CW 8	160	None	1.0×10^4	1.00, 40
	CW 9	80	None	None	0.25, 10
AT sets	AT 1	80	None	None	None
	AT 2	160	None	None	None
	AT 3	240	None	None	None
	AT 4	None	None	None	None
	AT 5	None	None	1.0×10^4	None
	AT 6	None	None	1.0×10^6	None
	AT 7	80	None	None	0.25, 10, aseptic

Table 2 Duncan analysis results of individual target compound for the batch test

DEET				PAR			
Light intensity	low	medium	high	Light intensity	low	medium	high
p=0.208*	0.089**	0.065	0.129	p<0.01	0.969	0.989	0.987
Aeration	none	intermittent	full	Aeration	none	intermittent	full
p<0.01	-0.003	0.116	0.169	p<0.01	0.994	0.984	0.967
E.coli abundance	none	1×10^4	1×10^6	*E.coli* abundance	none	1×10^4	1×10^6
p=0.214	0.061	0.099	0.124	p<0.01	0.974	0.991	0.981
Plant biomass	low	medium	high	Plant biomass	low	medium	high
p<0.01	0.207	0.029	0.046	p<0.01	0.991	0.984	0.969

CAF				TCS			
Light intensity	low	medium	high	Light intensity	low	medium	high
p<0.01	0.647	0.922	0.892	p<0.01	0.924	0.968	0.975
Aeration	none	intermittent	full	Aeration	none	intermittent	full
p<0.01	0.666	0.862	0.933	p<0.01	0.982	0.979	0.996
E.coli abundance	none	1×10^4	1×10^6	*E.coli* abundance	none	1×10^4	1×10^6
p<0.01	0.735	0.817	0.909	p<0.01	0.929	0.957	0.981
Plant biomass	low	medium	high	Plant biomass	low	medium	high
p<0.01	0.678	0.811	0.972	p<0.01	0.939	0.961	0.967

* p, statistical factor significance to the removal of target compound. p>0.05, no significance; p<0.05, significant; p<0.01, highly significant.

** 0.129 (high light intensity) > 0.089 (low light intensity) > 0.065 (medium light intensity), meaning high light intensity level has the best effect on DEET removal compared with the other two levels. A higher value indicates more removal.

Table 3 Duncan analysis results of average PPCP removal for the batch test

Average removal in each CW			
Light intensity	low	medium	high
$p<0.01$*	0.657	0.736	0.746
Aeration	none	intermittent	full
$p<0.01$	0.637	0.735	0.766
E.coli abundance	none	1×10^4	1×10^6
$p<0.01$	0.675	0.716	0.748
Plant biomass	low	medium	high
$p<0.01$	0.696	0.704	0.739

* *p*, statistical factor significance to the removal of target compound. $p>0.05$, no significance; $p<0.05$, significant; $p<0.01$, highly significant.

Table 4 Pearson's r values and *p* values in concentration correlation analysis between target PPCP compounds and COD, TOC, ammonium, nitrite, nitrate and phosphate in continuous flow CW & CW-ST systems

			COD	TOC	Ammonium	Nitrite	Nitrate	Phosphate
CW system	DEET	Pearson's r	0.651*	0.694*	0.466	-0.125	0.348	0.622*
		p value	0.022	0.012	0.126	0.699	0.267	0.031
	PAR	Pearson' r	0.770**	0.794**	0.683*	-0.088	0.451	0.832**
		p value	0.003	0.002	0.014	0.784	0.141	0.001
	CAF	Pearson's r	0.680*	0.684*	0.524	-0.115	0.679*	0.543
		p value	0.015	0.014	0.080	0.722	0.015	0.068
	TCS	Pearson's r	0.767**	0.818**	0.727**	-0.225	0.554	0.859**
		p value	0.004	0.001	0.007	0.482	0.062	3.4E-04
CW-ST system	DEET	Pearson's r	0.820**	0.739**	0.731**	-0.141	0.334	0.714**
		p value	0.001	0.006	0.007	0.662	0.289	0.009
	PAR	Pearson's r	0.821**	0.875**	0.712**	-0.355	0.819**	0.841**
		p value	0.001	1.9E-04	0.009	0.257	0.001	0.001
	CAF	Pearson's r	0.746**	0.674*	0.697*	-0.056	0.323	0.643*
		p value	0.005	0.016	0.012	0.864	0.306	0.024
	TCS	Pearson's r	0.707*	0.776**	0.748**	-0.302	0.853**	0.874**
		p value	0.010	0.003	0.005	0.340	4.2E-04	2.0E-04

