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Enhanced pharmaceutical removal from water in a three step bio-ozone-bio process

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ABSTRACT

Individual treatment processes like biological treatment or ozonation have their limitations for the removal of pharmaceuticals from secondary clarified effluents with high organic matter concentrations (i.e. 17 mg TOC/L). These limitations can be overcome by combining these two processes for a cost-effective pharmaceutical removal. A three-step biological-ozone-biological (BO_3B) treatment process was therefore designed for the enhanced pharmaceutical removal from wastewater effluent. The first biological step removed 38% of ozone scavenging TOC, thus proportionally reducing the absolute ozone input for the subsequent ozonation. Complementariness between biological and ozone treatment, i.e. targeting different pharmaceuticals, resulted in cost-effective pharmaceutical removal by the overall BO_3B process. At a low ozone dose of 0.2 g O_3/g TOC and an HRT of 1.46 h in the biological reactors, the removal of 8 out of 9 pharmaceuticals exceeded 85%, except for metoprolol (60%). Testing various ozone doses and HRTs revealed that pharmaceuticals were ineffectively removed at 0.1 g O3/g TOC and an HRT of 0.3 h. At HRTs of 0.47 and 1.46 h easily and moderately biodegradable pharmaceuticals such as caffeine, gemfibrozil, ibuprofen, naproxen and sulfamethoxazole were over 95% removed by biological treatment. The biorecalcitrant carbamazepine was completely ozonated at a dose of 0.4 g O_3/g TOC. Ozonation products are likely biodegraded in the last biological reactor as a 17% TOC removal was found. No appreciable acute toxicity towards *D. magna*, *P. subcapitata* and *V. fischeri* was found after exposure to the influents and effluents of the individual BO_3B reactors. The BO_3B process is estimated to increase the yearly wastewater treatment tariff per population equivalent in the Netherlands by less than 10%. Overall, the BO_3B process is a cost-effective treatment process for the removal of pharmaceuticals from secondary clarified effluents.

1. Introduction

Human consumption of pharmaceuticals has increased in the past years and is expected to rise even more due to the growing world population and increased average age (Van Der Aa et al., 2011). After administration pharmaceuticals are excreted and disposed into the sewer (Fent et al., 2006). This results in elevated pharmaceutical concentrations in wastewater, as is illustrated by measurements of numerous studies over the past decades (Mompelat et al., 2009; Sacher et al., 2008). Many pharmaceuticals are persistent in conventional wastewater treatment plants (WWTPs) (Rivera-Utrilla et al., 2013; Verlicchi et al., 2012). Pharmaceutical levels in WWTP effluents jeopardize the aquatic environment (Escher et al., 2011; Fent et al., 2006; Küster et al., 2010; Zhao et al., 2007) and reach drinking water resources (Mompelat et al., 2009). As a result, they end up in the water cycle, and this stresses the need for their removal from WWTP effluents.

Ozone treatment has been studied for pharmaceutical removal (Luo et al., 2014). Ozonation is an effective technique to oxidize pharmaceuticals (Huber et al., 2003). Ozone targets electrophilic compounds that contain double bonds, aromatic structures or amine groups which are often found in the chemical structure of pharmaceuticals (Nakada et al., 2007). Ozonation leads to shorter and more oxidized products which are not further broken down by ozone, but are more susceptible to biodegradation (Snyder et al., 2006). High removal rates can be obtained by ozonation, but process efficiency reduces when other compounds than the pollutants

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of interest are present (Mohapatra et al., 2014). WWTP effluents contain orders of magnitudes more harmless organic matter than residual pharmaceutical concentrations, both being oxidized by ozone, resulting in degradation processes competition for ozone. The biodegradability of recalcitrant compounds typically increases after ozonation (Alvares et al., 2001). However, toxic by-products can be formed during ozonation (Illés et al., 2014), resulting in the need of a subsequent treatment step after ozonation (Rivera-Utrilla et al., 2013). Activated carbon (AC) is a commonly proposed post-ozone treatment step as it effectively removes organic compounds from water through adsorption and does not generate toxic by-products (Knopp et al., 2016). A costly downside of AC is the regeneration or replacement of AC when saturated.

An alternative or addition to physical-chemical techniques is biological treatment, i.e. employing microorganisms to degrade pharmaceuticals and ozonation by-products. In general, biological treatment requires low energy and chemical inputs. However, complex molecules like pharmaceuticals present at low concentrations are challenging to biodegrade (Luo et al., 2014). For many pharmaceuticals insufficient degradation rates result in incomplete removal in biological processes applied at WWTPs (Joss et al., 2006). Moreover, specific micropollutant degrading microorganisms are easily outcompeted by other microorganisms that depend on easily degradable substrates present at higher concentrations (Li et al., 2014). Nonetheless, biological treatment was found suitable to remove toxic by-products that were formed during ozonation, thus lowering the toxicity (de Souza et al., 2010; Li et al., 2015).

Combining biological and ozone treatment can be an alternative set of processes for enhanced pharmaceutical removal. Combinations of biological processes with advanced oxidation processes, including ozonation, are known to have beneficial effects over single process technologies (Scott and Ollis, 1995), and have been suggested as the most promising option to prepare wastewater for reclamation (Gadipelly et al., 2014; Gomes et al., 2017). Effective degradation and mineralisation of the degradation products was achieved in a combined ozone-biological process for the widely prescribed antibiotic tetracycline (Gómez-Pacheco et al., 2011).

In the study presented here, the capacities of biological and ozone treatment processes are combined for pharmaceutical removal in a post-treatment process at the WWTP. A three-step biological-ozone-biological (BO_3B) treatment process is designed for the cost-effective removal of pharmaceuticals. The first biological treatment step aims at organic matter removal, thereby lowering the ozone dose required for oxidation of biorecalcitrant pharmaceuticals in the subsequent ozone treatment. The second biological treatment aims at the removal of potentially toxic by-products formed during ozonation. Applied ozone dose and the hydraulic retention time (HRT) of the bioreactors are the studied key-parameters to adjust the process performance. The objective of this study is therefore to test the influence of the applied ozone dose and HRT on the BO_3B process performance. Both chemical and toxicological parameters are used to assess the BO_3B process efficiency. An optimal combination of biological and ozone treatment is hypothesized to result in a cost-effective BO_3B process to remove pharmaceuticals and the toxicity they impose at minimal energy input.

