Laboratory- and full-scale studies on the removal of pharmaceuticals in an aerated constructed wetland: effects of aeration and hydraulic retention time on the removal efficiency and assessment of the aquatic risk

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Abstract

Pharmaceutical residues in wastewater pose a challenge to wastewater treatment technologies. Constructed wetlands (CWs) are common wastewater treatment systems in rural areas and they discharge often in small water courses in which the ecology can be adversely affected by the discharged pharmaceuticals. Hence, there is thus a need for studies aiming to improve the removal of pharmaceuticals in CWs. In this study, the performance of a full-scale aerated sub-surface flow hybrid CW treating wastewater from a healthcare facility was studied in terms of common water parameters and pharmaceutical removal. In addition, a preliminary aquatic risk assessment based on hazard quotients was performed to estimate the likelihood of adverse effects on aquatic organisms in the forest creek where this CW discharges. The (combined) effect of aeration and hydraulic retention time was evaluated in a laboratory-scale batch experiment. Excellent removal of the targeted pharmaceuticals was obtained in the full-scale CW (> 90 %) and as a result the aquatic risk was estimated low. The removal efficiency of only a few of the targeted pharmaceuticals was found to be dependent on the applied aeration (namely gabapentin, metformin and sotalol). Longer the hydraulic retention time increased the removal of carbamazepine, diclofenac and tramadol.

Key words

Sub-surface flow, hybrid, Forced Bed Aeration, hazard quotient, LECA

Introduction

Many pharmaceuticals show such persistence to biodegradation that their presence in surface waters is used as an indicator of wastewater contamination (Vystavna et al., 2013). The environmental concentrations of pharmaceuticals are usually very low, in the range of ng/L but some commonly used substances which are poorly removed during wastewater treatment can occur at µg/L levels (Ashton et al., 2004; Lindqvist et al., 2005; Loos et al., 2009). It is likely that the highest concentrations of pharmaceuticals are detected in small streams where limited dilution occurs.

The chronic effects that pharmaceutical residues can pose in the environment are difficult to identify and quantify. Therefore, the data on the ecotoxicity of pharmaceuticals is mostly derived from experiments in the laboratory and only a small part of this data is targeting effects after chronic exposure, i.e. long-term exposure at low concentration (Quinn et al., 2008). An initial estimate of the aquatic risk of the pharmaceuticals can be calculated via hazard quotients (HQs). The HQs compare the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC) for a specific organism observed in the laboratory experiments (Santos et al. 2007). If the ratio MEC / PNEC is higher or equal to 1, the particular pharmaceutical can have adverse ecological effects (Gros et al. 2010).

Constructed wetlands (CWs) are mostly used at rural and remote locations as wastewater treatment systems for single households and small communities. They discharge in small rivers and water courses which often have high biodiversity (Matamoros et al., 2016) making them vulnerable to anthropogenic pollution. The configurations vary from surface flow systems to sub-surface flow systems and hybrids where several (different types of) CWs are applied in the treatment chain (Kadlec & Wallace, 2009). The configuration, the operation and the ambient environmental conditions within the CW are likely to affect the pharmaceutical removal efficiency. Several studies on pharmaceutical removal efficiencies in different types of CWs have already been performed (for review see Verlicchi & Zambello, 2014) but there is still need to explore factors that could improve the removal efficiency. For example, dissolved oxygen content is likely to play an important role in the removal of pharmaceuticals. Improved removal efficiency of e.g. diclofenac, ibuprofen and ketoprofen has been observed during discontinuous feeding which replenishes the oxygen in the substrate pores as studied in horizontal sub-surface CWs (Ávila et al., 2013; Zhang et al., 2012). Ávila et al. (2014) studied the effect of active aeration on pharmaceutical removal in vertical sub-surface flow CWs, and concluded that the actively aerated saturated CW performed similarly to the typical unsaturated CW. However, their research included only a limited number of pharmaceutical substances and therefore, further research is needed to conclusively define the effect of active aeration on different types of pharmaceuticals.

