Investigation of benzotriazoles' fate in *Lemna minor* systems

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Abstract: : The removal of five benzotriazoles (1H-benzotriazole, BTR; 4-methyl-1H-benzotriazole, 4TTR; 5-methyl-1H-benzotriazole, 5TTR; xylytriazole, XTR and 5-chlorobenzotriazole, CBTR) from water and treated wastewater using duckweed *Lemna minor* bioreactors was investigated. Batch experiments were initially carried out in triplicates to study the role of photodegradation, hydrolysis, and plant uptake on target compounds removal and the relevant kinetics constants and half-lives of the target compounds were calculated. Afterwards, a continuous flow lab-scale system comprised from one treatment line with three duckweed mini ponds in series was used to investigate the removal of target compounds from secondary treated wastewater. The system operated for a period of three months with a total hydraulic retention time of 8.3 days. All analyses of micropollutants were carried out using an HPLC-DAD system. According to the results, the tested substances were significantly removed in batch experiments with active *Lemna minor*. Calculation of half-lives values revealed significant differences according to the compound, ranging between 1.6 ± 0.3 d (CBTR) and 25 ± 3.6 d (4TTR). The target benzotriazoles were significantly removed in the continuous-flow system. The lowest removal was noticed for 4TTR (26%), while the highest for CBTR (72%).

Keywords: micropollutants, constructed wetlands, plant uptake, elimination

1. Introduction

Benzotriazoles (BTRs) are commonly used in corrosion-inhibiting products, cooling fluids, vulcanization accelerators and dishwashing detergents. During the last decade, several articles have been published concerning the occurrence and fate of these compounds in Sewage Treatment Plants (STPs) and the mechanisms affecting their removal in biological wastewater treatment systems. Despite their frequent detection in water and wastewater (Stasinakis *et al*., 2013; Molins-Delgado *et al*., 2015), so far, there is only one study available concerning their removal in constructed wetland systems. Specifically, Matamoros *et al*. (2010) studied the removal efficiency of three BTRs in a surface flow constructed wetland planted with *Typha latifolia* and *Phragmites australis* and in a vertical flow constructed wetland and reported that their removal was affected by seasonality.

Among different plant-based systems, ponds with duckweed *Lemna minor* have been applied with success in different countries for the removal of nutrients and heavy metals, combining efficient wastewater treatment and important biomass production (Haarstad *et al*., 2012; Iatrou *et al*., 2015). To the best of our knowledge, so far, no information is available for the fate of BTRs in *Lemna minor* ponds and the contribution of different mechanisms on their removal.

Based on the above, the main objectives of this study were to investigate the removal of five benzotriazoles; namely 1H-benzotriazole (BTR), 4-methyl-1H-benzotriazole (4TTR), 5-methyl-1H-benzotriazole (5TTR), xylytriazole (XTR) and 5-chlorobenzotriazole (CBTR), from water and treated wastewater in batch and continuous-flow experiments using *Lemna minor* bioreactors.

2. Materials and methods

2.3. Batch experiments

Three different batch reactor systems were used to investigate the aqueous removal of target compounds in SIS medium due to hydrolysis, photodegradation and plant uptake. The total period of the experiments was 36 d and samples were taken at different time intervals, The temperature was set at 24.0 ± 0.5 ˚C, the pH was 7.0 ± 0.2 and the initial concentration of target compounds was 150 μg L^{-1}. Experiment A was conducted in the absence of *Lemna minor* under dark conditions to investigate the hydrolysis of BTRs. Experiment B was conducted under light conditions (study of photodegradation); whereas in Experiment C, 2 gr of *Lemna minor* were added in each flask to investigate the contribution of plant uptake on BTRs' removal.

Table 1. Removal of target BTRs in batch experiments A, B and C. (The total duration of the experiments was 36 d; Experiment A was conducted under dark conditions; Experiment B under light conditions; Experiment C under light conditions and presence of *Lemna minor*)

	Experiment A	**Experiment B**	**Experiment C**
BTR	6.4 ± 1.6	36.2 ± 4.0	100 ± 0
4-TTR	1.0 ± 1.6	6.1 ± 8.0	48.2 ± 4.1
5-TTR	0	14.3 ± 3.0	100 ± 0
CBTR	94.6 ± 4.7	96.9 ± 0.6	100 ± 0
XTR	21.4 ± 1.8	24.1 ± 3.4	100 ± 0

2.2. Continuous-flow system

The continuous flow set-up comprised from one treatment line with three duckweed mini ponds in series (Iatrou *et al*., 2017). The system operated with a total hydraulic retention time (HRT) of 8.3 days, under 16/8h light/darkness, respectively. The fed up of duckweed system was conducted using secondary biologically treated wastewater, originating from University Campus STP. Influent wastewater was spiked with target micropollutants in order to achieve a concentration of around 30 μg L^{-1} at the inlet of the lab-scale system and samples were taken from the outlet of each mini pond.

2.3. Analysis and Calculations

To control the operation of continuous-flow system, (COD), and ammonia-N (NH_4-N) were determined in a regular basis at Points A to D, according to Standard Methods. The determination of BTRs was conducted using a Shimatzu LC20-AD prominence liquid chromatographer associated with a SPD-M20A prominence diode array detector (LC-DAD). For the analysis of samples from continuous-flow systems, solid phase extraction was previously applied according to Mazioti *et al*. (2015).