2. Materials and methods

2.1. Feed solution and inoculum

Secondary clarified effluent was obtained from WWTP Bennekom (Bennekom, the Netherlands) as feed solution for the experimental work. WWTP Bennekom is a conventional activated sludge (CAS) system with different redox conditions for biological nitrogen and phosphate removal and has a capacity to treat 20.000 population equivalents. Two batches of effluent were taken during the experiment. The effluent had an average total organic carbon (TOC) concentration of 17.3 ± 3.3 mg/L and a pH of 7.6 ± 0.2, other effluent characteristics are given in tables S1 and S2 of the Supplementary Information (SI). Inocula from three locations were taken and mixed. The inoculum mixture consisted of MBR sludge from a hospital wastewater treatment facility (Pharmafilter, Reinier de Graafziekenhuis, Delft, the Netherlands), primary and secondary sludge of WWTP Bath (Bath, the Netherlands) treating a mixture of industrial and domestic wastewater, and biomass from the BioGAC polishing step of WWTP Horstermeer (One-Step filter, Nederhorst den Berg, the Netherlands).

2.2. Chemicals

The pharmaceutical stock solution was prepared in HPLC grade methanol and consisted of caffeine, carbamazepine, diclofenac, gemfibrozil, ibuprofen, metoprolol, naproxen, sulfamethoxazole and trimethoprim. Pharmaceuticals were selected based on consumption patterns, occurrence in the environment, physico-chemical properties and whether they can be analysed (De Voogt et al., 2009). To avoid the influence of methanol on the experiments, spikes of the stock solution were evaporated till dryness under a gentle nitrogen stream whereafter secondary clarified effluent was added to obtain the feed solution with a pharmaceutical concentration of approximately 200 μg/L For experimental and analytical reasons the spiking concentration is above the environmental relevant concentrations, as also done in other research (de Wilt et al., 2018; Jewell et al., 2016).

2.3. Experimental setup BO_3B process

The experimental setup, consisting of a biological reactor (BR1), an ozone reactor (O_3R) and a second biological reactor (BR2), were operated in series (Fig. 1). An adaptation period of 5 months was applied for the physical and biological stabilization of the BO_3B process before various process parameters were tested. Thereafter different ozone doses in O_3R, and different HRTs of the bioreactors

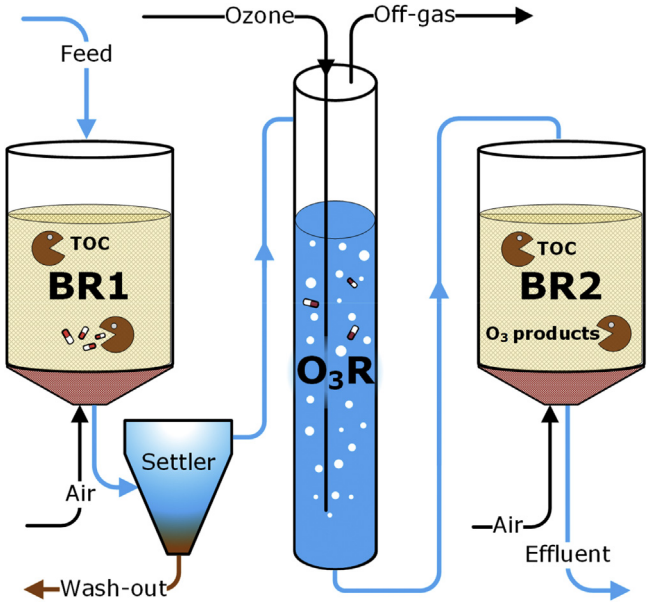

Fig. 1. Schematic of the experimental set-up. BR1 represents the first biological reactor, O_3R the ozone reactor and BR2 the second biological reactor.

were studied during a 5-month experimental period. HRTs of respectively 1.46, 0.47 and 0.3 h were tested by changing the flow rate. Four ozone doses were tested by varying the ozone concentration in the injected gas mixture, 0.1, 0.2, 0.4 and 0.5 g O_3/g TOC. Each time a parameter was changed the BO_3B process was operated for 4–7 days before samples were taken and new settings were applied.

2.3.1. Biological reactors BR1 and BR2

Two identical lab-scale reactors (BR1 and BR2) were operated in continuous mode. Sand with a diameter of 1.2–1.6 mm was obtained from a drinking water treatment plant sandfilter (Vitens, de Meern, the Netherlands) and functioned as the carrier material in the reactors. Sand and the inoculum mixture were mixed and formed the filter bed of the reactors. BR1 and BR2 were inoculated with 2.8 and 3.3 kg of filter bed material, respectively, the effective reactor volume was approximately 1,4 L. The initial dry matter (DM) and organic matter (OM) content of the filter bed was 749.5 g DM/kg and 38.0 g OM/kg, respectively. After wash-out of surplus filter bed material during the adaptation period these contents decreased to 734.9 g DM/kg and 30.8 g OM/kg at 23 weeks of operation. Reactors were fed from the top by continuous dripping. The influent was dispersed evenly over the filter bed surface by a fine porous plate placed on top of the filter bed. Effluent was discharged at the bottom of the reactors. A net with marbles on the bottom retained the filter bed. BR1 was operated with a subsequent 1.5 L settler, preventing washed-out biomass to enter the O_3R. BR1 was fed with the feed solution as described in section 2.1, BR2 with the effluent of the O_3R. In both reactors aerobic conditions were obtained by a counter-current air flow. Fluorescein was used in a conservative tracer pulse test to determine the HRT. Fluorescein concentrations in the effluent were analysed at a frequency of 10 s.

