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Performance evaluation of selected aquatic plants and iron-rich media for removal of PPCPs from wastewater in constructed wetlands

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ABSTRACT

Excessive use of pharmaceuticals and personal care products (PPCPs) and their release into the water environment have become a major challenge since most of the wastewater treatment options are not equipped to treat these micro-contaminants. Some advanced technologies are reported to be effective for PPCPs treatment in wastewater but cost of those technologies remains as major drawback. Constructed wetlands (CWs) are a low-cost technology for wastewater treatment; however, their performance in term of PPCPs removal has not yet been fully investigated. This study aimed to evaluate the performance of selected aquatic plants and iron-rich media for removal of PPCPs from wastewater. Four aquatic plants were selected in the preliminary testing, where vetiver was recognized to be the most appropriate plant since it responded well to high dose of PPCPs. The experimental results revealed the increased efficiency of PPCPs removal at a low PPCPs dose. The removal of acetaminophen, amoxicillin and β-estradiol were found to be 97.5%–98.4%, 73.7%–92.2% and 75.0%–89.2%, respectively.

Keywords: Constructed wetland; Fenton reaction; Pharmaceuticals and personal care products; Iron-rich media

1. Introduction

In the recent years, pharmaceuticals and personal care products (PPCPs) have increasingly drawn attention due to their universal consumption and indiscriminate discharge to the aquatic environment. PPCPs residuals drained into sewer or on-site sanitation system are usually released into surface water, while most of the wastewater treatment plants (WWTPs) lack the efficient PPCPs removal facility [1]. Some technologies such as: membrane process [2], advanced oxidation process [3], ultraviolet [4] as well as process optimization (e.g., increasing sludge residence time) [5] have been recognized to be effective in removal of PPCPs; however, costs of these technologies remain a major drawback.

Constructed wetlands (CWs) have been reported to be effective in removing PPCPs to some extent [6], but details of their removal are still lacking. The micro-environment of CWs is considered as a leading mechanism in PPCPs removal, which allows different metabolic pathways. Moreover, other processes such as: photolysis, hydrolysis, biodegradation and sorption onto suspended solids are also expected to add further contribution in PPCPs removal [7].

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In response to environmental stress, plants produce reactive oxygen species such as: superoxide anions, hydroxide radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). The behavior of plants with environmental stress (such as: drought, salinity and heavy metals) and changes in endogenous H_2O_2 in plant tissue have been widely studied [8–10]. In previous researches, plants are stated to have positive response to PPCPs stress [11,12]. However, there is a lack of understanding on H_2O_2 production and PPCPs removal by plants. The exogenous H_2O_2 possesses potential to be utilized in Fenton process (Eqs. (1) and (2)), particularly for PPCPs removal. The iron species, which act as effective catalyst in Fenton reaction, can be supplied by selecting appropriate iron-rich media [13,14].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + {}^{\bullet}OH \tag{1}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2 + H^+ \tag{2}$$

In this study, the removal of PPCPs from wastewater by accelerating the Fenton reaction using iron-rich media (containing laterite soil) in CWs was proposed. The performance of plants in exogenous H_2O_2 production in response to stress (PPCPs exposure) and potential of utilizing exogenous H_2O_2 by iron-rich media have not yet been well understood. Therefore, the experiments aimed to: (i) characterize endogenous and exogenous H_2O_2 generation and PPCPs removal in selected aquatic plants and (ii) examine performance of iron-rich media in PPCPs removal in lab-scale CWs.

