Removal of acetaminophen from wastewater by constructed wetlands with *Scirpus validus*

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ABSTRACT
Since most of the existing wastewater treatment options lack the ability to treat micro-contaminants, the increased use of pharmaceuticals and personal care products (PPCPs) and release as human waste have become a serious concern in recent years. Constructed wetlands (CWs) are a low-cost technology for wastewater treatment, however, its performance in term of PPCPs removal has not yet been fully investigated. This study aimed to characterize the removal factors and efficiency of acetaminophen (ACT) removal by CWs. The results revealed the decreased concentrations of ACT with increasing hydraulic retention times (HRT) of 0, 3, 5 days. The contribution of removal factors was found to be varied with initial ACT concentration. At the low ACT concentration (i.e. 1 ppb), plant uptake was the dominant, followed by microbial and photolytic removal. In contrast, at the high ACT concentration (i.e. 100 ppb), microbial and photolytic removal were found as dominant factors. On the other hand, hydrogen peroxide (H₂O₂) concentration was found at higher level in the plant shoot than in the root probably due to occurrence of the Fenton reaction resulting in PPCPs removal.

Keywords: Acetaminophen, Constructed wetland, Fenton reaction, Pharmaceuticals and personal care products, Wastewater

1. Introduction
Pharmaceuticals and personal care products (PPCPs) have increasingly drawn attention due to their universal consumption as well as indiscriminate discharge to the aquatic environment [1]. PPCPs residuals are usually drained into sewer or on-site sanitation system, while most wastewater treatment plants (WWTPs) lack the PPCPs removal facility, resulting in the release of these contaminants into surface water [2].

Because acetaminophen (ACT) is the most widely used pharmaceutical, trace amounts of ACT is often detected in treated wastewater and contaminating surface water or groundwater [3]. The concentrations of ACT are reported 1-6 μg/L and up to 10 μg/L in European sewage treatment plant effluents and natural water source in USA, respectively [4]. The contamination of ACT is dealt with a great concern since it could be easily transformed into by-products. Huguet et al. [5] reported the formation of intermediate compound as 1, 4-benzoquinone from the treated water. The transformation of ACT into disinfectant-by-product during disinfection of drinking water [6], was found to be detrimental to liver, and inducing nephrotoxicity [7].

Recent treatment technologies such as: ozonation [8], reverse osmosis [9], and advanced oxidation process [10], as well as process optimization (e.g. increasing sludge residence time) [11] have been considered to be useful to cope with the problems, however, the costs of these technologies are still a major drawback [12].

Constructed wetlands (CWs) have been reported to be effective in removing PPCPs to some extent [13], but the mechanisms involved in the PPCPs removal are poorly known. The existence of micro-environments in CWs which allow different metabolic pathways are considered to be a leading mechanism in PPCPs removal. Other processes such as: photolysis, hydrolysis, biodegradation, and sorption onto suspended solids also contribute to PPCPs removal [14, 15], however the involvement of these factors is still poorly understood [16, 17].

On the other hand, reactive oxygen species (ROS) such as hydroxyl radical (·OH) and hydrogen peroxide (H₂O₂) generated by plants
in response to environmental stress might have also involved in detoxification process. H$_2$O$_2$ is essential for the Fenton reaction, coupling suitable catalyst, can increase PPCPs treatment efficiency. The objectives of this research were:

i) To evaluate the removal efficiency of ACT by laboratory-scale constructed wetlands planted with *Scirpus validus*.

ii) To investigate factors responsible for ACT removal such as: plant uptake, media adsorption, microbial and photolytic effect and others.

iii) To study the relationship between H$_2$O$_2$ in the plant tissues and ACT removal efficiencies.

### 2. Materials and Methods

#### 2.1. Preparation of Plant, Media and Chemicals

*Scirpus validus* was collected from naturally growing areas, which was acclimatized in nutrient solution for three weeks, and homogenous plants were selected for the experiment. Media (i.e. sand, small gravel and coarse gravel) were sieved, washed and dried prior being used. The porosities of sand, small gravel and coarse gravel were found as 0.337 ± 0.021, 0.443 ± 0.013, 0.524 ± 0.004, respectively.