* $p<0.05$, significant correlations

** $p<0.01$, highly significant correlations

Table 5 Pearson's r values and *p* values in concentration correlation analysis between target PPCP compounds in continuous flow CW & CW-ST systems

			DEET	PAR	CAF	TCS
CW system	DEET	Pearson's r	1	0.705*	0.717**	0.706*
		p value	n.a. ***	0.011	0.009	0.010
	PAR	Pearson's r	0.705*	1	0.784**	0.979**
		p value	0.011	n.a.	0.003	3.0E-08
	CAF	Pearson's r	0.717**	0.784**	1	0.806**
		p value	0.009	0.003	n.a.	0.002
	TCS	Pearson's r	0.706*	0.979**	0.806**	1
		p value	0.010	3.0E-08	0.002	n.a.
CW-ST system	DEET	Pearson's r	1	0.704*	0.953**	0.626*
		p value	n.a.	0.011	2.0E-06	0.030
	PAR	Pearson's r	0.704*	1	0.665*	0.981**
		p value	0.011	n.a.	0.018	2.1E-08
	CAF	Pearson's r	0.953**	0.665*	1	0.599*
		p value	2.0E-06	0.018	n.a.	0.040
	TCS	Pearson's r	0.626*	0.981**	0.599*	1
		p value	0.030	2.1E-08	0.040	n.a.

* $p<0.05$, significant correlations

** $p<0.01$, highly significant correlations

*** n.a. not available

Figure captions

Figure 1 Schematic representations of the batch experiment

Figure 2 Schematic representations of the continuous flow CW and continuous flow CW-ST

Figure 3 Removals of the target PPCP compounds in batch and ATs tests

Figure 4 Concentrations of target PPCP compounds in the final treated water by (A) the continuous flow CW and (B) continuous flow CW-ST systems