2. Materials and methods

2.1. Experimental setup

Overall experiments were carried out in three stages. The first stage involved selection of appropriate aquatic plant among four promising aquatic plants namely: cattail (*Typha* sps.), vetiver (*Vetiveria zizanioides*), reed (*Phragmites australis*) and bird of paradise (*Strelitzia reginae*) collected from naturally growing area and examined for their H_2O_2 production potential and PPCP removal. The experiment was undertaken in 12 reactors, each with a size of 0.5 m (length) × 0.5 m (width) × 0.6 m (height) size, which were fed separately with a synthetic wastewater. The synthetic wastewater was prepared by spiking of selected PPCPs namely: (i) acetaminophen (ACT), (ii) amoxicillin (AMX) and (iii) β-estradiol (β-EST) at concentrations of 1 ppb (1 μg/L) and 1,000 ppb (1,000 μg/L) to distilled water. Control reactors feeding with synthetic wastewater in the absence of plants were also operated. Overall experiments were undertaken in batch mode at the hydraulic retention times (HRTs) of 3 and 7 d.

The second-stage experiments examined the potential of iron-rich media and associated Fenton reaction in PPCPs removal. The experiments used four cylindrical reactors; each with a size of 1 m (height) and 0.15 m (diameter) was filled with iron-rich media (60 cm) at the bottom and sand (5 cm) from the top. The synthetic wastewater spiked at 10 ppm (10,000 μg/L) of ACT, AMX and β-EST was fed in the three reactors separately. One reactor was fed by a hospital wastewater enriched with 10 ppm (10,000 μg/L) of each selected PPCPs. The experiments were conducted in batch mode at the HRT of 1, 3, 5 and 7 d.

Following the second-stage experiment, the third stage involved experiments with selected plants and PPCPs for 30 d. Four reactors, each with a dimension of 0.8 m (height) and 0.45 m (diameter) were filled up with porous media (65 cm), and 5 cm of coarse sand on the top and vetiver plants (selected from first-stage experiment) were planted. The reactors were fed with the hospital wastewater in a continuous mode at the flow rate of 9 mL/min. The HRT was maintained at 3 d, and effluent samplings were done at 6 d intervals.

2.2. Chemical and reagent preparation

ACT, AMX, β-EST (analytical grade), ion-exchange resin AG1-X2 200-400 mesh, horseradish peroxidase (POD; HRP) 5U, filter glass microfiber GF/B 4.7 cm (Whatman) and *N,N*-diethyl-*p*-phenylenediamine (DPD) from Aldrich Sigma, UK, were used. The cartridge Oasis HLB Plus (225 mg, 60 μm) was used for solid phase extraction of PPCPs.

2.3. Aquatic plant and iron-rich media

The collected aquatic plants were acclimatized in a nutrient solution for 3 weeks before the experiments. Laterite soil containing iron concentration (Fe_2O_3) of 13% was mixed with crushed shellfish, activated carbon with cement as a binding agent to form iron-rich media (Fig. 1). The average size of the iron-rich media was 4 cm in diameter and 3 cm height.

2.4. Sampling and analytical method

Collection of water samples and analysis of PPCPs were according to Gros et al. [15] and Tabtong et al. [16]. The determination of endogenous H_2O_2 (plant) was according to Uchida et al. [17]. During the detection of endogenous H_2O_2, composite samples of plant shoot and root (each 1.5 g wet weight) were homogenized in liquid nitrogen by mortar and pestle. Each sample was suspended in 5 mL of 0.2 M perchloric acid and centrifuged at 1,200× g in 4°C for 5 min. Supernatant was neutralized with 4 M potassium hydroxide to pH 7.5 with total volume 10 mL and centrifuges the solution at 3,000× g in 4°C for 15 min to remove insoluble potassium perchlorate. Subsequently, 800 μL of aliquot of supernatant was applied to 0.12 g column of anion-exchange resin, and the column was washed with 3.2 mL of deionized water before collecting

Fig. 1. Iron-rich media.

1 mL of elute. In spectrophotometer cuvette, the elute was added with 400 μL of 12.5 mM 3-dimethylaminobenzoic acid, 80 μL of 1.3 mM 3-methyl-2-benzothiazolinone hydrazone and 20 μL HRP, respectively, and the reaction mixture was incubated at 25°C for 5 min and stopped reaction by cooling in ice bath for 15 min. The absorbance was read at 590 nm and compared with calibration curve ranging from 0 to 0.05 mM for H_2O_2 concentration.

