Performance Evaluation of Selected Plants and Iron Rich Media for Removal of PPCPs from Wastewater in Constructed Wetlands

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Abstract
Excess use of pharmaceutical and personal care products (PPCPs), and its release as human waste has 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 remain 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 plants were selected in the preliminary testing, where, vetiver was recognized to be most appropriate plant since it responded well to high dose of PPCPs (mainly: acetaminophen), and possess robust root system. This study revealed the increasing rate of PPCPs removal at low dose. The removal of acetaminophen (ACT), amoxicillin (AMX) and β-Estradiol (β-EST) were found to be 97.5-98.4%; 73.7-92.2% and 75.0-89.2%, respectively. The occurrence of Fenton reaction in use of iron-rich media was observed since H_2O_2 concentration of water, and plant leaves in reactors using media was low.

Keywords
Constructed wetland; Fenton reaction; pharmaceuticals and personal care products

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 in PPCPs removal facility (Ellis, 2006). Some technologies such as; ozonation (Andreozzi et al., 2005), reverse osmosis (Kimura et al., 2009), and advanced oxidation process (Esplugas et al., 2007), as well as process optimization (e.g. increasing sludge residence time) (Carballa et al., 2007) have been recognized to be effective in removal of PPCPs, however, costs of these technologies remain a major drawback (Fent et al., 2006).

Constructed wetlands (CWs) have been reported to be effective in removing PPCPs to some extent (Verlicchi and Zambello, 2014), but details of removal are still lacking. The micro-environments of CWs, is considered as a leading mechanism in PPCPs removal which allow 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 (Zhang et al., 2014).

Plant produces reactive oxygen species (ROS) such as; super oxide anions, hydroxide radical (*OH), hydrogen peroxide (H_2O_2) are reported in response to environmental stress, which are also being used as signaling agent in plants. On the other hand, the elevated H_2O_2 possess potential to be utilized in advanced oxidation process particularly for PPCPs removal via. Fenton reaction (Equation-i)

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{-} + {}^{*}OH \text{ ………………(Eq. i)}$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2 + H^{+} \text{ ………………..(Eq. ii)}$$

In this regard, accelerating the Fenton reaction by using iron rich media in CWs is proposed to remove PPCPs from wastewater. The release of H_2O_2 by plants and its utilization by iron-rich media have not yet been well studied. This study aimed to i) characterize H_2O_2 generation and PPCPs removal of selected aquatic plants, ii) examine performance iron-rich media in PPCPs removal in lab-scale CWs.

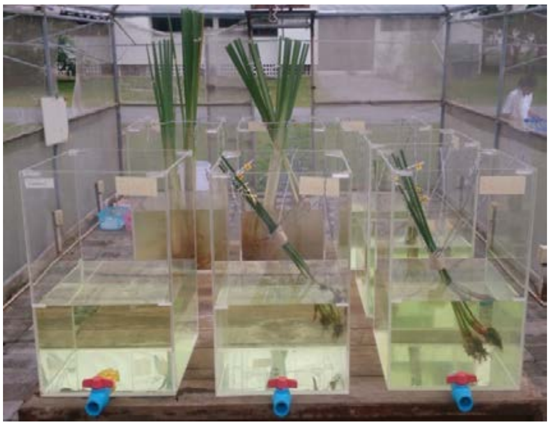
MATERIALS AND METHODOLOGY

Experimental set up

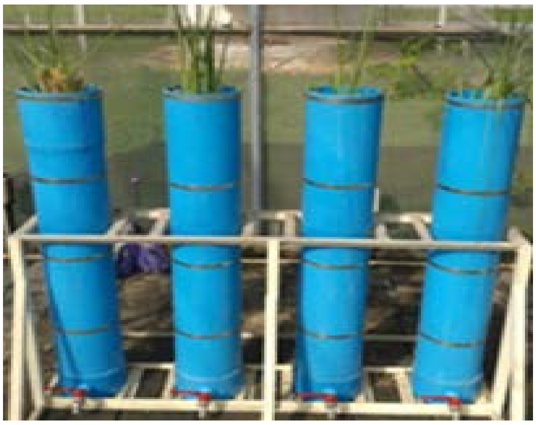
The experiments were carried out in 3 stages. The first stage was performed in reactor of 0.5 m (length) x 0.5 m (width) x 0.6 m (height) size. Four promising plants namely; cattail *(Typha sps),* vetiver *(Vetiveria zizanioides*), reed (*Phragmites australis*) and bird of paradise (*Strelitzia reginae)* were selected, and placed in separate reactors. The reactors were fed separately with four types of PPCPs i) acetaminophen (ACT), ii) amoxicillin (AMX) and iii) β-estradiol (β-EST), at concentration of 1 ppb (1 μg/L) and 1000 ppb (1000 μg/L) (Figure 1a). The experiments were run in batch and hydraulic retention times (HRT) were maintained for 0, 3, 7 days.