The main objective of this study is to evaluate the removal efficiency in a full-scale sub-surface flow constructed wetland treating wastewater from a healthcare facility and analyze the ecological impact of the effluent discharge in an effluent-dominated stream. In addition, the effects of active aeration and hydraulic retention time (HRT) on the removal efficiency are studied in a separate batch experiment.

2 Materials and methods

2.3 Full-scale constructed wetland

The full-scale CW investigated in this study was built in 2015 and it is located at a health care facility in the Province of Antwerp in Belgium. The CW comprises a vertical sub-surface flow (VSSF) part followed by a horizontal sub-surface flow (HSSF) part having a total surface area of 240 m² (40 x 6 m) and a depth of 110 cm. Both parts are saturated. The design capacity of the system is 340 inhabitant equivalent but at the time of sampling the complete capacity of the system was not in use and therefore, the HRT of the system was long, approximately 10 d (design HRT 3 - 4 d). The CW receives wastewater from a septic tank at intervals and flow rate dependent on water consumption. The effluent flow rate varied during the sampling period from 6 m^3d^{-1} to 16 m^3d^{-1} based on 5 daily measurements during 5 consecutive days (hydraulic loading rate 0.025-0.067 m/d). The CW discharges effluent in a small forest creek where dilution occurs only by rainfall. The creek runs in a sandy ground and is shaded by the forest trees. The water depth in the creek was 0 - 10 cm (partly dry) and its flow rate low (partly stagnant).

The CW bed (both VF and HSSF) contains porous light expanded clay aggregate (LECA; ∅ 8/16 mm, Argex) granules. The HSSF part is partly filled with tobermorite (calcium silicate hydrate mineral) to increase the phosphorous removal. The CW is planted with *Phragmites* *australis* and *Iris pseudacorus*. Aeration is provided in the CW with the Forced Bed Aeration technology (FBA®, Rietland). The aeration time is controlled automatically based on the flow rate of the incoming wastewater (4 h/d per 10 m^3/d) and the capacity of the air pumps is 150 m^3/h.

During the sampling period the weather was dry and the average temperature was ~10°C. Grab samples were taken from a reservoir tank where influent is collected after the septic tank and from an effluent collection well at the end of the CW from where the effluent is directly discharged into the creek. One influent and one effluent mixed sample were obtained per day and one such sample was based on 5 grab samples taken every 2 – 3 hours during day time. The sampling campaign lasted for 5 days. In addition, two grab samples were taken from the creek on the third sampling day (at noon) at distances 50 m and 100 m from the effluent discharge point.

2.4 Batch experiment

A microcosm scale batch experiment was set up in order to investigate in more detail the role of HRT and active aeration on the removal efficiency of selected pharmaceuticals. The substrate (1.3 L per setup) was put in a plastic container (∅ 20 cm, h ~5 cm) where influent (0.5 L) was added. The substrate (LECA) and influent were fetched from the full-scale CW and stored at 4 °C until the start of the batch experiment (2 days). Four treatments were applied in the microcosms (Table 1). Aeration was applied by means of one aquarium air pump (Hozelock 320) and air stones. The experiment was conducted inside at constant temperature (20°C) and the setups were covered to prevent light penetration. Effluent samples were obtained by draining the whole liquid volume from the microcosms.

Table 1 – Treatments during batch experiment

Treatment	Applied HRT (d)	Aeration applied
HRT2-AIR	2	Yes
HRT6-AIR	6	Yes
HRT2-NO-AIR	2	No
HRT6-NO-AIR	6	No

2.5 Analysis methods

2.5.1 Common water quality parameters

Dissolved oxygen (DO) and pH were measured once in the full-scale CW using a multimeter HQ40d (Hach). The measurements were conducted in the influent, at 3 locations along the length of the CW and in the effluent once during the experiment. The mixed influent and effluent samples obtained during the sampling campaign of the full-scale CW were analyzed for chemical oxygen demand (COD), ammonium (NH_4^+) and nitrate (NO_3^-) by using kits according to manufacturer's instructions (LCI500, LCK305, LCK340; Hach, Belgium). The influent and effluent samples from the batch experiment were analyzed for DO and pH (HQ40d, Hach).

