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Improved biodegradation of pharmaceuticals after mild photocatalytic pretreatment

Arnoud de Wilt [1], Maricor J. Arlos[2], Mark R. Servos[2], Huub H. M. Rijnaarts[1], Alette A. M. Langenhoff [1] & Wayne J. Parker[3]

[1]Sub-department of Environmental Technology, Wageningen University & Research, Wageningen, The Netherlands; [2]Department of Biology, University of Waterloo, Waterloo, ON, Canada; and [3]Department of Civil and Environmental Engineering, University of Waterloo, Waterloo, ON Canada

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Correspondence
Alette A. M. Langenhoff, Sub-department of Environmental Technology, Wageningen University & Research, 6700 AA, Wageningen, The Netherlands.
Email: alette.langenhoff@wur.nl

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Abstract

The combination of photocatalysis and biodegradation was investigated for the removal of nine selected pharmaceuticals as a means to reduce loadings into the environment. The combined process, consisting of a resource-efficient mild photocatalysis and a subsequent biological treatment, was compared to single processes of intensive photocatalysis and biological treatment. The UV-TiO_2 based photocatalysis effectively removed atorvastatin, atenolol and fluoxetine (>80%). Biological treatment after mild photocatalytic pretreatment removed diclofenac effectively (>99%), while it persisted during the single biological treatment (<50%). Moreover, the biodegradation of atorvastatin, caffeine, gemfibrozil and ibuprofen was enhanced after mild photocatalytic pretreatment compared to biological treatment alone. The enhanced biodegradation of these pharmaceuticals appeared to be triggered by the biodegradation of photocatalytic products. Mild photocatalysis followed by biological treatment is an effective and resource-efficient combination for pharmaceutical removal that could substantially reduce the loading of pharmaceuticals into the environment.

Introduction

The occurrence of pharmaceuticals in the aquatic environment has become a worldwide environmental concern due to observed impacts that include the disruption of endocrine systems, feminisation of fish and development of antimicrobial resistant organisms (Schwarzenbach *et al*., 2006). After being administered to the patient, pharmaceuticals largely end up in excreta and are transported via sewage to municipal wastewater treatment plants (WWTPs). WWTPs are commonly designed for cost-effective removal of bulk organic matter, nitrogen and phosphorus and typically make use of biological treatment processes. There are limitations regarding the removal of pharmaceuticals in biological treatment processes currently employed at WWTPs as pharmaceuticals are often incompletely removed (Joss *et al*., 2006; Verlicchi *et al*., 2012). Advanced oxidation processes (AOPs) making use of photocatalysis, ozone, or hydrogen peroxide (the latter often as Fenton's Reagent that includes ferrous sulphite as a catalyst), can effectively eliminate pharmaceuticals (Luo *et al*., 2014). The main advantage of AOPs is the complete oxidation of organic contaminants in a wide variety of applications (Klavarioti *et al*., 2009). However, AOPs have disadvantages over biological processes. AOPs require either continuous energy and/or chemical inputs that are significantly higher than those required for biological processes. Toxic by-products can be formed during AOP treatment which can be more toxic than the parent compounds (Illés *et al*., 2014). Furthermore, the various AOP reaction mechanisms are mostly nonspecific, targeting not only the compounds of concern but also other compounds present in the matrix thereby reducing the elimination efficiency of AOPs for target compounds such as pharmaceuticals (Mohapatra *et al*., 2014).

Using the strengths of AOP and biodegradation in a combined technology could possibly result in better overall pharmaceutical removal. Scott and Ollis (1995) concluded in their review of technologies for the removal of organic contaminants in water that two-step treatment technologies combining chemical and biological processes can have advantages over single processes. The benefits of employing a combination of processes can be obtained for wastewaters containing: (1) recalcitrant compounds; (2) biodegradable wastes with small amounts of recalcitrant compounds; (3) inhibitory compounds; and (4) intermediate dead-end products (Scott and Ollis, 1995).