2.3.2. Ozone reactor O3R

A glass-column ozone reactor (inner Ø 3.6 cm, height 216 cm) with an effective liquid volume of 1.6 L operated in a counter-current mode was continuously fed with effluent of BR1. A gaseous ozone/air mixture was injected at the bottom of the column though a diffuser to create fine bubbles. An air flow of 1 L/h was used to generate ozone (Ozon Netech NT-BT 2G) and continuously analysed (Ozone analyser BMT 964) before injection into the reactor. Ozone in the reactor off-gas was measured by a spectrophotometer (KRATOS Spectroflow 783 UV/Vis Absorbance Detector model 9000-7831, path length = 6 mm, $\lambda = 254$) and residual ozone in the liquid effluent by the indigo method (Bader and Hoigné, 1981). In both streams ozone was not detected during the experiment. The applied ozone dose was expressed as g O_3/g TOC.

2.4. Nutrient limitation experiments

Batch experiments were performed to investigate whether the BO_3B process was nutrient limited. Two series of duplicate serum bottles (250 mL) were fed with 150 mL feed solution and inoculated with 3.7 g of BR1 filter bed material. The filter bed material was taken after 25 weeks of reactor operation. One series of batches was amended with a mixture of macro nutrients and trace elements (de Wilt et al., 2018). The aerobic batches were closed with cotton-wool stoppers, incubated at 20 °C on a shaker plate and sampled at 0, 46, 53, 69, 77 and 94 h.

2.5. Analytical methods

Liquid samples for pharmaceutical and TOC analysis were taken from the influents and effluents of the reactors (30 mL) and batch experiments (7.5 mL). Samples for pharmaceutical analysis were directly centrifuged at 3620 g, after which the supernatant was frozen and stored at −10 °C prior to extraction. Pharmaceuticals were extracted by SPE and analysed by LC-DAD according to the procedure described by (de Wilt et al., 2018). Samples for TOC determination were analysed on a Shimadzu TNM-L ROHS TOC-L. Further details on the analytical method are given in the SI (Text S1).

2.6. Toxicity assays

Standardized bioassays on three trophic levels involving *Daphnia magna*, *Pseudokirchneriella subcapitata* and *Vibrio fischeri* were conducted as they have been widely used for determining toxic effects of pharmaceuticals in wastewater (Emmanuel et al., 2005; Escher et al., 2005; Foekema et al., 2012). The aquatic bioassays to test for acute toxicity were conducted on the feed solution and effluents of each BO_3B treatment step operated at an ozone dose of 0.2 g O_3/g TOC and an HRT of 1.46 h. *D. magna* immobilization was determined with the Daphtoxkit F™ magna tests (MicroBioTests Inc.) according to the ISO Standard 6341. *D. magna* immobilization was studied after 48 h exposure. *P. subcapitata* growth inhibition and toxicity towards *V. fischeri* were tested according to the methods described by He et al. (2016) but using $K_2Cr_2O_7$ as positive control to validate the protocol for the *P. subcapitata* assay.

3. Results and discussion

3.1. General performance

Nutrient removal, pH changes and wash-out of the filter bed were investigated to assess the general performance of the biological reactors. Consistent with the prevailing aerobic conditions in BR1, ammonium and nitrite were completely nitrified to nitrate. No further conversion of nitrate was found over O_3R and BR2. Phosphate concentrations were halved over the BO_3B process, which mainly occurred in BR1. No remarkable changes in pH were found as the pH in the effluents of BR1, O_3R and BR2 was 7.7, 8.1 and 7.7, respectively. Because of nutrient and trace element limitation concerns in the feed solution, the effect of nutrient and trace element addition was studied in batch experiments. TOC and pharmaceutical removal was identical between batches with or without the additional nutrients and trace elements (data not shown). This demonstrated that the feed solution (i.e. the secondary clarified effluent) contained sufficient nutrients and trace elements to support biological TOC and pharmaceutical removal. During the adaptation period of 5 months inclination of the filter bed by several centimetres and wash-out of surplus particulate OM was observed. Initially the OM content of BR1 was 50.7 g OM/kg DM, whereas this was reduced to 41.8 g OM/kg DM after the adaptation period. Thereafter, no appreciable wash-out was observed during the 5 month experimental period. No major changes in the TOC and pharmaceutical removal efficiency of BR1 were observed during the adaptation period. This implies that the biomass was well adapted to pharmaceuticals and no noticeable further adaptation occurred. Incidental clogging of major flow paths affecting the reactor hydraulics was observed throughout the entire 10 months of operation. In most cases the clogging was overcome by the reactor itself by water build-up after which new flow paths were formed.

3.2. The BO_3B process

We designed the BO_3B process as a cost-effective alternative to direct ozonation for the removal of pharmaceuticals from WWTP

effluents. As implementation of ozone treatment is often associated with high operational costs, the BO_3B process aims at reducing ozone inputs. A key parameter determining the cost-effectiveness of ozonation is the OM concentration, i.e. TOC or dissolved organic carbon (DOC), as it reacts with ozone and OH radicals and thereby decreases the removal efficiency of target compounds (von Sonntag and von Gunten, 2012). Hence, Lee et al. (2013) found that the removal of pharmaceuticals by ozonation was consistent when the ozone dose was normalized to the OM concentration in the liquid (i.e., g O_3/g DOC) for various WWTP effluents with different origins. Direct ozonation of the feed solution used in this study, containing a moderately high OM concentration of 17.3 ± 3.3 mg TOC/L, would require relatively high absolute ozone doses. Therefore we aimed at TOC removal in the first biological treatment step (BR1). At the most intensively studied HRT of 1.46 h a TOC elimination of 38 ± 4% was observed over BR1. Thus, by applying a TOC normalized ozone dose, the absolute ozone dose in the subsequent O_3R could be proportionally reduced, thereby increasing the cost-effectiveness of O_3R to remove the present pharmaceuticals. In comparison, studies on post-treatment by ozonation of Swiss, Japanese and U.S. wastewaters used feed solutions with lower OM concentrations; 7.0–7.7 (Huber et al., 2005), 2.9–4.2 (Nakada et al., 2007), 4.2–6.0 (Hollender et al., 2009), 2.4–4.8 (Zimmermann et al., 2011) and 4.7–7.1 mg DOC/L (Lee et al., 2013). However, for other wastewaters originating from the U.S., Australia and Germany, moderate to high OM concentrations are found, 6.6–10.3 mg TOC/L (Wert et al., 2009) and 15–26.4 (Lee et al., 2013) and 23.0 mg DOC/L (Ternes et al., 2003). This demonstrates the high variety in wastewater matrices and indicates that the benefit of biological OM removal prior to ozonation is not limited to this study only. The TOC removal over O_3R was 6%. However, the average TOC removal at ozone doses ranging from 0.1 to 0.5 g O_3/g TOC was 13 ± 6% and did not correlated with the ozone dose. This low and unsteady TOC removal during ozonation is also found by others (Bahr et al., 2007). The average TOC removal over BR2 was 17 ± 3%, which is a result of the increased biodegradability after ozonation (Snyder et al., 2006). Literature has shown that ozone oxidises both pharmaceuticals and biorecalcitrant OM (Hollender et al., 2009; Simpson, 2008). Therefore, we postulate that most likely ozonation products originating from both pharmaceuticals and biorecalcitrant OM attributed to the observed TOC removal over BR2.