Second stage experiments were carried out in cylindrical reactors of 1 m (height), 0.15 m (diameter) (Figure 1b). The reactors were filled with iron-rich porous media (60 cm) at the bottom and sand (5 cm) was added from the top. Plant selection based on the 1^{st} stage of experiment and synthetic wastewater was fed in 3 reactors spiked at 10 ppm concentrations of ACT, AMX, and β-EST separately, and 1 reactor by real hospital wastewater spiked at 10 ppm of ACT, AMX, and β-EST. The feeding was done in batch mode with HRTs 0, 1, 3, 5 and 7 days.

At the third stage, reactors of 0.8 m (height) and 0.45 m (diameter), were filled (0.65 m) by porous media, and 5 cm top by coarse sand (Figure 1c). Plant selected from the first stage experiment, was grown and hospital wastewater spiked at concentration of 10000 ppb (10000 μg/L) as fed in a continuous mode for 30 days of operation period. Samples were collected at 6 days intervals.



a) First stage experiments



b) Second stage experiments

c) Third stage experiments

d) Porous media prepared by laterite soil and other composites

Figure 1. Experimental set-up

Chemical and reagent preparation

ACT, AMX, β-EST (analytical grade), ion exchange resin AG1-X2 200-400 mesh, horse radish peroxidase 5U (POD), 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 (SPE).

Experiment was run using synthetic and real hospital wastewater. The synthetic wastewater was prepared spiking of selected PPCPs at concentration of 1 and 1000 ppb in distilled water. On the other hand, real wastewater, collected from a local hospital, was enriched by 10,000 ppb (10 ppm) of each selected PPCPs. The wastewater was prepared in 100 L volume, and fed to reactors separately.

Plant and media preparation

Four plants namely; typha *(Typha spp)*, vetiver *(Vetiveria zizanioides*), reed (*Phragmites australis*) and bird of paradise (*Strelitzia reginae)* were selected. These plants were collected from naturally growing areas and acclimatized in nutrient solution for three weeks, afterwards, homogenous plants were selected for experiments. The iron rich porous media was prepared by crushed shellfish, activated carbon and laterite soil (Figure 1d).

Sampling and analytical method

Sample collection and analysis of PPCPs

Collection of water samples and analysis of PPCPS were followed according to Phong et al. (2016), which was retrieved from Gros et al. (2013); and Tabtong et al. (2015). The plant samples extraction for PPCP analysis was retrieved from Shenker et al. (2011), and the detail process is mentioned in Phong et al. (2016). The determination of H_2O_2 (plant) was according to Uchida et al. (2002), which is further described in Phong et al. (2016). Moreover, H_2O_2 in water was followed N,N-diethyl-p-phenylenediamine (DPD) method according to Bader et al. (1988).

RESULTS AND DISCUSSION

First stage experiment (Hydroponic conditions)

Hydrogen peroxide (H_2O_2) levels in plants

H_2O_2 concentrations were varied with plants in relation to PPCPs types and doses (Figure 2), which lacks of clear trends. The highest H_2O_2 concentration was observed in bird of paradise, which was followed by reed, vetiver and cattail. H_2O_2 concentrations were noticed to be higher at low dose of PPCPs (1 ppb) except vetiver and cattail which were higher under ACT. The occurrence of low levels of H_2O_2 at high level of PPCPs dose is likely due to its involvement in PPCPs removal.

The concentrations of H_2O_2 was observed relatively high while feeding with ACT, whereas, an elevated level of H_2O_2 was observed in vetiver plants under high dose of ACT (1000 ppb). This condition could suggest that ACT is a reactive and more stressful PPCP to plant, whereas, vetiver plants react ACT stress more sensitively.