Similarly, the exogenous H_2O_2 determination according to Bader [18], involved pouring of a water sample of 27 mL into a 50-mL beaker, in which 3 mL of buffer solution was added and stirred sufficiently. After 10 s, the solution was transferred to a photometric cell; meanwhile, DPD reagent (50 μL) and POD reagent (50 μL) were reacted with the solution. The mixture was analyzed within 45 ± 5 s at the spectrum 551 nm by a spectrophotometer. The concentration of NH_4^+ was determined following the standard methods [19]. All analyses were done duplicate or triplicate to ensure data accuracy.

3. Results and discussion

3.1. First-stage experiment

3.1.1. Endogenous hydrogen peroxide (H_2O_2) levels in plants

The initial concentrations of endogenous H_2O_2 in bird of paradise, reed, vetiver and cattail were 1.26 ± 0.43, 0.69 ± 0.25, 0.69 ± 0.51 and 0.64 ± 0.18 μmol/g fresh weight (FW), respectively. Such concentrations were found to be changed and varied with plants (Fig. 2) while plants exposed to different levels of PPCPs; however, H_2O_2 concentration remained below 3 μmol/g FW. In general, concentrations of endogenous H_2O_2 were reported to be varied among plant species [11].The highest endogenous H_2O_2 concentration was observed in bird of paradise, followed by reed, vetiver and cattail. On the other hand, endogenous H_2O_2 in plants were not much varied with conditions of low (1 ppb) and high (1,000 ppb) doses of PPCPs exposures. A high level of endogenous H_2O_2 could lead to the toxicity and damage of plant cells [9] that could have caused the plants to maintain limited level of endogenous H_2O_2.

There is lack of literatures explaining the behavior of plants in response to PPCPs types. The higher level of endogenous H_2O_2 concentrations under ACT than AMX and β-EST (Fig. 2) was considered to be due to reactive and stressful nature of ACT. On the other hand, the lower level of H_2O_2 in other plants suggested less stressful of AMX and β-EST. However, the stressful phenomena need to be studied further.

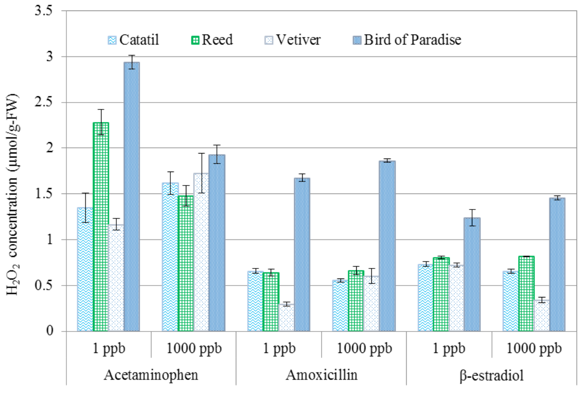

Fig. 2. Endogenous H_2O_2 concentrations in plants after 7 d of exposure to different PPCPs.

3.1.2. PPCPs removal by plants

The removal of PPCPs (%) by aquatic plants under low and high doses of feeding in relation to HRTs is shown in Fig. 3. It shows that more than 95% removal of ACT for both low and high doses was observed in 7 d, whereas similar levels of removal by majority of aquatic plants under a low dose of ACT feeding were achieved within 3 d.

The removal of AMX was found comparatively lower than ACT, which fluctuated in wide range (31%–96%) in high dose (1,000 ppb), and 54%–96% feeding in low dose (1 ppb). Under the low dose, majority of aquatic plants except cattail removed AMX almost 95% in 7 d. The limited efficiency of AMX removal by some plants under high dose likely to be associated to recalcitrant characteristic of AMX [20]. The removal efficiencies of β-EST were also 95%–99% in 7 d (under low dose, i.e., 1 ppb). The removal of high dose (1,000 ppb) of β-EST was well performed by vetiver and reed. In a separate study, Ikehata et al. [21] also concurred the removal of β-EST was by the Fenton reaction. Since the experiment was operated in a plastic greenhouse with entry of sunlight for the photosynthetic reasons, some portion of PPCPs was likely to be degraded by sunlight.