ACT (analytical grade), ion exchange resin (AG1-X2 200-400 mesh), horseradish peroxidase (5U) and filter glass microfiber (GF/B 4.7 cm; Whatman) from Aldrich Sigma, UK were used.

#### 2.2. Analytical Method

The pre-treatment and analysis of water samples were followed according to Gros et al. [18]. A liter of water sample was collected, subsequently acidified to pH 3 by EDTA to minimize microbial activity and filtered by GF/B. Solid-phase extraction (SPE) was conducted with Oasis HLB sorbent cartridges (HLB Plus 225 mg 60 μm). The cartridges were pre-conditioned with 6 mL DI water (pH = 3.5) and samples were percolated through the cartridges at flow rate of 5 mL/min. After percolation, the cartridges were washed with 2 mL of DI water-methanol (95:5) and the eluent was discarded. The cartridges were finally wrapped by aluminium foil and stored in freezer.

The plant samples extraction method was retrieved from Shenker et al. [19]. Plant samples (1.5 g mixture of fresh shoot and root) were collected and extracted with 20 mL of methanol, assisted by ultrasonic probe (15 min, 50 kHz). After extraction, the mixture was agitated (30 min, 300 rpm), centrifuged (25 min, 5000 rpm) and filtered by GF/B. The sample was diluted with DI water to 500 mL and loaded with Oasis HLB sorbent cartridges. The media was collected in term of composite samples and analyzed by HPLC-MS/MS.

ACT (analytical grade), ion exchange resin (AG1-X2 200-400 mesh), horseradish peroxidase (5U) and filter glass microfiber (GF/B 4.7 cm; Whatman) from Aldrich Sigma, UK were used.

### 2.3. Experimental Set Up

The experiments were conducted in acrylic reactors with dimension of 0.5 m long × 0.5 m wide × 0.6 m deep. Synthetic wastewater was prepared at two concentrations i.e. 1 ppb and 100 ppb of ACT, which was fed at volume of 30 L in batch mode. *Scirpus validus* was selected as plant species for the experiment. The experiment was designed with retention time of 3 and 5 days.

All experimental reactors were run under hydroponic condition except reactor D (media consisting of sand, small gravel and coarse gravel). Reactor A consisting of plants was exposed to sunlight. Reactor B without plants and media was used to quantify the effect of microbial removal. The microbes were inoculated by contacting of plant root to the synthetic wastewater for 10 seconds. Reactor C, consisting of plants was protected from sunlight. Plants were grown together with the media in reactor D, but photolytic removal was expected to occur at limited extent due to difficulty of sunlight to pass through the water and media. A control reactor without media and ACT was operated in parallel. Samples of water, plant and media were collected with corresponding retention time for ACT and H$_2$O$_2$ analyses. The temperature ranged 29 - 34°C in green house during the experiment. The details of experimental design are provided in Table 1 and Fig. 1.

The mass balance (Eq. (1)) was applied to determine contribution level of each removal factor. The fractions removed by photolytic, microbial, plant uptake, media adsorption, and residual fraction in effluent were determined separately. Total weights of the plant and media were taken into consideration in calculation of total ACT.

\[
\text{Total mass ACT} = (\text{Photolytic} + \text{Microbial}) + \text{Plant uptake} + \text{Media adsorption} + \text{Remaining in effluent} + \text{Other processes}
\]
3. Results and discussion

3.1. Reduction of ACT Concentration from Treated Wastewater

The changes in ACT levels with the initial ACT concentrations of 1 ppb and 100 ppb under different operating conditions are shown in Fig. 2. The concentrations of ACT were found to reduce with increasing HRT except in reactor D fed at the high ACT concentration. The lowest ACT reduction was observed in reactor B, indicating the limited contribution of microbial and photolytic removal. In reactor D, the reduction of ACT was found to be moderate which could be due to the low hydrophobicity of ACT [22].