2.5.2 Analysis of pharmaceuticals

Twelve pharmaceuticals from 7 different therapeutic classes were targeted in this study. The selected pharmaceuticals were atenolol (ATL), bisoprolol (BSP), carbamazepine (CBZ), diazepam (DZP), diclofenac (DCF), gabapentin (GBP), metformin (MFM), metoprolol (MTP), sotalol (STL), telmisartan (TST), tramadol (TMD) and valsartan (VST). The analysis of the target pharmaceuticals was done using an LS-MSMS system (Thermo Fisher Scientific LTQ Orbitrap) after purification and concentration of the samples using solid phase extraction (SPE). SPE was done using commercially available SPE cartridges filled with Oasis HLB material from Waters (Milford, MA, USA). The analytical procedure is described in detail elsewhere (Auvinen et al., 2017).

2.6 Data analysis

Statistical analyses on the pharmaceutical removal efficiencies were performed by using the SPSS Statistics 24 software. Since the data had partly non-normal distribution as observed by using the Shapiro-Wilk test, the data sets were further analyzed by using a non-parametric test (Kruskal-Wallis H test) with Bonferroni post hoc test to define the significance of the differences. Spearman's rank order correlations were run to determine the correlation between removal efficiency and DO concentration. The significance level was set at $p = 0.05$.

2.7 Aquatic risk assessment

The hazard quotients (HQs) were calculated based on the measured environmental concentration (MEC) and predicted no-effect concentration (PNEC) according the following equation:

$$HQ = \frac{MEC}{PNEC}$$

The PNEC was estimated based on chronic toxicity data using an assessment factor of 1000 applied to the lowest EC50 value reported (Vestel et al., 2016) or NOEC values with an assessment factor of 10 (Jin et al., 2012). The variation in the HQs was calculated based on the lowest and the highest MEC in the effluent/creek.

The preliminary risk assessment based on HQs was done using small water organisms and plant species (*Brachionus calyciflorus, Lemna minor, Desmodesmus subspicatus*, *Ceriodaphnia dubia* and *Daphnia magna)* as model organisms. The PNEC-values calculated based on literature data are shown in Table 2.

Table 2 – PNEC values obtained based on literature data. *The factor has been taken into account when defining PNEC value.

Type	Species	*Factor applied	PNEC (µg/L)	Reference
Algae	*D. subspicatus*	1000	74	Cleuvers, 2003
Invertebrate/rotifer	*B. calyciflorus*	10	38	Ferrari et al., 2003
Invertebrate/crustacean	*C. dubia*	10	2.5	Ferrari et al., 2003
Invertebrate/crustacean	*D. magna*	1000	76.3	Ginebreda et al., 2010
Plant	*L. minor*	1000	25.5	Cleuvers, 2003

3 Results

3.1 Water quality based on conventional parameters during full-scale treatment

The full-scale CW performance was monitored during the sampling campaign for COD, NH_4^+ and NO_3^- and on-site measurements pH and DO were measured on one day (Table 3). The high pH in the effluent water is likely to be caused by the tobermorite mineral in the substrate. Due to the oxic conditions in the CW (10.6±0.1 mg/L), the COD and NH_4^+ removal were high (98 % and >98 %, respectively). The denitrification efficiency was limited, likely due to the aeration applied, and hence, approximately 50 % of NH_4^+-N in the influent was discharged as NO_3^--N.

Table 3 – Conventional water quality parameters during full-scale treatment. Average values ± standard deviation (n=5; except for pH and DO in CW n=3 and for pH and DO in influent and effluent n=1). N/A: not analyzed.

	pH	DO (mg/L)	COD (mg/L)	NH_4^+ (mg N/L)	NO_3^- (mg N/L)
Influent	7.5	0.7	486±128	68±7	<5

CW	7.7±0.5	10.6±0.1	N/A	N/A	N/A
Effluent	8.6	11.3	11±1	<2	33±3
Removal efficiency (%)	-	-	98	>98	-

3.2 Dissolved oxygen concentration and pH during the batch experiment

The pH did not change markedly during the batch experiment (Table 4). The DO was much higher in the effluent of the aerated microcosms (7.7±1.0 mg/L) than in the microcosms without aeration (0.9±0.5 mg/L).