Overall, ACT and β-EST were removed efficiently by the four selected aquatic plants, which demonstrated their ability to produce endogenous H_2O_2 (Fig. 2). In principle, endogenous H_2O_2 is originally produced in the plants but, due to the root–water interaction, can be released into surrounding water [22]. For the second-stage experiment, due to the presence of low level of endogenous H_2O_2 especially even at exposing at high dose of PPCP, vetiver plant was selected. This condition indicated that vetiver plant has some mechanisms, which can regulate their internal H_2O_2 [22], and is able to maintain a less oxidative stress.

3.2. Second-stage experiment

3.2.1. PPCPs removal by vetiver plant with iron-rich media

The synthetic and hospital wastewaters with initial PPCPs concentration of 10 ppm were fed in batch mode in this stage of experiments. The removal efficiencies of PPCPs in the reactors containing iron-rich media with vetiver plants are presented in Table 1. It shows that removal of ACT was highest at the HRT of 7 d (i.e., 98.4% and 97.5%), while the AMX and β-EST removal were 73%–92%. PPCPs from the hospital wastewater (spiked with three types of PPCPs combined) were also removed in a similar or even at higher levels than the wastewater spiked with a single PPCP. In a separate study, Wang and Chu [23] reported the higher rate of ACT removal than AMX and β-EST.

3.3. Third-stage experiment (continuous feeding for 30 d)

3.3.1. Removal of ACT

The results of ACT removal based on the experiments operated for 30 d at the HRT of 3 d are presented in Fig. 4.