3.3. Pharmaceutical removal in the BO_3B process

Pharmaceutical removal over the BO_3B process operated at an ozone dose and HRT of respectively 0.2 g O_3/g TOC and 1.46 h is depicted in Fig. 2. In general, pharmaceuticals were effectively removed over the three step process displaying removal efficiencies of >60% to complete removal. Compounds known to be susceptible towards biodegradation such as caffeine, gemfibrozil, ibuprofen and naproxen were well removed (>95%) in the first bioreactor (BR1). Of the moderately biodegradable compounds sulfamethoxazole was efficiently removed (>99%), whereas metoprolol and trimethoprim were only partially removed during biological treatment, respectively 32% and 42%. Unsurprisingly, the recalcitrant compounds carbamazepine and diclofenac showed limited removal in BR1 (<14%). Carbamazepine, diclofenac and trimethoprim were targeted by ozonation in the subsequent O_3R, demonstrating removal efficiencies of 77%, 80% and 49%, respectively. The other compounds were removed by <10%. The small remaining fractions of diclofenac and trimethoprim after ozonation were completely removed during the second biological treatment step (BR2), whereas metoprolol was poorly removed (21%). In addition, a slight increase (4%) in the carbamazepine concentration was found after BR2 which could indicate carbamazepine production. This

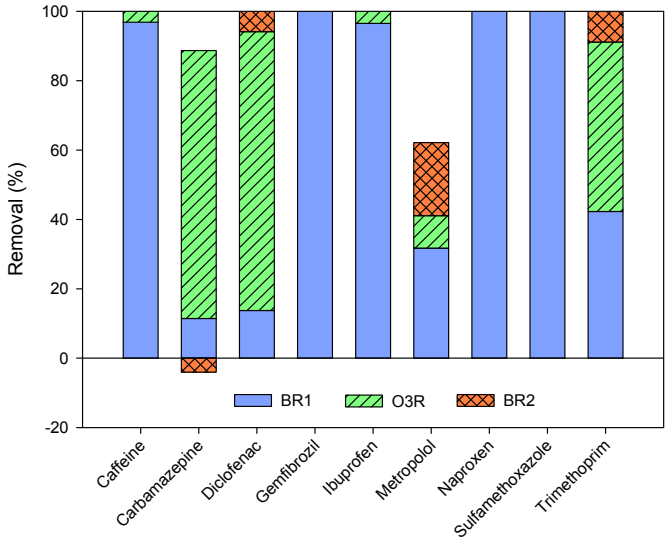

Fig. 2. Pharmaceutical removal over the BO_3B process at an ozone dose of 0.2 g O_3/g TOC and an HRT of 1.46 h.

concentration increase was also observed in other test campaigns of this study. Back-transformation of conjugated metabolites to the parent compound is found for carbamazepine during biological treatment (Radjenović et al., 2009; Vieno et al., 2007). Back-transformation of ozonation products is to our knowledge not described in the literature, thus no firm explanation other than an analytical deviation could be found for this slightly increased carbamazepine concentration.

The pharmaceutical removal patterns of this study were compared to the literature on biological treatment and ozonation. BR1 and BR2 were qualitatively compared to CAS systems, as they were inoculated with biomass derived from CAS systems. Taking into account that there is a high variance in reported removal efficiencies of individual compounds among various studies, removal efficiencies of caffeine, ibuprofen and naproxen in CAS processes are generally above 75% (Deblonde et al., 2011; Luo et al., 2014; Sipma et al., 2010; Verlicchi et al., 2012). This corresponds well with the fate of these pharmaceuticals in BR1 of this work. Similar to the moderate removal of metoprolol and the low removal of carbamazepine and diclofenac in BR1, reported removal efficiencies are typically around 40% and below 35%, respectively. In contrast, gemfibrozil, sulfamethoxazole and trimethoprim are relatively well removed in this work compared to their reported removal efficiencies in the literature of approximately 60%, 50% and 30%, respectively (Deblonde et al., 2011; Luo et al., 2014; Sipma et al., 2010; Verlicchi et al., 2012). Especially the removal of gemfibrozil and sulfamethoxazole in BR1 was high (>99%). Biodegradation is the predominant removal mechanisms in biological treatment (Alvarino et al., 2014). Therefore, the biodegradation rates in our study were higher compared to CAS systems, since we employed a lower HRT, a lower amount of biomass and higher pharmaceutical concentrations. For other biological treatment systems, e.g. sand filters, elevated removal efficiencies for individual pharmaceuticals have been observed compared to CAS systems (Göbel et al., 2007; Reungoat et al., 2011). A better removal in a sand filter than in CAS systems was found by Reungoat et al. (2011) for i.a. trimethoprim. Nevertheless, in that sand filter the removal efficiencies of caffeine, gemfibrozil, metoprolol and sulfamethoxazole were low, ~30%, ~50%, <10%, no removal, respectively, compared to CAS systems and BR1 of this work. Correspondingly, Göbel et al. (2007) found a high (74%) trimethoprim removal during sand filtration, however no

effective elimination was found for sulfamethoxazole and other antibiotics. In sand filtration and activated sludge treatment aerobic conditions were found to correlate positively to pharmaceutical removal (Alvarino et al., 2014; Göbel et al., 2007; Matamoros et al., 2007). Surprisingly, the compounds for which better anaerobic removal is reported such as trimethoprim and sulfamethoxazole were well removed in BR1. Hence, the observed pharmaceutical removal suggested that BR1 was an effective barrier to biodegradable compounds.