Table 4 – DO and pH during the batch experiment. Average values ± standard deviation (n=3)

	pH	DO
Influent	7.5	3.6
HRT2-AIR	6.5±0.0	7.9±0.6
HRT6-AIR	6.4±0.2	7.5±1.1
HRT2-NO-AIR	7.0±0.0	1.2±0.2
HRT6-NO-AIR	7.0±0.1	0.6±0.5

3.3 Occurrence of pharmaceuticals in the influent

The selected pharmaceuticals occurred in the influent at varying levels (Figure 1, Table 5). The lowest average concentration (40±20 ng/L) was measured for DZP and the highest one (50.66±32.74 µg/L) for MFM. TST and VST were not detected in any of the samples. The concentrations fluctuated also greatly from day to day (standard deviation near average concentration) due to daily variations in water consumption for e.g. bathing. In general, the influent pharmaceutical concentrations are so high that they are comparable to concentrations occurring in hospital effluent (Auvinen et al., 2017).

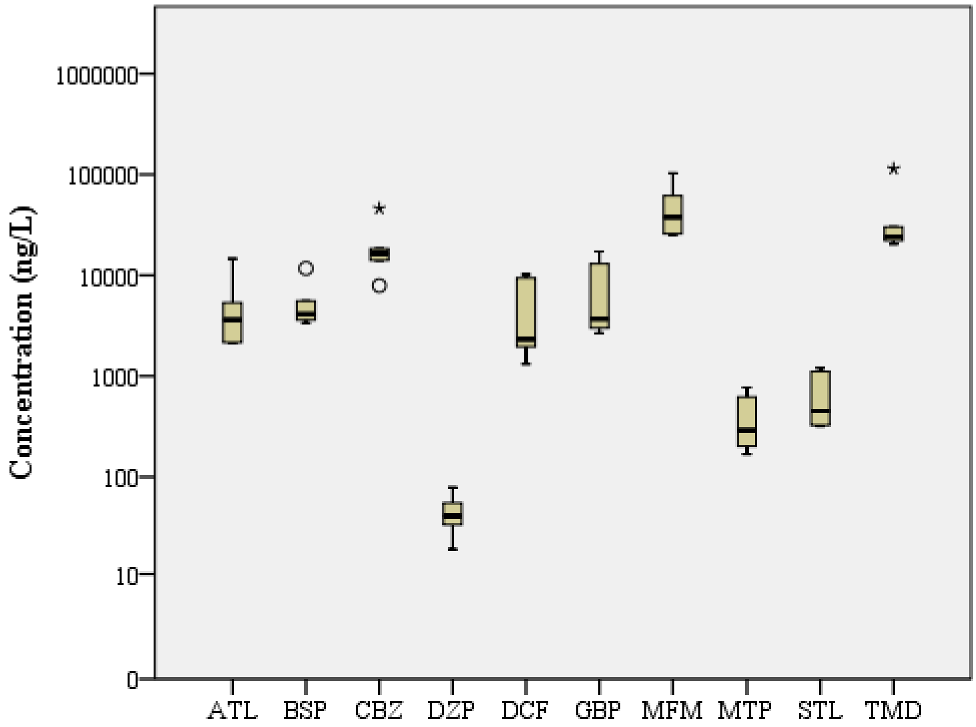

Figure 1 – Box plots on the influent pharmaceutical concentrations of the full-scale CW (n=5). Note the logarithmic scale. The tick marks ○ and * mark the outliers.

3.4 Removal of selected pharmaceuticals during full-scale treatment

Very efficient removal of the selected pharmaceuticals was achieved during the full-scale treatment (in general >90 %) (Table 5). Although MFM and TMD were present in the influent at the highest concentrations, their efficient removal in the CW lowered their concentrations in the effluent to ≤ 30 ng/L. The highest average concentration observed in the effluent was for CBZ (1280±300 ng/L). The average concentrations of ATL, BSP and DCF were below 100 ng/L and the average concentrations of DZP, GBP, MTP and STL were below the detection limit (10 ng/L). In the creek only CBZ and TMD were detected in the two grab samples (1380±520 ng/L and 60±20 ng/L, respectively). On the day when the creek water was sampled, only CBZ and TMD were detected in the effluent.