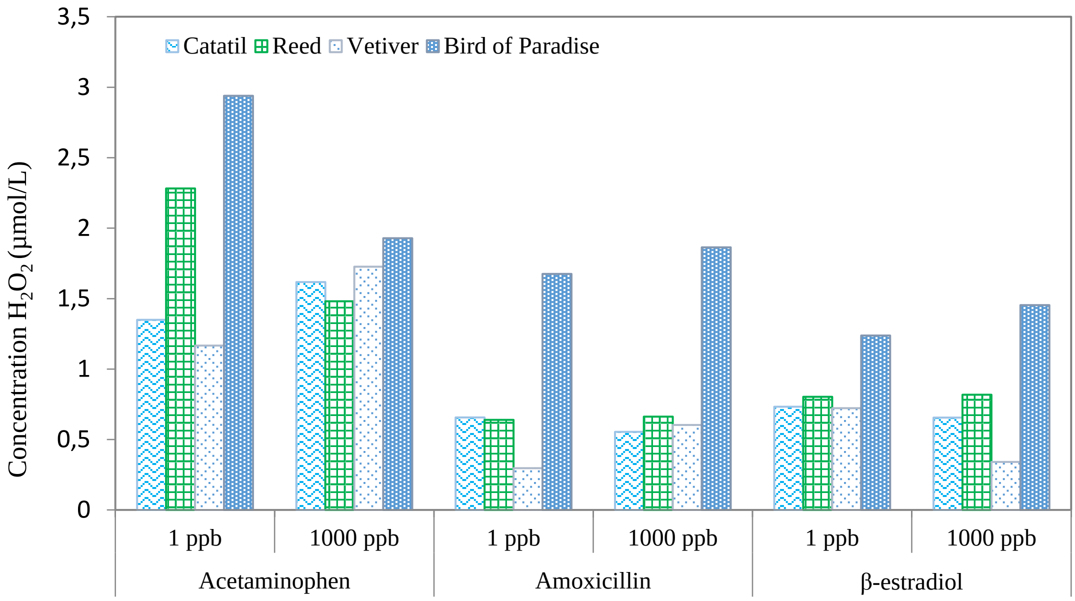


Figure 2. H_2O_2 concentrations in plants after 7 days of exposure to different PPCPs