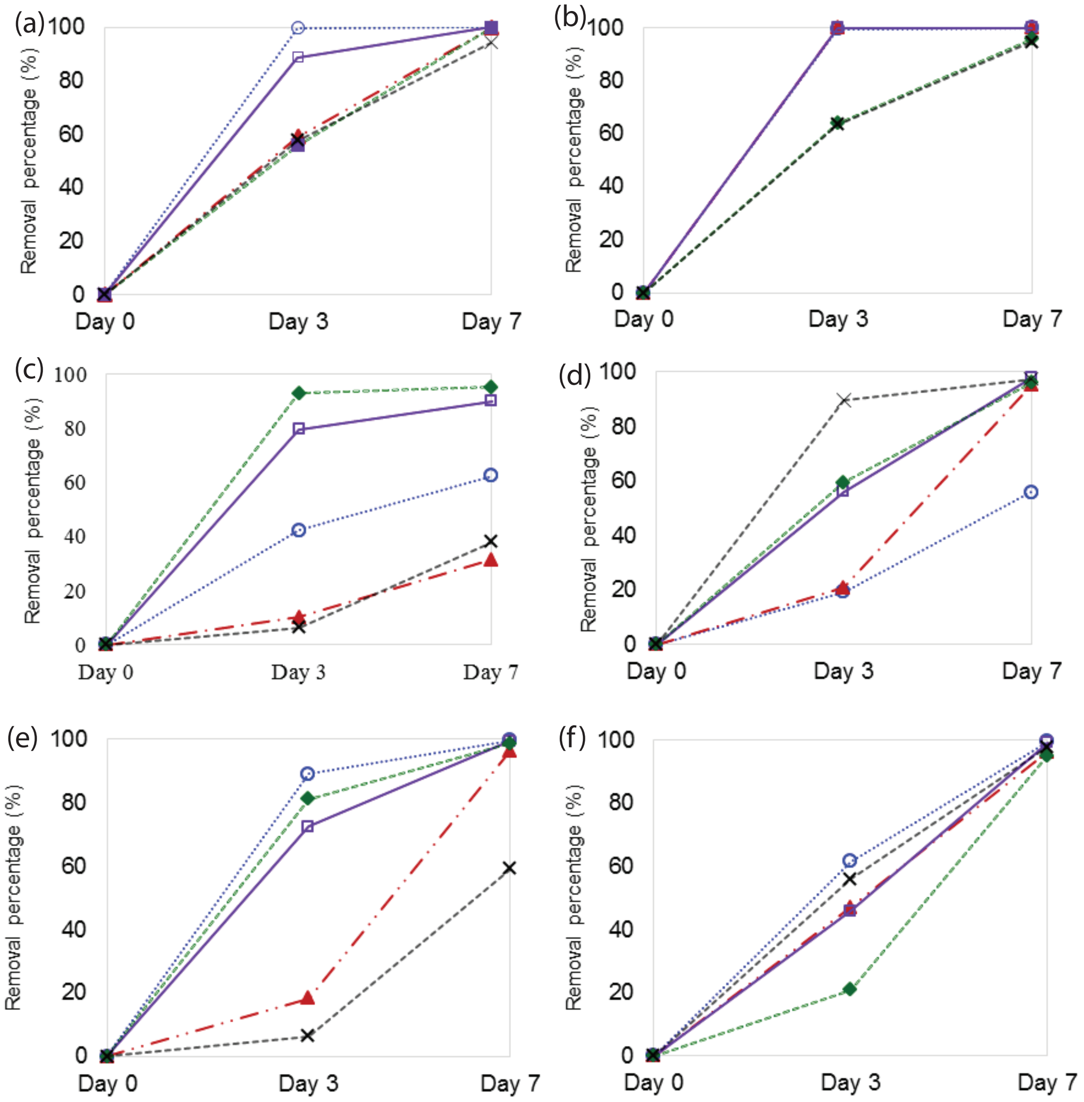

Fig. 3. Removal of PPCPs by different plants: (a) ACT (1000 ppb), (b) ACT (1 ppb), (c) AMX (1000 ppb), (d) AMX (1 ppb), (e) β-EST (1000 ppb), and (f) β-EST (1 ppb).

Table 1
PPCPs removal percentage with iron-rich media

PPCPs	Removal percentage (%)			
	Day 1	Day 3	Day 5	Day 7
Acetaminophen	45.6	92.9	96.3	98.4
Amoxicillin	73.0	73.0	73.4	73.7
β-estradiol	44.4	53.5	68.0	75.0
Acetaminophen[a]	89.1	93.0	93.5	97.5
Amoxicillin[a]	69.7	79.1	83.7	92.2
β-estradiol[a]	42.9	54.5	61.8	89.2

[a] Hospital wastewater with three types of PPCPs.

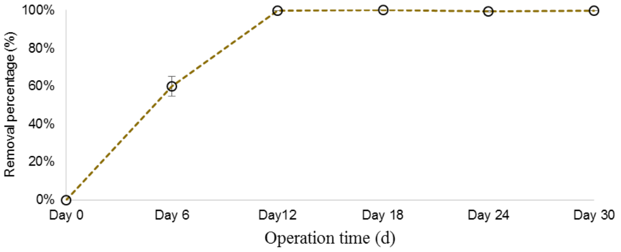

Fig. 4. Removal of ACT in 30 d operation.

The ACT removal was found to increase with increasing operation time, resulting in more than 99% removal in 12 d of operation, which was congruent with the second-stage experiment findings (Table 1). The removal of ACT in this study seems to be permanent since there were no revert of ACT in water. In a different study, Phong et al. [12] reported increment of ACT concentration in aqueous solution caused by desorption from media (sand and gravel) after a certain period in a continuous run experiment. This is explained as incomplete mineralization of ACT. The lack of further