3.5 Effect of active aeration and hydraulic retention time on the removal efficiency

Aeration improved the removal of GBP significantly (Figure 2). The removal of MFM and STL was improved significantly at HRT 2 d (Figure 2) but at HRT 6 d the removal was statistically equally efficient with or without aeration. The removal efficiencies of GBP, MFM (at HRT 2 d) and STL (at HRT 2 d) correlated also well with the DO concentration in the effluent (r_s=0.8, p<0.05). The concentration of TST was below the detection limit (10 ng/L) in all effluent samples. The variable removal efficiencies observed for VST are likely to be caused by the low influent concentrations (20±10 ng/L) and subsequent difficulties in quantification.

The removal efficiency of CBZ was improved with increasing HRT. The longer HRT improved the removal of DCF only during aeration and oppositely, the longer HRT enhanced the removal of TMD when aeration was not applied.

Table 5 – Removal of selected pharmaceuticals during full-scale treatment

	ATL	BSP	CBZ	DZP	DCF	GBP	MFM	MTP	STL	TMD
Influent (ng/L)	5570±5220	5670±3480	20580±14800	40±20	5040±4370	7910±6740	50660±32740	410±270	680±440	42180±40320
Effluent (ng/L)	90±120	10±10	1280±300	<10±0	50±90	<10±0	<10±0	<10±0	<10±0	30±10
Removal efficiency (%)	98	~100	94	78	99	~100	~100	98	99	~100