PPCP removal by plants

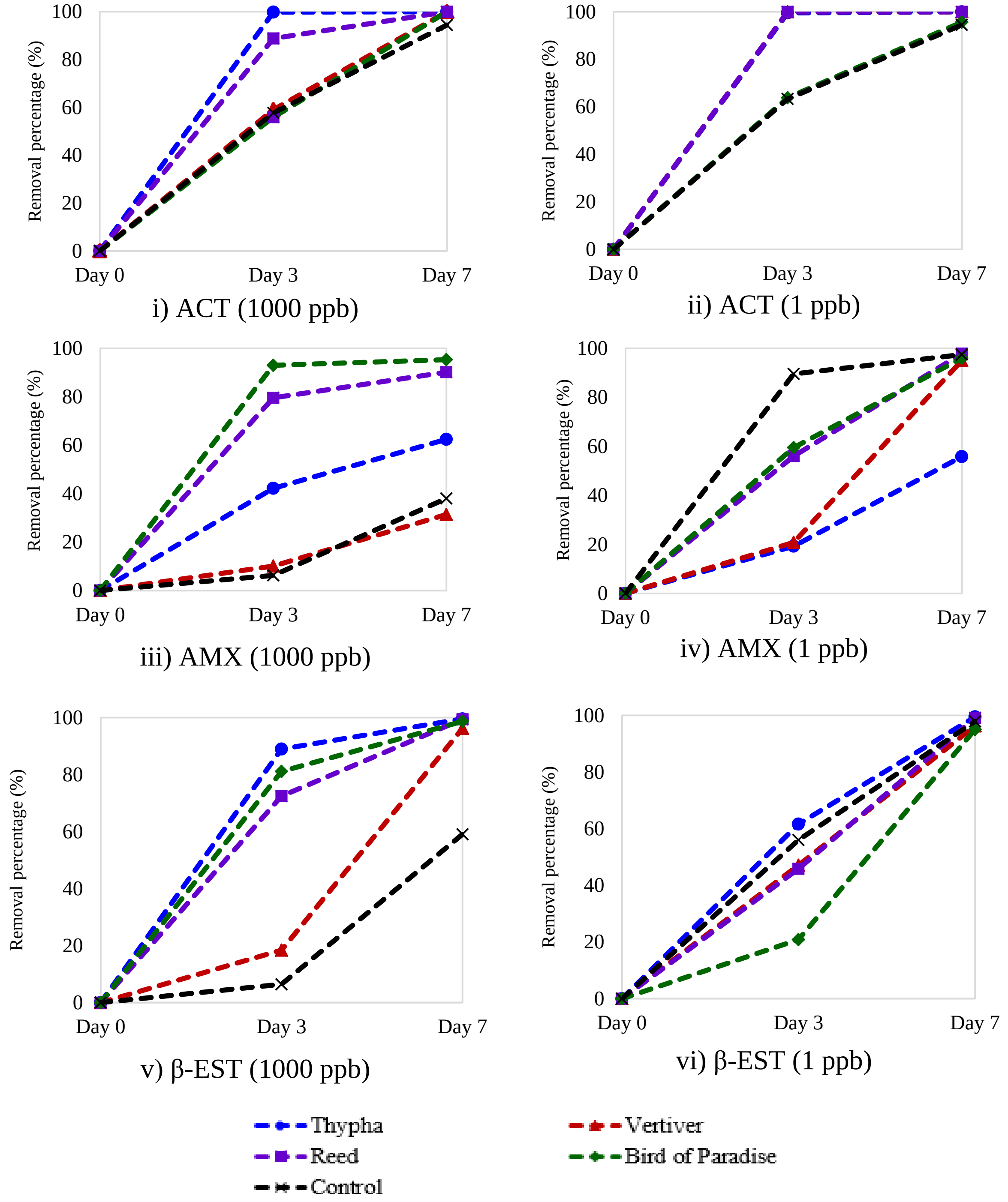


Figure 3. Removal of PPCPs by different plants.

The removal of PPCPs (%) under low and high dose of feeding in relation to HRTs is shown in Figure 3. It shows the > 95% removal for both low and high doses of ACT in 7 days, whereas, majority of plants had achieved the similar level of ACT within 3 days, under a low dose of feeding.

The removal of AMX was observed comparatively lower than ACT, which fluctuated in wide range (31-96%) in high dose (1000 ppb), and 54-96% feeding in low dose (1 ppb). In condition of low dose of feeding, majority of plants except typha removed AMX almost at

95% in 7 days. The poor performance of plant in removal of AMX is more likely due to recalcitrant characteristic of AMX (Zhang et al., 2014). On the other hand, the removal of β-EST was occurred at smoothly, which was removed for 95-99% in 7 day (under low dose i.e.1 ppb). The removal of high dose (1000 ppb) of β-EST was well performed by reed and vetiver plants.

In overall, ACT and β-EST were removed efficiently by all 4 chosen plants. Similarly, all plants demonstrated well potential of H_2O_2 production. For the 2nd stage of experiment, vetiver plant was selected mainly due to increased level of H_2O_2 production with high dose of ACT stress and presence of robust root system.

Second stage of experiment (comparative study: with and without of using media)
H_2O_2 concentrations in water