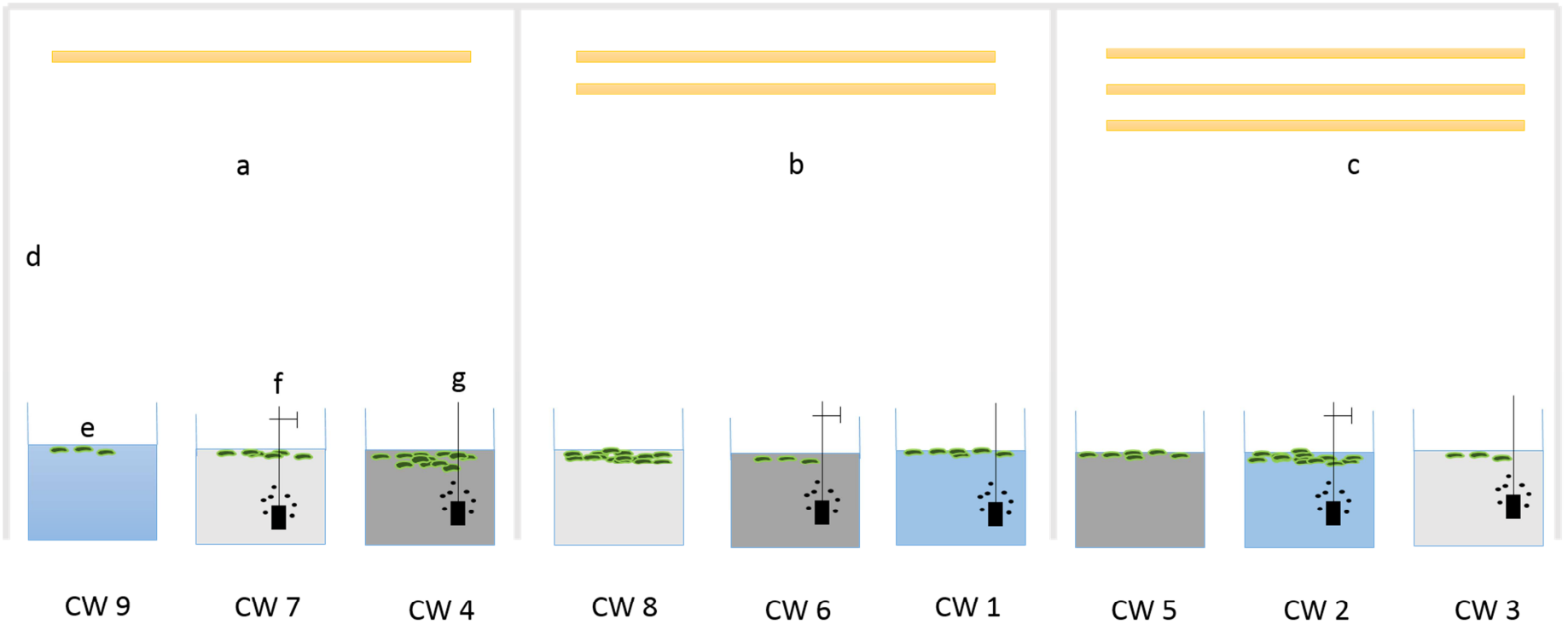

Figure 1. Schematic representations of the batch experiments. a. low light intensity chamber. b. medium light intensity chamber. c. high light intensity chamber. d. reflective fabric. e. Greater duckweed. f. intermittent aerator. g. full aerator.

CW colour: blue: no bacteria; light grey: 1.0×10^4 CFU/100 mL bacterial abundance; dark grey: 1.0×10^6 CFU/100 mL bacterial abundance