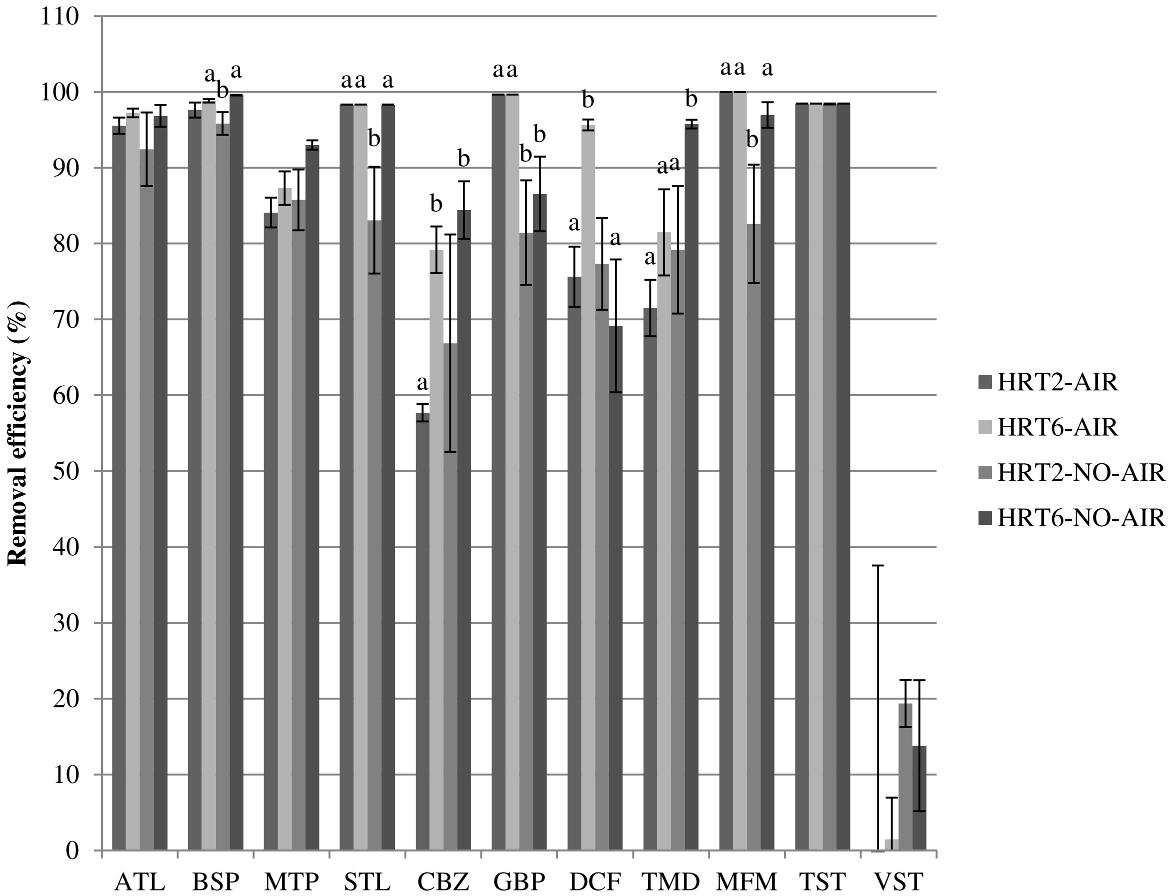

Figure 2 – Dependency of the removal efficiencies of the selected pharmaceuticals on aeration and HRT (average ± standard deviation; n=3 except for HRT2-NO-AIR n=2 due to loss of sample). Statistically significant differences are marked with differing letters a and b.

3.6 Aquatic risk assessment

The HQs are calculated only for CBZ due to the very low concentrations of other pharmaceuticals present in the effluent. The HQs, which was based on minimum and maximum concentrations detected in effluent and the creek, ranged from 0.01 to 0.7; not indicating possible toxicity to the target organisms by CBZ discharge alone.

4 Discussion

In contrast to earlier experiments on beta-blockers (ATL, BSP, MTP, STL) in activated sludge systems (Wick et al., 2009), excellent removal of these compounds was obtained in the current study. Also in earlier studies on CWs, lower removal efficiency of ATL, BSP and STL has been noted, MTP and STL being the more recalcitrant types (11 – 80 %; Conkle et al. (2008). Dordio et al. (2009) studied the removal of ATL in unplanted microcosms filled with LECA granules and concluded that the efficient removal obtained (82 %) over 4 days was primarily caused by adsorption of ATL to the LECA granules because new material (no biofilm) was used in the study. It is thus possible that the combination of biodegradation and adsorption onto the LECA granules enabled the excellent removal efficiency observed in the current study. The full-scale CW is only recently (in late 2015) taken into operation and this has possibly an effect on the adsorption capacity of the LECA (not saturated, biofilm not fully developed). The removal efficiency of STL further depended on aeration and correlated positively with the DO concentration of the effluent. The removal efficiency of STL was however not dependent on the aeration when HRT of 6 d was used instead of 2 d. Anoxic biotransformations are in general slower than oxic ones and hence, a longer HRT is needed to obtain the same treatment efficiency.

GBP has earlier been reported to be quite efficiently removed in CWs (88 %; Chen et al., 2016). Based on the batch experiment, GBP is readily biodegradable in oxic conditions and it can be removed even at a short HRT. In a previous study, where hospital wastewater was treated in an aerated pilot-scale sub-surface flow CW, GBP was only removed by 33 -37 % (Auvinen et al., 2017). It is possible that the high organic loading applied in the aforementioned study restricted the removal of GBP.

Although many studies on CWs report low removal efficiency for DCF (< 50 %; e.g. Matamoros & Bayona, 2006), excellent removal of pharmaceuticals has earlier been observed especially in hybrid systems. Ávila et al. (2010), who studied the removal of DCF in a CW system comprising of two horizontal sub-surface flow CWs in series, showed that DCF was removed by > 97 % at a similar hydraulic loading rate (0.028 m/d) as applied in the current study. Similarly, a removal efficiency of 89 % of DCF was observed in a hybrid CW where vertical sub-surface flow CW is followed by a horizontal sub-surface flow CW and a surface flow CW (Ávila et al., 2015). The reason for the better removal in hybrid systems can be related to the presence of both anoxic and aerobic conditions occurring in these types of CWs, which are likely to be essential for the degradation process of DCF (Ávila et al., 2014). Based on the batch experiment it seems that HRT also plays a role in the removal efficiency of DCF in aerobic conditions. It is possible that the oxic pathway necessary for the degradation is limiting the removal in the microcosms without aeration and hence, the removal is not improved even at longer HRT.