The effective oxidation of diclofenac, carbamazepine and trimethoprim during ozone treatment at 0.2 g O_3/g TOC and an HRT of 1.46 h is in good agreement with their high reported ozonation rate constants (k_{O3}) of 6.8, 3 and 2.7×10^5 M^{-1} s^{-1}, respectively (Zimmermann et al., 2011). The limited metoprolol removal during ozonation corresponds well with its moderate k_{O3} of 2×10^3 M^{-1} s^{-1}. The results of this work are in accordance with the work on ozonation of secondary clarified effluent by Hollender et al. (2009). Similarly, this can be attributed to the high ozonation rate constants, the neutral pH and the absence of ozone scavengers like nitrite (<0.05 mg/L). Although the removal efficiency decreased at lower ozone doses for most of their tested compounds, diclofenac, carbamazepine and trimethoprim were well removed (>95%) at 0.40 g O_3/g DOC. At this dose metoprolol removal was approximately 60%, whereas it was effectively removed (>95%) at 1.16 g O_3/g DOC. The observed removal by ozonation is typically a result of the breakdown of pharmaceuticals into smaller oxidized products rather than mineralisation (Snyder et al., 2006). For example, the formation of different quinazoline-containing products during carbamazepine ozonation (McDowell et al., 2005). Therefore considerable amounts of ozonation products will enter BR2.

3.4. Effect of ozone dose

Pharmaceutical removal over the BO_3B process at ozone doses varying from 0.1 to 0.5 g O_3/g TOC at an HRT of 1.46 h is depicted in Fig. 3. The recalcitrance of carbamazepine towards biodegradation and its high ozonation rate constant (>10^5 M^{-1} s^{-1}) allows this compound to be used as indicator for the O_3R performance at different ozone doses. At ozone doses of 0.4 and 0.5 g O_3/g TOC carbamazepine was completely removed over the BOB process, which was mainly contributed to ozonation. Incomplete removal of ~90% and ~75% was found for ozone doses of 0.2 and 0.1 g O_3/g TOC, respectively. These results are in good accordance with Lee et al. (2013), who reported a carbamazepine removal by ozonation of ~55%, 55–90% and >99% at ozone doses of 0.1, 0.25 and 0.5 g O_3/g DOC, respectively. For the other pharmaceuticals a complete removal was obtained even at the lowest ozone doses, except for

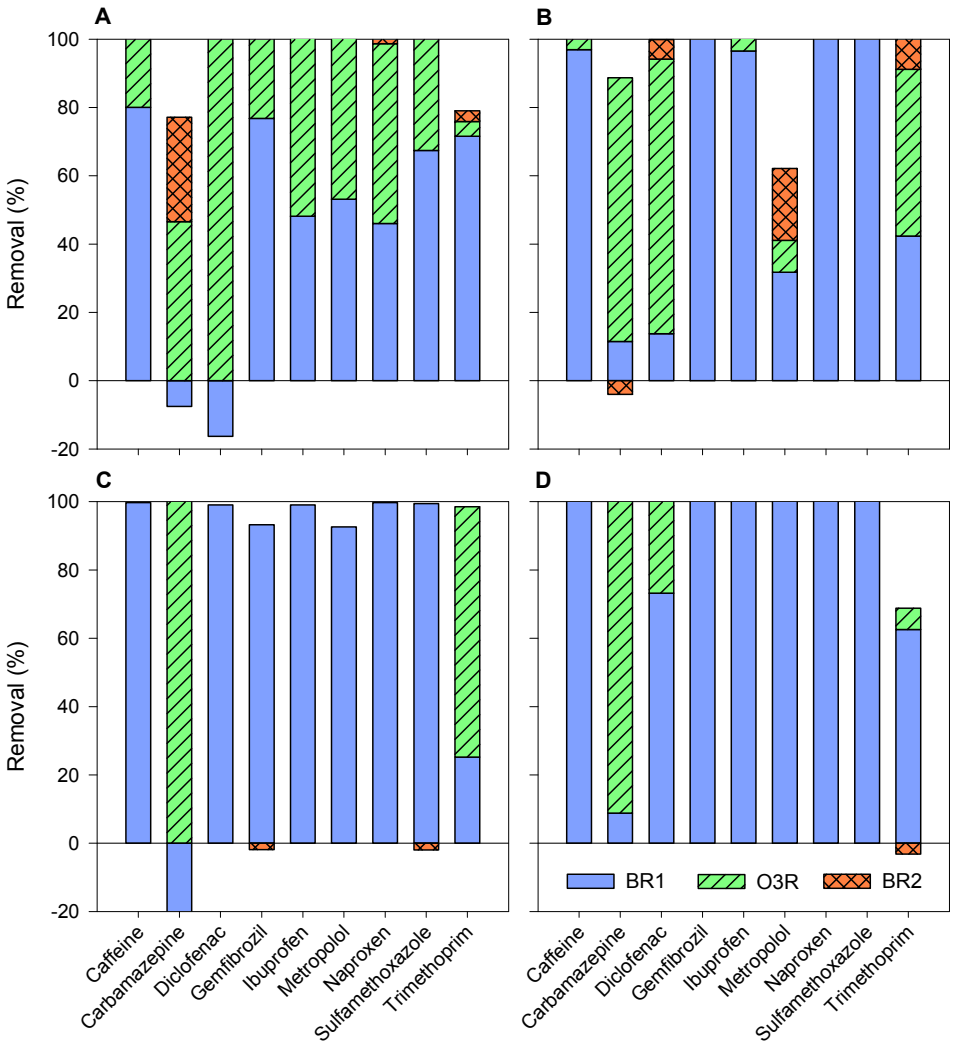

Fig. 3. Pharmaceutical removal over the BO_3B process at an HRT of 1.46 h and ozone doses of (A) 0.1, (B) 0.2, (C) 0.4 and (D) 0.5 g O_3/g TOC.