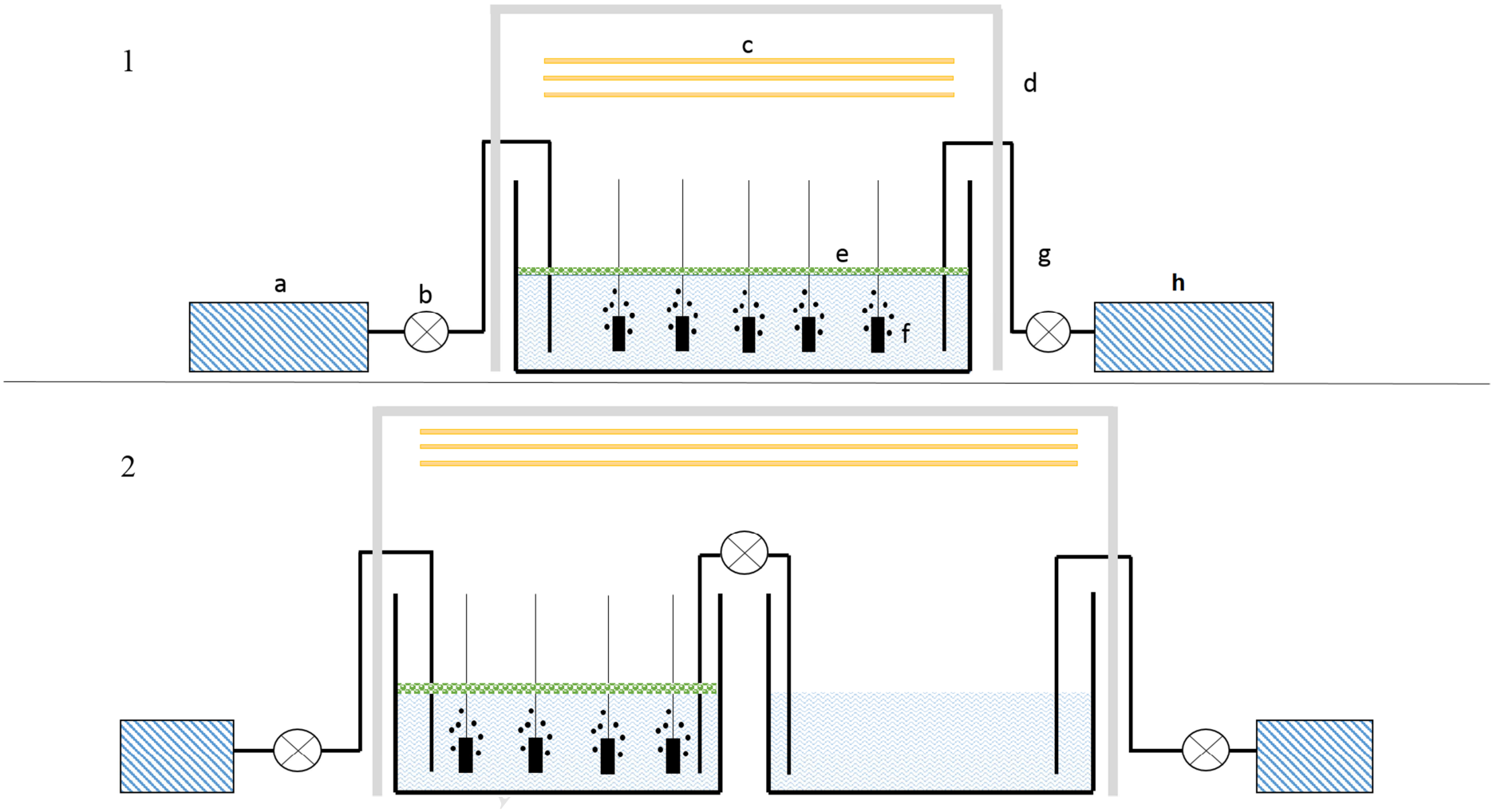

Figure 2 Schematic representations of the (1) continuous flow CW and (2) continuous flow CW-ST. a. inflow tank. b. peristaltic pump. c. lights. d. reflective fabric. e. Greater duckweed. f. aerators. g. peristaltic pump tubing. h. outflow tank.