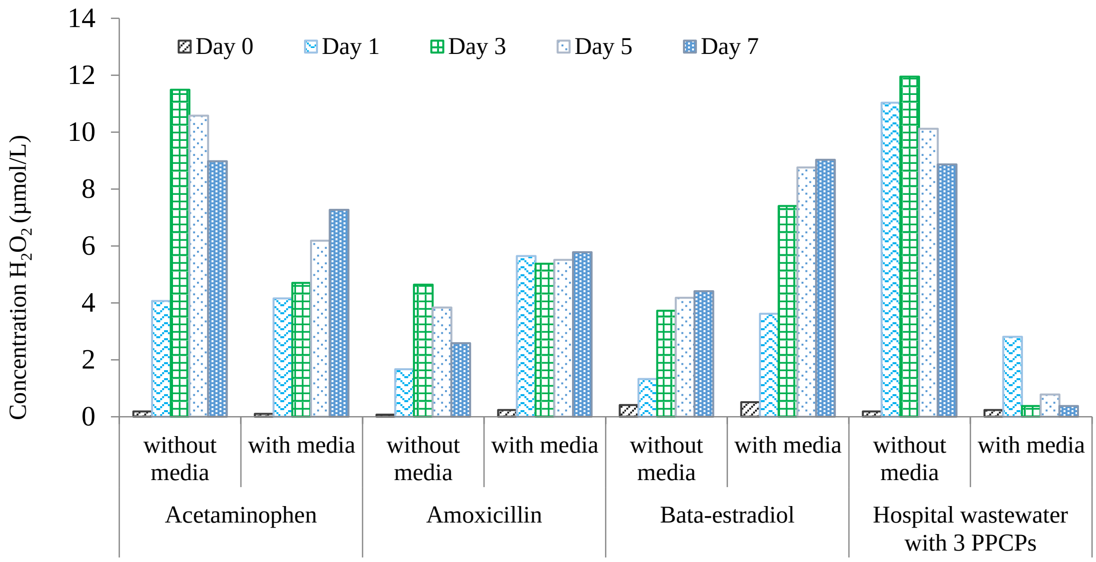


Figure 4. H_2O_2 concentrations in water in condition of with and without media use

Comparative results of H_2O_2 levels in water, with and without use of media is presented in Figure 4. In general, high levels of H_2O_2 were observed in reactors operated without media, in-which, H_2O_2 were increased in the consecutive sampling event (3 day), and then declined in the subsequent sampling day (5 days and 7 days). In contrast, H_2O_2 concentrations in reactors containing iron rich media were raised continuously for 7 days, except reactor fed with hospital wastewater. The existence of a relatively low level of H_2O_2 in use of media use was likely due to Fenton reaction catalyzed by iron.

H_2O_2 concentrations in plant
Similar to water, high concentrations of H_2O_2 were observed in plants in reactors without media (Figure 5). It shows the increased concentrations of H_2O_2 with increasing HRTs (i.e. 5 and 7 days), which was likely due to accumulation of H_2O_2 produced in response to stress. On the other hand, low levels of H_2O_2 observed in reactor having media, which was resulted due to advance Fenton reaction.

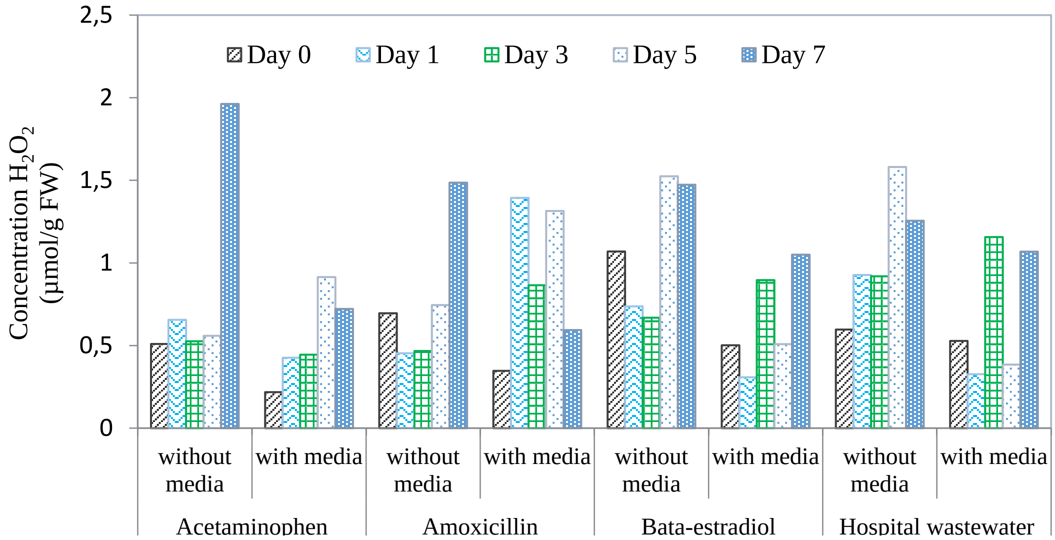


Figure 5. H_2O_2 in plants in conditions of with and without using media

PPCPs removal by vetiver plant under use of porous media

The removal of PPCPs in reactors containing iron-rich media with vetiver plants is shown in Table 1. Synthetic and hospital wastewater with initial concentration of 10 ppm were fed in batch mode in this experiments. It shows that removal of ACT was undertaken at the highest level in 7 days (i.e. 98.4 % and 97.5%). This removal was fairly higher than AMX and β-EST removal which varied 73 -92%. PPCPs from hospital wastewater (spiked with 3 types of PPCPs combined) were also removed in a similar or even at higher levels than wastewater spiked with a single PPCPs.