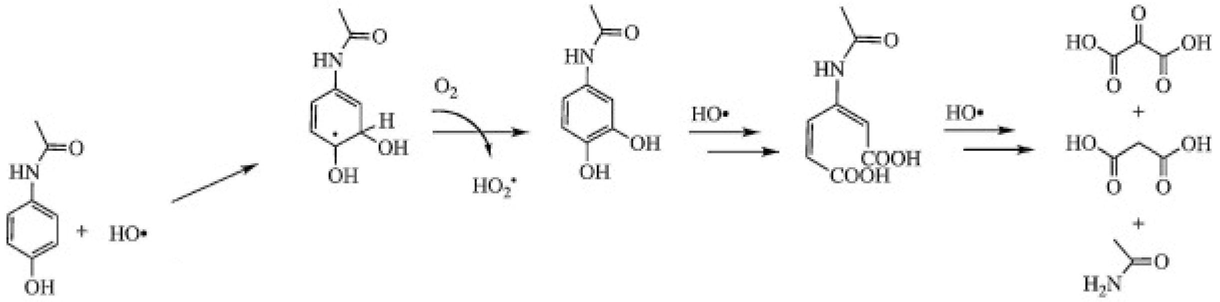

Fig. 5. ACT degradation pathways.

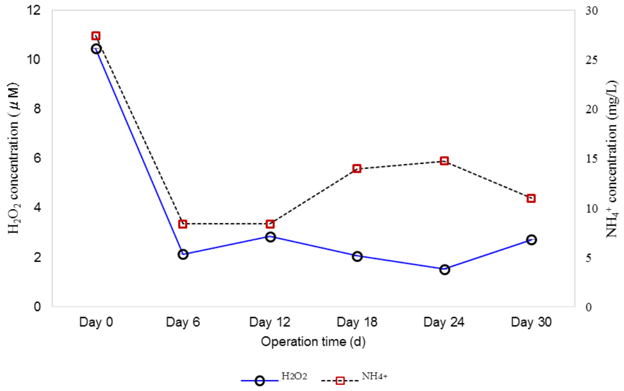

Fig. 6. Exogenous H_2O_2 and NH_4^+ concentration in plant rhizosphere (continuous mode).

increment of ACT concentration after 12 d of operation in this study suggests the occurrence Fenton reaction with the iron-rich media.

3.3.2. Degradation of ACT to end product

Since removal of PPCPs may not ensure its complete degradation, different techniques have been proposed in removal of PPCP, but increasing concern is about incomplete degradation of PPCPs. Similarly, there are reports that partially degraded PPCPs compounds are more harmful than its original form [24].

Limited information exists in the degradation of PPCPs after treatment. Moctezuma et al. [25] proposed a chain of reaction in degradation of ACT under UV/H_2O_2 system with involvement of $^{\bullet}$OH group (Fig. 5). In this study, the intermediate product was proposed to be degraded further by the $^{\bullet}$OH group and producing NH_4^+ (Eq. (3)), whereas the current work did not analyze the intermediate products.

$$CH_3\text{-}CO\text{-}NH_2 + {}^{\bullet}OH \rightarrow CH_3\text{-}COO^- + NH_4^+ \quad (3)$$

In order to examine the degradation of ACT in this experiment, the changes in concentrations of NH_4^+ and exogenous H_2O_2 were monitored and expressed in Fig. 6. The decreased concentrations of NH_4^+ and exogenous H_2O_2 were observed with progressing HRTs until 12 d. After 12 d of HRT, the exogenous H_2O_2 concentration decreased further but NH_4^+ concentration was observed to be slightly higher. The decreased level of exogenous H_2O_2 indicated the occurrence of Fenton reaction (Eqs. (1) and (2)), and the elevated NH_4^+ level could result from the complete degradation of ACT [26,27]. This result was compatible with the stable trend of ACT removal efficiency after 12 d (Fig. 4).

4. Conclusions

Based on the experimental results obtained from this study, the following conclusions are made:

- Despite similar levels of endogenous H_2O_2 production and PPCPs removal showed by primarily selected four aquatic plants, due to the presence of low level of endogenous H_2O_2 especially even at exposing at high dose of PPCP, vetiver was selected as the most appropriate one.
- In the reactors with iron-rich media, ACT was removed more efficiently (i.e., 98.4% and 97.5%) than AMX and β-EST (73%–92%).
- Iron-rich media coupling Fenton reaction was found to be more promising since it favored the advanced degradation of ACT, yielding inorganic and less toxic final products such as NH_4^+–N.

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