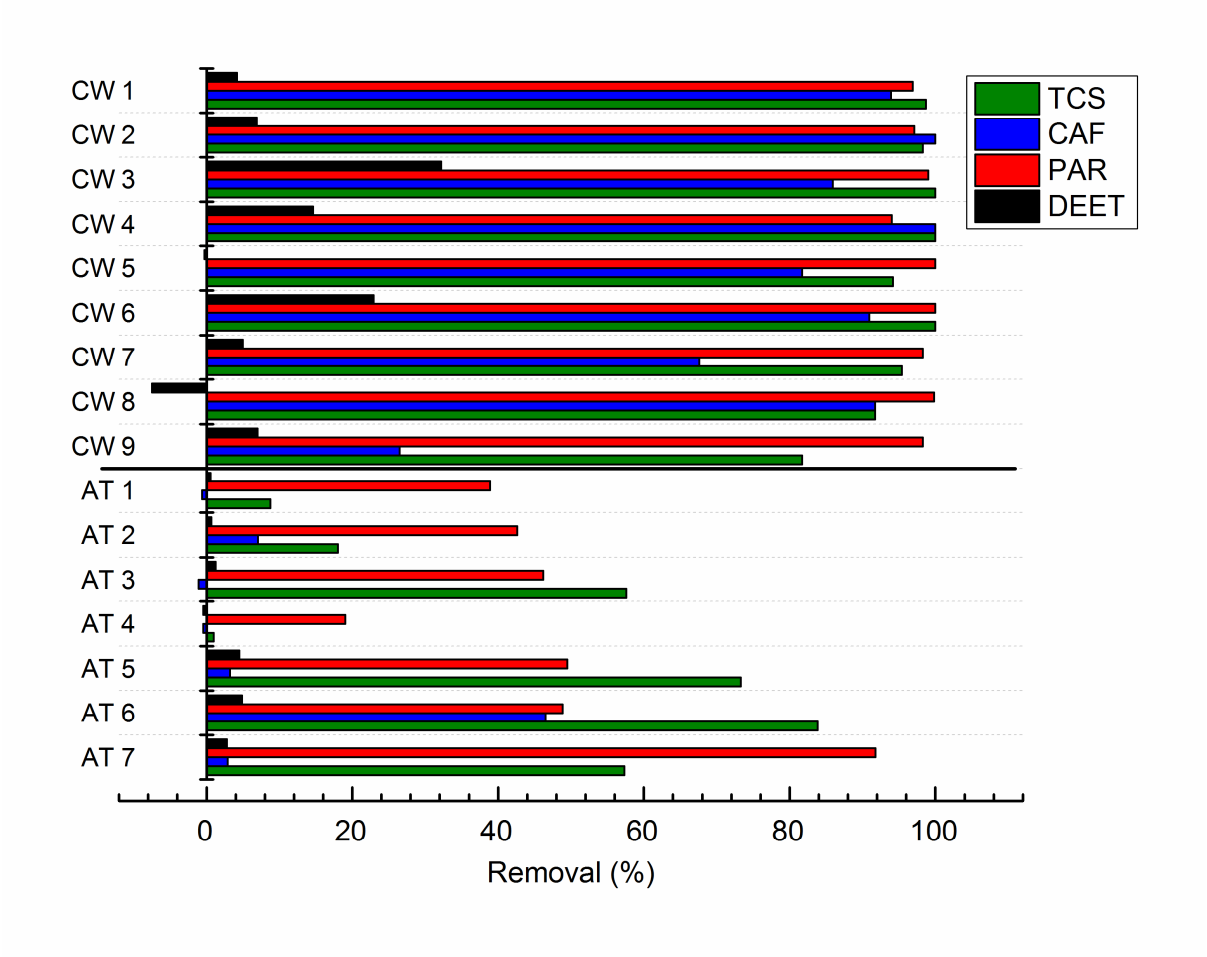

Figure 3 Removals of the target PPCP compounds in batch and ATs tests

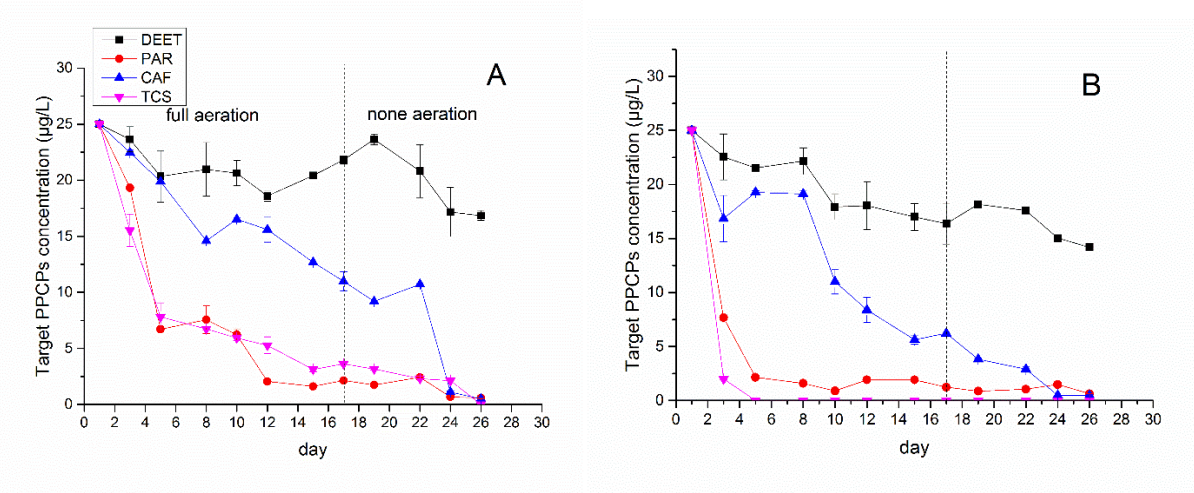

Figure 4 Concentrations of target PPCP compounds in the final treated water by (A) the continuous flow CW and (B) continuous flow CW-ST systems

(Day 1 to 17, full aeration; Day 17 to 26, none aeration)

1 Greater duckweed was used to remove target PPCPs at high organic load (300 mg/L)

2 Orthogonal design was employed to find the optimal factor levels favouring removal

3. More than 90% of paracetamol, caffeine and triclosan were removed in present study

4. Adjunction of stabilization tank significantly enhanced their removal ($p<0.05$)

5. COD and TOC removals achieved 89.3% and 91.2% using wetland-stabilization tank system