Table 1. PPCPs removal percentage with porous media.

	Removal percentage (%)			
PPCPs	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*	89.1	93.0	93.5	97.5
Amoxicillin*	69.7	79.1	83.7	92.2
β-estradiol*	42.9	54.5	61.8	89.2

*Hospital wastewater with 3 types PPCPs

A promising results could be observed in PPCPs removal, but treated effluent still contains considerably high levels of PPCPs, especially β-EST (215.2 ppb and 40.56 ppb in synthetic and hospital wastewater, respectively). This could be associated with the recalcitrant characteristic of β-EST.

Third stage of experiment (continuous feeding for 1 month)

Removal of ACT

The results of PPCPs removal based on the experiments operated for 1 month (30 days) with hydraulic retention time (HRT) of 3 days is presented in Figure 6. It shows the increased removal ACT with increasing operation time, whereas, it was removed >99% in 12 days of operation. The results was congruent with the findings from second stage of the experiments.

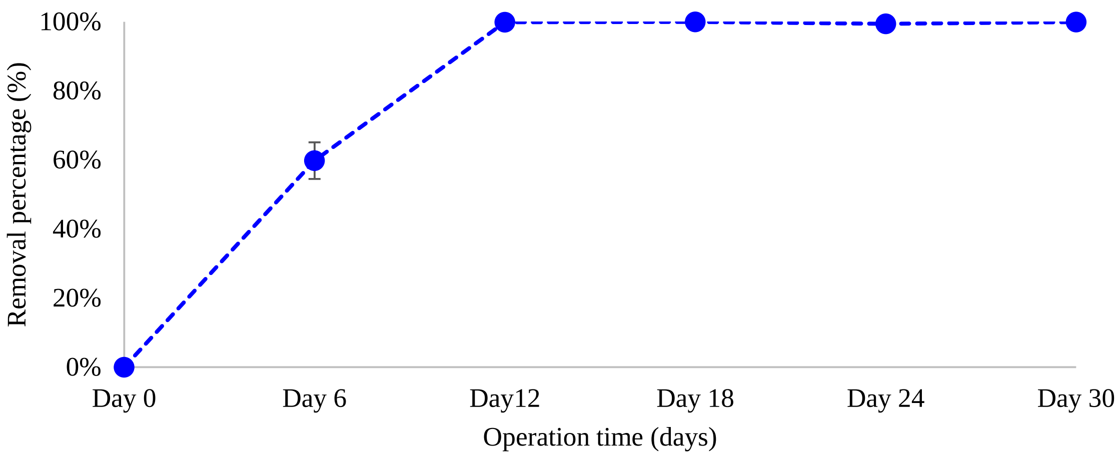


Figure 6. Removal of ACT in 30 days operation.

Degradation of ACT to end product

The degradation of PPCPs following to the treatment option is considered important since the incomplete degraded compound of PPCPs may harmful than its original form. It is stated that any form of oxidation processes, the degradation products of PPCPs must be analyzed since these could be more toxic than the parent compound (Noguera-Oviedo and Aga, 2016).

However, limited studies and information exists in the degradation of PPCPs after treatment. Moctezuma et al. (2012) has proposed a chain of reaction in degradation of ACT under UV/H_2O_2 system with involvement of $^{\bullet}OH$ group (Figure 7). In this study, the intermediate product was proposed to be degraded further by the $^{\bullet}OH$ group and produce NH_4^+ (Eq. iii).