The removal efficiency of BTRs in batch and continuous-flow experiments was calculated. Kinetic constants and half-lives were estimated in batch experiments using first-order kinetics.

3. Results and discussion

3.1. Batch experiments

Excepting 4TTR, up to the end of the experiments (36 d), all the compounds had been totally eliminated from the medium in the presence of *Lemna minor* (Table 1). However, significant differences were observed among target compounds. BTR was slightly decreased under dark conditions (Experiment A), while its removal enhanced in presence of light reaching 36.2 ± 4.0 % at the end of Experiment B (Day 36), and in the presence of *Lemna minor* reaching 100% at Day 12 of Experiment C. 4TTR, 5TTR and XTR were not removed or removed slightly during Experiment A and B, while they were significantly removed in the presence of biomass. As a result, in Experiment C, the observed removal of 4TTR reached 48.2 ± 4.1% at the end of the experiment, while 5TTR and XTR were fully eliminated up to Day 8. Finally, CBTR removal was progressively increased under different experimental conditions, reaching 100% in Day 8 of Experiment C.

Regarding the half-lives of target compounds, in Experiment C that included all studied mechanisms (hydrolysis, photodegradation and plant uptake), the lower half-life value was calculated for CBTR (1.6 ± 0.3 d), while the higher for 4TTR (25 ± 3.6 d). On the other hand the half-life of BTR, 5TTR and XTR was 4.0 ± 0.9 d, 2.7 ± 0.9 d and 2.0 ± 0.5 d, respectively.

3.2. Continuous-flow experiments

The performance of the duckweed system was satisfactory; the average COD and NH_4-N removal was equal to 54 ± 13% and 84 ± 1%, respectively. Regarding micropollutants, the existence of different ponds in series resulted to a gradual decrease of their concentrations, revealing the contribution of all ponds on their elimination. The total measured percentage removal in the system was ranged between 26 ± 10% for 4TTR and 72 ± 10% for CBTR (Figure 1).

To the best of our knowledge, this is the first study reporting BTRs removal in duckweed continuous-flow wastewater treatment systems, while there is limited information for the removal in other types of constructed wetlands. In the only available study in the field, Matamoros *et al*. (2010) studied the removal efficiency of BTR, 4TTR and 5TTR in surface flow constructed wetland with a total HRT of one month and reported that the highest removal was observed during summer and ranged between 50% (5TTR) and 65% (4TTR). Apart from *Lemna minor* systems, few articles are also available for the removal of BTRs using other plants such as hydroponic

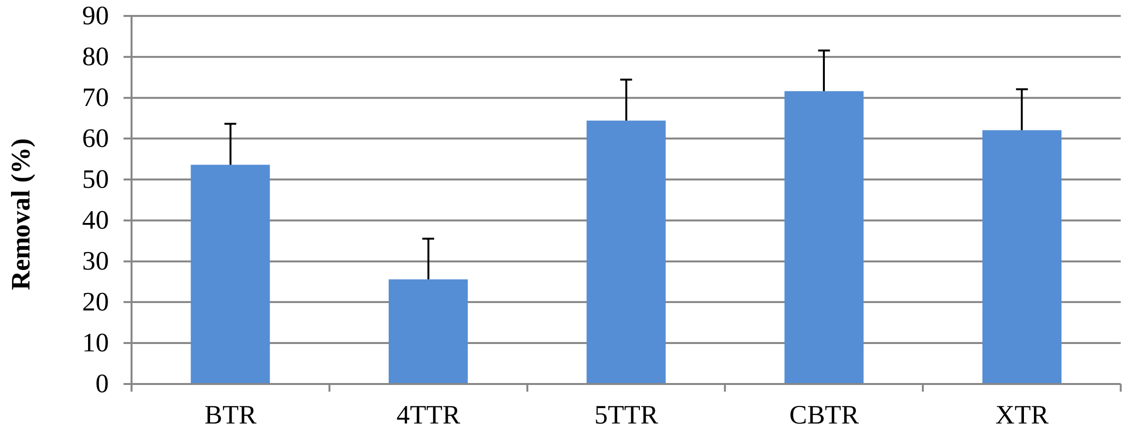

Figure 2. Total removal of BTRs in continuous-flow experiments (the total hydraulic retention time was 8.3 d).

sunflower (Castro *et al*., 2004). Their disappearance from water in hydroponic cultures seems to be due to transpiration and uptake, followed by phytotransformation. So far, the transformation products of BTRs in hydroponic cultures have not be fully identified; however, it has been reported that more polar compounds are formed comparing to the parent BTRs (Castro *et al*., 2004).

4. Conclusions

In batch experiments with *Lemna minor*, full elimination of BTRs was observed, excepting 4TTR that was removed by 48.2 ± 4.1% up to the end of the experiment (36 d). The estimated half-life values ranged between 1.6 ± 0.3 d (CBTR) and 25 ± 3.6 d (4-TTR).The operation of a continuous-flow *Lemna minor* system consisted of three mini ponds and a total hydraulic residence time of 8.3 d showed partial removal of target substances, ranging between 26% (4TTR) and 72% (CBTR).

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