The low level of ACT reduction in reactor A (without aluminum foil cover) indicated the minor role of photolytic degradation in ACT, which was similar to the findings of Yamamoto et al. [16]. The higher rate of ACT removal in reactor C (99.5%) compared to reactor A (55.7%), was likely due to transformation of ACT into by-products. The exact mechanism of ACT transformation in a dark environment has not yet been fully understood, but the existence of dark condition is hypothesized to be similar to bed media, in which, according to Huguet et al. [5], transformation of ACT into by-products could occur. Unlike other reactors, ACT concentration in the effluents of reactor D was found to increase at the HRT of 5 days. Since this reactor was operated with media, the increase of ACT concentration was hypothesized to be due to desorption from the media. The poor performance of sand and gravel as media in ACT removal was also mentioned by Lin [23].

The removal data of ACT presented in Fig. 2 shows that 30 -
40% of the fed ACT was removed in 3 days for both low and high concentrations in reactor B, slightly lower than the value reported by Yamamoto et al. [16], which was 50% microbial removal. Lam et al. [24] found less role of biodegradation in the overall PPCPs removal (including ACT).

Overall data from Fig. 2 show that the highest ACT removal efficiencies occurred in the reactor C (i.e. 92 - 99%) under dark condition for both low and high ACT concentrations. These results were hypothesized to be due to ACT transformation under dark condition [5]. Further analysis of the transformed by-products is strongly recommended to confirm this hypothesis. The ACT removal efficiencies of reactor A and reactor D were in range of 60 - 70%, while the lowest (below 50%) was found in reactor B. It appears from Fig. 2 that there might not be further ACT concentration reduction after HRT of 5 days, but further experiments at HRT longer than 5 days are suggested to confirm the data.

3.2. Factors Contributing to ACT Removal

The ACT concentrations in plants (Scirpus validus) under the low and high ACT concentrations are expressed in Fig. 3. The ACT concentrations were found to be 0.119 and 0.204 μg/g fresh weight (FW) of plant in reactor A under 1 ppb and 100 ppb, respectively, higher than among the other reactors which suggested plant uptake as a dominant mechanism in ACT removal. The results were found in close agreement with Zhang et al. [25, 26] who described the potential of Scirpus validus in the removal of PPCPs such as carbamazepine, naproxen, diclofenac and clofibric acid.

Although the plants in reactor C had less accumulation of ACT than the plants in reactor A, ACT concentrations in effluents of reactor C were lower than reactor A. The exposure of plants to sunlight in reactor A, could have accelerated plant uptake ability as compared to reactor C. The photolysis removal was observed to be less effective, but it could have prevented the transformation of ACT into other by-products in reactor A, which were likely to cause high ACT concentration in the effluent of reactor A.

Similarly, the ACT concentrations in plant samples were observed at lower level in reactor D than reactor A, but the effluent ACT concentrations of reactor D were lower. This was likely due to the combined effects of microbial removal and adsorption as well as transformation of ACT into by-products [5].

The first order of kinetic of ACT uptake by plants is presented in Table 2, which was observed in similar to Zhang et al. [26].

3.3. Mass Balance of ACT Removal

The contributions of removal factors such as; plant uptake, microbial removal, photolytic removal and media adsorption were calculated based on total ACT removal (Fig. 4).

The data of Fig. 4 show that at low ACT concentration 1 ppb in reactor A, plant uptake had the highest removal percentage (i.e. 68%) of total removal, whereas, 29% was remained in the effluent while 3% was treated by microbial and photolytic removal. For the same reactor but fed at the high ACT concentration of 100 ppb, plant uptake was limited to 2%, which suggested the maximum accumulation capacity of the plant.