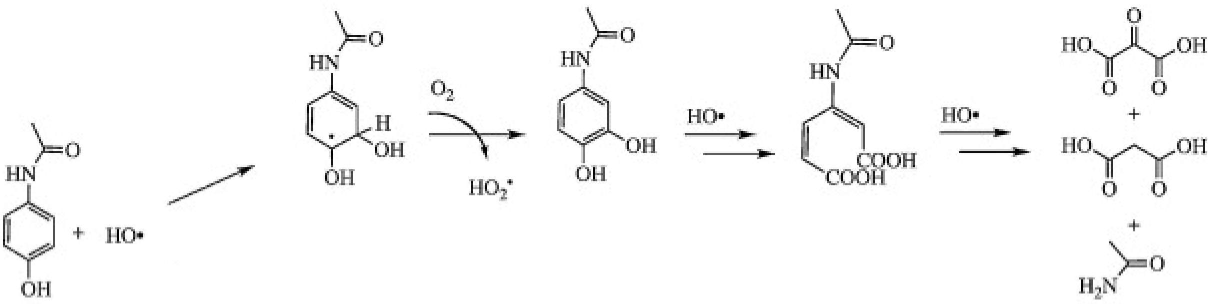


Figure 7. ACT degradation pathways.

$$CH_3\text{-}CO\text{-}NH_2 + \cdot OH \rightarrow CH_3\text{-}COO^- + NH_4^+ \text{ …………(Eq. iii)}$$

In order to examine the degradation of ACT in this experiment, the changes in concentrations of NH_4^+ and H_2O_2 were monitored and expressed in Figure 8. It shows the decreased concentration of NH_4^+ and H_2O_2 with progressing HRTs until 12 days. After 12 days of HRT, H_2O_2 concentration went decreasing but NH_4^+ was observed to be higher. The decreased level of H_2O_2 may indicates the occurrence of Fenton reaction (Eq. i, & Eq ii), and the elevated NH_4^+ level is resulted from the complete degradation of PPCPs.

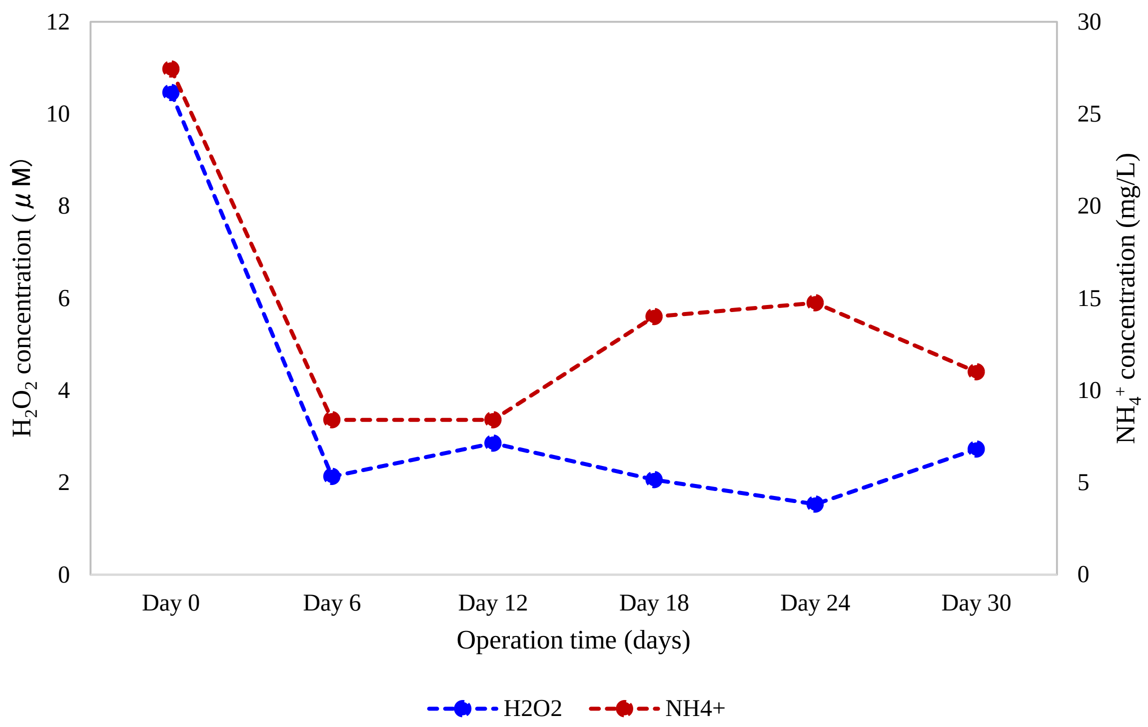


Figure 8. H_2O_2 concentration in plant rhizosphere (continuous mode).