trimethoprim, even though BR1 performed poorly in that testing campaign. Thus, ozonation effectively contributed to the pharmaceutical removal in the BO_3B process at an ozone dose of 0.1 g O_3/g TOC (e.g. >99% diclofenac and >40% carbamazepine removal). This is better than the <30% pharmaceutical removal at an ozone dose of 0.1 g O_3/g DOC reported by Reungoat et al. (2010) which can possibly be explained by the difference in using DOC or TOC to normalize the ozone dose as the amount of DOC is smaller than the amount of TOC. In general, the removal of diclofenac, metoprolol and trimethoprim was unsteady over BR1 and O_3R in the different test campaigns. Although their biodegradability is typically reported as moderate or low (Deblonde et al., 2011; Luo et al., 2014; Sipma et al., 2010; Verlicchi et al., 2012), these compounds were effectively biodegraded during some of our test campaigns. In all cases, the combination of BR1 and O_3R effectively removed diclofenac, independently of the applied ozone dose. Metoprolol is less susceptible to ozonation and unsteadily removed in O_3R when BR1 showed low removal (i.e. at 0.1 and 0.2 g O_3/g TOC). The low metoprolol removal by ozonation is similar to the findings of Hollender et al. (2009) who found ~90% and ~60% metoprolol removal at 0.62 and 0.40 g O3/g DOC, respectively. Trimethoprim removal did not correlate well with the applied ozone dose. At the intermediate ozone doses it was removed by ozonation, whereas it was not removed at highest and lowest ozone doses. Regarding the reported high trimethoprim ozonation rate constant ($>10^5\ M^{-1}\ s^{-1}$) removal at 0.5 g O_3/g TOC was expected. No other explanation than a possible analytical error at the highest ozone dose could be found to explain this finding. Ozonation of bromide-containing water can lead to the formation of bromate, which is suspected to be carcinogenic to humans (von Gunten and Hoigne, 1994). In our study bromide was not found above the detection limit (5 µg/L) in any of the samples. Hence, no toxic effects of bromate is expected as drinking water standards are 10 µg/L. These findings are in good accordance with the low bromide concentrations detected in drinking water intake of typically <25 µg/L (Von Gunten and Salhi, 2003).

3.5. Effect of HRT

Pharmaceutical removal over the BO_3B process at varying HRTs from 0.3 to 1.46 h at an ozone dose of 0.2 g O_3/g TOC is depicted in Fig. 4. The easily biodegradable pharmaceuticals caffeine, ibuprofen and naproxen were used to assess the influence of HRT on the BO_3B process pharmaceutical removal. At HRTs of 0.47 and 1.46 h the removal of these and most other compounds is highly similar, resulting in an efficient removal. Only metoprolol behaved different as it was better removed at an HRT of 0.47 h. This agrees well with the results of the ozone dose tests (Fig. 3.) in which the biological removal of metoprolol was unsteady over the different test campaigns. Similarly, trimethoprim removal over BR1 also fluctuated. In contrast to metoprolol, trimethoprim was effectively removed in O_3R and BR2. The limited biological removal of caffeine, ibuprofen and naproxen at an HRT of 0.3 h compared to the longer HRTs suggests that 0.3 h is too short for an effective biological treatment. Matamoros et al. (2007) reported a similar trend of decreasing pharmaceutical removal efficiencies at increasing hydraulic loading rates for a sand filter. At a loading rate of 70 mm day^{-1}, corresponding to an HRT of 4–6 h, caffeine, diclofenac, ibuprofen and naproxen were removed at 98, 76, 90 and 80%, respectively. However, at a loading rate of 160 mm day^{-1} the removal efficiencies decreased to approximately 66, 58, 50 and 54%, respectively. Assuming a linear relation for the sand filter between hydraulic loading rate and HRT, whereby these results can be quantitatively compared to those at the HRT of 1.46 h of this study, this suggest that the pharmaceutical removal of BR1 was relatively high. Escolà Casas and Bester (2015) studied and reviewed diclofenac removal in various sand filters (i.e. slow and fast filtration) and found HRT related reaction rate constants k ($\ln\frac{C_{out}}{C_{in}} = k \times HRT$) of 0.004, 0.04, 0.37 and 1.92 h^{-1} at HRTs of 5.7, 9.01, 0.108 and 0.13 h, respectively. The HRT related reaction rate constants of diclofenac in this study are 0.10, 0.96 and 0.59 h^{-1} at HRTs of 1.46, 0.47 and 0.3 h, respectively, and thereby at the higher end compared to the reported rate constants by Escolà Casas and Bester (2015). An HRT based rate constant calculation is a simplification of reality, neglecting heterogeneity aspects of filter beds such as redox, substrate and biomass gradients. However, the high variety among reported rates suggests that the difference between studies (e.g. inoculation of the sand filter, type of wastewater and operational conditions) justifies the comparison of HRT based rate constants. In the literature oxygen or biomass levels are reported to be rate limiting in sand filters (Escolà Casas and Bester, 2015; Matamoros et al., 2007). In this study an overdose of oxygen was supplied and a surplus of well-adapted biomass was present at inoculation resulting in the wash out of biomass. Therefore, it is hypothesized that the contact time between biomass and pharmaceuticals is the rate limiting factor. Even though the pharmaceutical removal over BR1 was limited at