In reactor C, the contribution of plant uptake was found only 19% in 1 ppb of ACT, which was further reduced to 1% under 100 ppb of ACT due to the maximum accumulation capacity. On other hand, the existence of dark environment had accelerated the transformation of ACT to by-products. The effect of photolytic removal was not significant but could play supportive role for other mechanisms responsible for ACT removal. Yamamoto et al. [16] described the moderate contribution of photolysis process in ACT removal. The contribution of microbial and photolytic removal was found to be moderate from 30 to 50% of total ACT removal for both 1 ppb and 100 ppb concentrations. Regarding the contribution of media, ACT removal by media adsorption was not found effective and was totally negligible at the 1 ppb concentration, which was congruent to the findings of Lorphensri et al. [27], but was elevated to 9% at the 100 ppb concentration. Media adsorption in combination with microbial removal could be the effective to boost up the overall ACT removal efficiency [23].

Fig. 3. Concentrations of ACT in plant samples; a) ACT feeding concentration at 1 ppb, b) ACT feeding concentration at 100 ppb
Table 2. First Order Kinetic of ACT Uptake by Scirpus validus

<table>
<thead>
<tr>
<th>Type</th>
<th>ACT at 1 ppb conc.</th>
<th>r²</th>
<th>ACT at 100 ppb conc.</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor A</td>
<td>y = 0.0594x – 0.0547</td>
<td>0.9817</td>
<td>y = 0.1019x – 0.0943</td>
<td>0.9384</td>
</tr>
<tr>
<td>Reactor C</td>
<td>y = 0.0172x – 0.0104</td>
<td>0.6822</td>
<td>y = 0.0506x – 0.0482</td>
<td>0.9932</td>
</tr>
<tr>
<td>Reactor D</td>
<td>y = 0.0251x – 0.0286</td>
<td>0.9458</td>
<td>y = 0.0259x – 0.0143</td>
<td>0.6262</td>
</tr>
</tbody>
</table>

Fig. 4. Contribution of removal factors under different conditions.
Overall, at the low ACT concentration (i.e. 1 ppb), plant uptake was the dominant which had removed 19 - 68% of total ACT and it was followed by microbial and photolytic removal (3 - 32%). In contrast, at the high ACT concentration (i.e. 100 ppb), microbial and photolytic removal were found as dominant factors (i.e. 53% of total ACT removed).

3.4. Hydrogen Peroxide (H$_2$O$_2$) in Plants

It is generally known that in response to environmental stress, plants generate reactive oxygen species (ROS) including hydrogen peroxide (H$_2$O$_2$). The occurrence of H$_2$O$_2$ in Scirpus validus plants in relation to different levels of ACT stress is illustrated in Fig. 5.

In general, H$_2$O$_2$ was found at lower levels in roots than in shoots of the plants. The H$_2$O$_2$ concentrations were slightly higher at the high ACT concentration, which indicated the generation of H$_2$O$_2$ by plants under increased stress. The lower concentration in the roots was probably due to H$_2$O$_2$ utilization in the detoxifying reaction such as Fenton reaction. The increase in H$_2$O$_2$ concentrations was noticed for 3 days (reactor A and C), and then decreased on day 5, suggesting active response of the plants during the first 3 days of ACT exposure.

4. Conclusions

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

- ACT concentrations in the CWs with Scirpus validus plants were found to be removed in the range of 55 - 99.5% at the HRT of 5 days.
- At the low ACT concentration of 1 ppb, ACT removal by plant uptake was found to be dominant (19 - 68% of removal), followed by microbial and photolytic removal mechanisms (3 - 32%). Media adsorption was less active, but by-product transformation was found to be 3 - 41%.
- At the high ACT concentration of 100 ppb, plant uptake of ACT was found to be 1 - 2%, whereas removal by microbial and photolytic were found to be 53%, and media adsorption was 9%. The by-product transformation contributed 1 - 46% of ACT removal.
- H$_2$O$_2$ concentrations were observed at higher concentrations in shoot than in root of the plant, and were found to increase with increasing ACT concentration.

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References