MFM has also earlier been shown to be efficiently removed in CWs (Auvinen et al., 2017). In that study, MFM was removed promptly during aeration but a lag-phase occurred when aeration was not used. Similar behavior was observed in the current study, where the removal efficiency in the non-aerated microcosms was improved with increasing HRT.

Poor removal of TMD has been reported in earlier literature. Auvinen et al. (2017) observed negative removal efficiencies for TMD in a pilot-scale sub-surface CW. Breitholtz et al. (2012) studied full-scale free-surface flow CWs and observed removal efficiencies ranging from negative values to 26 %. They explained the low removal to be partly caused by the sub-zero temperatures and subsequent slow biotransformations. Although Auvinen et al., (2017) did not find a correlation between aeration and removal efficiency for TMD in their study, it is possible that the applied HRT (1 d) was too short to obtain significant removal with or without aeration. In the current study, the increase in HRT (from 2 d to 6 d) increased the removal of TMD when aeration was not applied indicating that the anoxic pathway is preferred but adequate HRT is necessary.

CBZ is generally considered as a recalcitrant component and therefore, its efficient removal in the current study is somewhat surprising. The applied aeration did not decrease its removal significantly, although some studies indicate better removal at low redox conditions (Matamoros et al., 2005). CBZ has also been observed to be removed by adsorption to LECA (Dordio, Estêvão Candeias et al., 2009) similarly to ATL (Dordio, Pinto, et al., 2009). The fact that the removal of CBZ was improved by increasing HRT can be linked to the improved adsorption efficiency and/or be due to better biodegradation during longer contact time. It is also possible that the observed unusually high effluent pH of the full-scale CW affected the adsorption behavior. In earlier experiments pH has been shown to affect the dissociation of the pharmaceutical and its subsequent attachment to soil/sediment by ion exchange (Lorphensri et al., 2006).

Because the CW discharges into a small forest creek where little to no dilution occurs, it was important to assess the effect of pharmaceuticals on the ecotoxicity in this creek. Due to the efficient removal of all targeted pharmaceuticals the initial estimation of the aquatic risk in the forest creek is insignificant for the model organisms. Final conclusions on the risk should only be drawn after further investigations where more pharmaceuticals are targeted and where the degradation products of the pharmaceuticals are taken into account. Also, the differences in water consumption and aeration regime during day and night may have an effect on the discharge and hence, an effect on the potential toxicity of the effluent.

5 Conclusions

The full-scale CW produced a high quality effluent in terms of COD, NH_4^+ and the targeted pharmaceuticals. The removal efficiency of all targeted pharmaceuticals was > 90 %; higher than generally seen in CWs. The excellent removal is expected to be caused by the hybrid design of the CW where oxic and anoxic zones are both present, long HRT (10 d) and the presence of LECA which has been shown adsorb (at least) ATL and CBZ efficiently.

Aeration in the laboratory-scale experiment was shown to increase the removal of only a few pharmaceuticals, namely GBP, MFM and STL. The removal of MFM and STL was equally efficient with and without aeration when the longer HRT (6 d) was applied. TMD was better removed when aeration was not applied at long HRT. DCF showed opposite behavior and its removal improved with increasing HRT as aeration was applied. Due to the overall efficient removal of the targeted pharmaceuticals, the aquatic risk was considered low in a preliminary assessment.

Further research should aim at validating the results obtained during the batch experiment. This could be done when the full-scale CW is in full operation and its HRT is decreased to the design HRT of 3 – 4 d. The adsorption on LECA could decrease with increasing biofilm growth during longer operation time and cause a decrease in the removal efficiencies. However, because of the large specific surface area of the porous LECA, the area occupied by biofilm is larger than in conventional CWs filled with gravel, possibly enhancing the treatment. In future research attention should also be paid to the discharge of pharmaceutical degradation products, such as quanylurea (from MFM) which could be present at high concentrations in the effluent (Scheurer et al., 2012). Although the water quality of the effluent based on common parameters meets the requirements, the removal of NO_3^- could possibly be improved by adjusting the aeration regime of the horizontal stage of the CW. The discharge of NO_3^- is an important issue in Flanders which is categorized as a nitrate sensitive area by the European Union (European Commission, 1991).

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