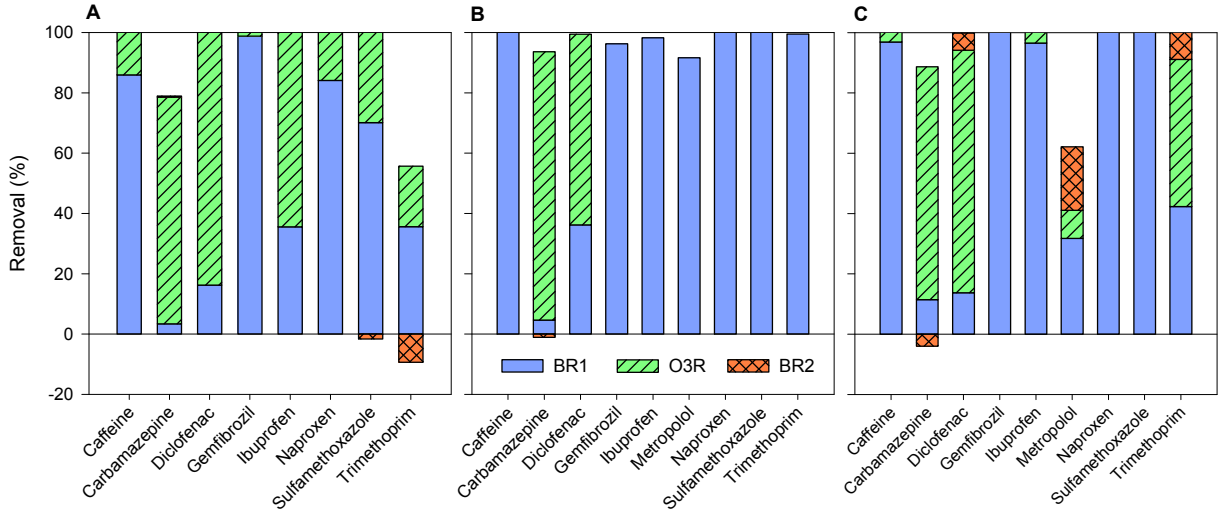

Fig. 4. Pharmaceutical removal over the BO_3B process at an ozone dose of 0.2 g O_3/g TOC and HRTs of 0.3 (A), 0.47 (B) and 1.46 (C) hours.

an HRT of 0.3 h, the removal over the entire BO_3B process was similar at the three HRTs. Remaining fractions of pharmaceuticals were effectively ozonated in O_3R resulting in a complete removal of most pharmaceuticals except for carbamazepine (~80%) and trimethoprim (~50%). TOC removal over BR1 reduced from 38%, to 31%–19% at HRTs of 1.46, 0.47 and 0.3 h, respectively. Similarly, Matamoros et al. (2007) found a decrease in TSS and BOD_5 at decreasing HRTs.

3.6. Lowest ozone dose and lowest HRT

The pharmaceutical removal at an ozone dose of 0.1 g O_3/g TOC and an HRT of 0.3 h is depicted in Fig. 5. A complete pharmaceutical removal is achieved, except for carbamazepine and trimethoprim. The pharmaceutical removal over BR1 in this campaign is higher compared to the HRT of 0.3 h at an ozone dose of 0.2 g O_3/g TOC (Fig. 4). This can be explained by the incidental changes in the outflow regime due to clogging of the BR1 filter bed. Especially at short HRTs this hydraulic behaviour can locally strongly influence the pharmaceutical removal in the reactor. Therefore an HRT longer than 0.3 h is recommended to achieve a continuous and high pharmaceutical removal. At an HRT of 0.3 h the pharmaceutical removal over O_3R was low. The low removal of carbamazepine is in the same range as its low removal over O_3R at an ozone dose of 0.1 g O_3/g TOC at an HRT of 1.46 h (Fig. 3). The ineffective ozonation of pharmaceuticals that are recalcitrant towards biological treatment, such as carbamazepine, indicates that an ozone dose of 0.1 g O_3/g TOC is too low for the effective removal of a broad pallet of pharmaceuticals and therefore higher doses are recommended for full scale implementation.

3.7. Toxicity

The pharmaceutical removal during the campaign in which toxicity was tested is depicted in Fig. S1 of the SI. Although the pharmaceutical concentrations in the BO_3B influent in this study were above environmental relevant concentrations, no significant inhibition was found for *D. magna*, *P. subcapitata* and *V. fischeri* in the toxicity bioassays (Figs. S2-S4 of the SI). The bioassays with *P. subcapitata* and *V. fischeri* demonstrated the highest toxicity, however inhibition did not exceed 25% and showed no significant difference between effluent samples of the individual steps of the BO_3B process. This is contrary to the increased toxicity towards *D. magna*, *P. subcapitata* and *V. fischeri* found after ozonation of other pharmaceuticals such as ketoprofen (Illés et al., 2014). However, this can be explained by the high concentrations (>mg/L) applied in that study compared to this study. Kaiser et al. (2014) found that transformation products after biological treatment of carbamazepine can be more toxic for *V. fischeri* than carbamazepine. As carbamazepine persisted in BR1 it is unlikely that many transformation products were formed resulting in low observed toxicities in this study. The three applied bioassays test for acute toxicity towards the test organisms. This limits an in-depth toxicological evaluation as chronic toxicity such as genotoxic effects are not studied in these bioassays. For instance, bio-transformation products of carbamazepine were found to have a higher genotoxicity potential than carbamazepine, however these products were effectively removed during ozonation (Brezina et al., 2017). Although the low observed acute toxic effects hampered the evaluation of individual BO_3B process steps in toxicity reduction, the combination of ozonation with a subsequent biological treatment (i.e. sand filtration) was found to effectively reduce toxicity (Stalter et al., 2010). Nevertheless, further ecotoxicological investigations are recommended as pharmaceuticals and their transformation products formed during biological and ozone treatment can pose diverse ecotoxicological risks (Luo et al., 2014).