CONCLUSIONS

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

- Despite similar levels of H_2O_2 production and PPCPs removal showed by primarily selected 4 plants, vetiver plants was selected as a most appropriate once since it produced high level of H_2O_2 in response to high dose of ACT and plants possess robust root and easy adaptation.
- Levels of H_2O_2 was found higher in water, and plant leaves in reactor without media. In contrast, low levels of H_2O_2 was observed in water and plants in use of media, which indicated the occurrence of Fenton reaction.
- PPCP removal was observed at higher rate under low dose of PPCPs exposure. ACT was removed more efficiently (i.e. 98.4 % and 97.5%) than AMX and β-EST (73 - 92%), whereas, positive role of iron-rich media was observed in PPCPs removal.
- Iron rich media coupling Fenton reaction was found to be more promising since it has favored the advanced degradation of ACT, yielding inorganic and less toxic final products such as NH_4^+-N.

REFERENCES

Andreozzi, R., Canterino, M., Marotta, R. & Paxeus, N. 2005. Antibiotic removal from wastewaters: The ozonation of amoxicillin. *Journal of Hazardous Materials*, 122**,** 243-250.

Bader, H., Sturzenegger, V. & Hoigné, J. 1988. Photometric method for the determination of low concentrations of hydrogen peroxide by the peroxidase catalyzed oxidation of N,N-diethyl-p-phenylenediamine (DPD). *Water Research*, 22**,** 1109-1115.

Carballa, M., Omil, F., Ternes, T. & Lema, J. M. 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Research,* 41**,** 2139-2150.

Ellis, J. B. 2006. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. *Environmental Pollution,* 144**,** 184-189.

Esplugas, S., Bila, D. M., Krause, L. G. T. & Dezotti, M. 2007. Ozonation and advanced oxidation technologies to remove endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. *Journal of Hazardous Materials,* 149**,** 631-642.

Fent, K., Weston, A. A. & Caminada, D. 2006. Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology,* 76**,** 122-159.

Gros, M., Rodríguez-Mozaz, S. & Barceló, D. 2013. Rapid analysis of multiclass antibiotic residues and some of their metabolites in hospital, urban wastewater and river water by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *Journal of Chromatography A,* 1292**,** 173-188.

Kimura, K., Iwase, T., Kita, S. & Watanabe, Y. 2009. Influence of residual organic macromolecules produced in biological wastewater treatment processes on removal of pharmaceuticals by NF/RO membranes. *Water Research,* 43**,** 3751-3758.

Moctezuma, E., Leyva, E., Aguilar, C. A., Luna, R. A. & Montalvo, C. 2012. Photocatalytic degradation of paracetamol: Intermediates and total reaction mechanism. *Journal of Hazardous Materials,* 243**,** 130-138.

Noguera-Oviedo, K. & Aga, D. S. 2016. Lessons learned from more than two decades of research on emerging contaminants in the environment. *Journal of Hazardous Materials,* 316**,** 242-251.

Phong, V. H. N., Koottatep, T., Chapagain, S. K., Panuvatvanich, A., Polprasert, C. & Ahn, K.-H. 2016. Removal of acetaminophen from wastewater by constructed wetlands with Scirpus validus. *Environmental Engineering Research,* 21**,** 164-170.

Shenker, M., Harush, D., Ben-Ari, J. & Chefetz, B. 2011. Uptake of carbamazepine by cucumber plants – A case study related to irrigation with reclaimed wastewater. *Chemosphere,* 82**,** 905-910.

Tabtong, W., Boontanon, S. K. & Boontanon, N. 2015. Fate and Risk Assessment of Perfluoroalkyl Substances (PFASs) in Water Treatment Plants and Tap Water in Bangkok, Thailand. *Procedia Environmental Sciences,* 28**,** 750-757.

Uchida, A., Jagendorf, A. T., Hibino, T., Takabe, T. & Takabe, T. 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Science,* 163**,** 515-523.

Verlicchi, P. & Zambello, E. 2014. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. *Science of The Total Environment,* 470–471**,** 1281-1306.

Zhang, D., Gersberg, R. M., Ng, W. J. & Tan, S. K. 2014. Removal of pharmaceuticals and personal care products in aquatic plant-based systems: A review. *Environmental Pollution,* 184**,** 620-639.