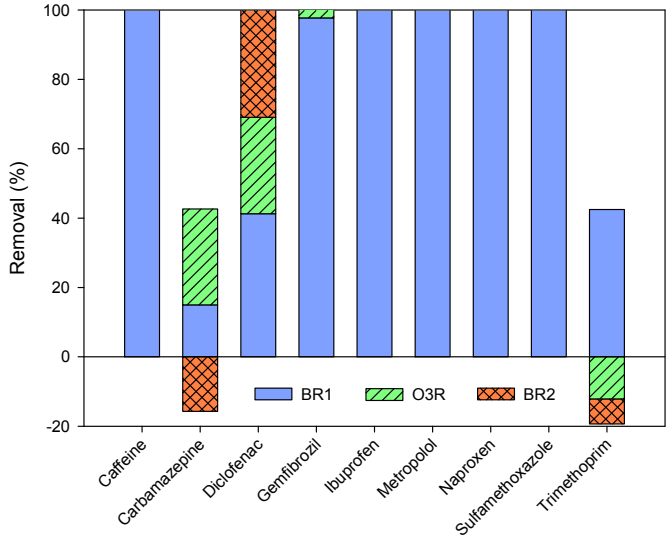

Fig. 5. Pharmaceutical removal over the BO_3B process at an ozone dose of 0.1 g O_3/g TOC and an HRT of 0.3 h.

3.8. Costs and energy demand

Production costs of ozone represent 20–40% of the total costs for large scale ozonation installations and range, depending on the energy price and the system requirements, between 1.4 and 2.5 €/kg O_3 (Xylem Water Solutions Netherlands B.V. 2018). The BO_3B process operated at an ozone dose of 0.2 g O_3/g TOC and HRT of 1.46 h effectively removed pharmaceuticals in this study. Moreover, 38% TOC removal was achieved during biological treatment prior to ozonation (i.e. BR1). Therefore the costs for ozone production in the BO_3B process are <0.005 €/m^3 considering a secondary clarified effluent with an average TOC concentration of 17.3 mg/L. Thereby the total costs for ozonation (investment and operational costs) are estimated to be < 0.03 €/m^3. The investment and operational costs for biological pre- and post-treatment (i.e. BR1 and BR2, respectively) are hypothesized to be smaller or equal to the costs for ozonation as the operational costs of sand filters is known to be low. The total costs for additional treatment by the BO_3B process are therefore estimated to be < 0.06 €/m^3. These costs are on the lower end of the costs estimated by Joss et al. (2008), who reported the additional investment and operational costs for ozonation and post-filtration to be 0.05–0.15 €/m^3 depending on the WWTP size and DOC concentration. According to the Dutch Water Authorities, the investment and operational costs for conventional wastewater treatment (excluding sewer transport costs) in the Netherlands are 0.45 €/m^3 (Unie van Waterschappen, 2015). Thus, the additional costs for BO_3B treatment are less than 15% of the total treatment costs. In addition, the yearly average wastewater treatment tariff is €55.69 per population equivalent (Unie van Waterschappen, 2015). Based on the <0.06 €/m^3 for BO_3B treatment the yearly additional costs are estimated to be < 5 € per population equivalent, which corresponds to a less than 10% tariff increase. The energy requirements for ozone production are approximately 15 and 35 kWh/kg O_3 when manufactured from oxygen and air, respectively (Fridman, 2008). Hence, the energy demand is about 0.03–0.07 kWh/m^3. Together with the energy demand of pumps and other equipment the total demand is estimated to be 0.1–0.15 kWh/m^3, which corresponds well with literature estimations of 0.1–0.3 kWh/m^3 (Joss et al., 2008). The energy demand for

conventional wastewater treatment in the Netherlands is about 0.39 kWh/m^3 of which 0.17 kWh/m^3 is needed for aeration (Unie van Waterschappen, 2015). Post-treatment by a BO_3B process would therefore increase the energy consumption by approximately 25–38%. Overall, this study demonstrates that even for secondary clarified effluents with high TOC levels ozonation can be a cost-effective treatment process when combined with biological pre- and post-treatment steps, i.e. the BO_3B process. Therefore, BO_3B treatment is found an effective process for the reduction of emission of potentially harmful compounds into the aquatic environment.

4. Conclusions

The combination of biological treatment and ozonation in the designed three-step BO_3B process is found an effective treatment process for the removal of pharmaceuticals from secondary clarified effluent with high TOC concentrations. Ozonation and biological treatment are complementary processes targeting different pharmaceuticals. Pharmaceutical removal over the BO_3B process exceeded 85%, except for metoprolol (60%), at a low ozone dose of 0.2 g O_3/g TOC and an HRT of 1.46 h in the biological reactors. Carbamazepine that persisted during biological treatment was completely ozonated at increased ozone doses of 0.4 g O_3/g TOC, whereas at a dose of 0.1 g O_3/g TOC it showed low removal (<40%). Easily and moderately biodegradable pharmaceuticals such as caffeine, gemfibrozil, ibuprofen, naproxen and sulfamethoxazole were >95% removed by biological treatment at HRTs of 0.47 and 1.46 h. At the lowest tested HRT of 0.3 h these pharmaceuticals were incompletely removed (35%–95%). The input of absolute amounts of ozone was effectively reduced by the elimination of TOC over the first biological step, i.e. BR1. At HRTs of 1.46, 0.47 and 0.3 h a decrease in TOC concentration was found over BR1of respectively 38%, 31% and 19%, resulting in a proportional reduction of ozone in the subsequent ozone reactor, i.e. O_3R. No appreciable acute toxicity towards *D. magna*, *P. subcapitata* and *V. fischeri* was found after exposure to the influents and effluents of the individual BO_3B reactors. However, further ecotoxicological investigations are suggested. The secondary clarified effluent contains sufficient nutrients and trace elements to support biological pharmaceutical removal. Costs associated to BO_3B treatment are estimated to increase the current treatment costs for conventional wastewater treatment by 15%. The yearly tariff per population equivalent for wastewater treatment in a country like the Netherlands is estimated to increase by less than 10%. The energy demand for wastewater treatment is expected to increase by 25–38%. Overall, the BO_3B process is a cost-effective treatment process for the removal of pharmaceuticals from secondary clarified effluents.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.watres.2018.03.028.

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