In the field of water and soil contamination, this principle has been studied and applied to demonstrate the advantages of combined processes for the removal of various contaminants (Fakhru'l-Razi *et al*., 2009; Yeung and Gu, 2011; Huang *et al*., 2012; Ye *et al*., 2012). A sequential

combination of photocatalytic and biological treatment processes doubled 2,4,6-trinitrotoluene (TNT) mineralisation as compared to biological treatment alone, whereas no TNT mineralisation was observed for the single photocatalytic process (Hess *et al*., 1998). Furthermore, the combined treatment resulted in more soluble and polar transformation products when compared to the single processes. For quinoline removal, the maximum specific growth rate increased by 15% and the inhibition constant doubled when changing from biodegradation only to sequential coupled photocatalysis and biodegradation (Yan *et al*., 2013). Photocatalytic pretreatment that reduced COD by 8–10%, enhanced the subsequent biodegradation of the dye intermediate H-acid (Mohanty *et al*., 2005). Chun and Yizhong (1999) demonstrated the advantage of combining photocatalysis with biodegradation for wastewater containing nonbiodegradable azo dyes. The biodegradability of the wastewater indicated by the BOD_5/COD ratio was enhanced from nil to 0.75, after a 20- to 30-minute photocatalytic oxidation. In soil remediation, combining chemical oxidation using Fenton's reagent with biodegradation, removed diesel more effectively than applying single processes (Sutton *et al*., 2014).

Specifically for micropollutant removal, the combination of chemical and biological removal has received less attention. Positive effects on the biodegradation of the widely applied antibiotic tetracycline were found after pretreatment by ozonation (Gómez-Pacheco *et al*., 2011) and photocatalysis (Xiong *et al*., 2017). In the study of Gómez-Pacheco *et al*. (2011) tetracycline in the influent inactivated the microbial population of biological waste water treatment, whereas AOP preoxidation resulted in 100% mineralisable TOC and stable biological treatment. Biodegradation and mineralisation of the broad-spectrum antibiotic sulfadiazine could be accelerated by 35 and 71%, respectively, when intimately coupled with photocatalysis (Pan *et al*., 2014). Also for the pesticide 2,4,6-trichlorophenol, a faster removal was found for sequentially and intimately coupled photocatalysis and biodegradation compared to the single processes (Wang *et al*., 2015). Complete degradation and detoxification of the herbicide atrazine were obtained by photocatalytic pretreatment followed by biodegradation. The single photocatalytic treatment resulted in complete atrazine removal but inefficiently mineralized and detoxified the transformation products (Chan *et al*., 2004).

The main aim of the current study was to gain insight into the influence of AOP pretreatment on the subsequent biodegradation of pharmaceutical compounds, as there are only a few reports describing this in literature. Because AOP processes are generally energy and/or chemically intensive, we developed a mild photocatalysis method requiring low energy input. The removal of nine commonly detected pharmaceuticals in wastewater was studied by combining this mild photocatalysis method and biodegradation in batch experiments. The pharmaceuticals were selected as representative compounds for various classes of pharmaceuticals and were applied in a mixture in the treated water. The combination of mild photocatalysis followed by biodegradation was tested and the pharmaceutical removal was compared to removal in the single processes of intensive photocatalysis and biodegradation.

Materials and methods

Chemicals

A pharmaceutical stock solution that contained a mixture of nine compounds (Table 1): atenolol, atorvastatin, caffeine, carbamazepine, diclofenac, fluoxetine, gemfibrozil, ibuprofen and naproxen was employed. The stock solution (2 g/L of each pharmaceutical) was prepared in HPLC grade methanol and stored at –20°C. Powdered TiO_2, commercially available as P25 (99.7% purity, Sigma-Aldrich, Canada) was used for the photocatalytic experiments. The photocatalytic properties of this material have been described previously (Roy *et al*., 2018). Suppliers for all other reagents and chemicals used in this study are described in detail elsewhere (Arlos *et al*., 2015).

Experimental set-up

Figure 1 displays the three experimental protocols that were studied: (1) intensive photocatalytic treatment (IP); (2) single process biological treatment (B); and (3) mild photocatalytic pretreatment followed by biological treatment (MP + B).

Photocatalytic experiments

Batch photocatalytic experiments were performed with the set-up described by Arlos *et al*. (2016). In short, the set-up consisted of a multiposition stir plate each with a six-cm collimated UV-LED (θ_{beam} = 4 cm, λ = 365 nm, power output = 1.67 mW). The irradiance at the water level was 0.390 mW/cm^2. Beakers (650 mL) wrapped in aluminium foil containing 600 mL of ultrapure water were amended with TiO_2 suspension to a final concentration of 0.5 g/L, similar used in comparable experiments (Tong *et al*., 2012). The batches were spiked with 150 µL of the pharmaceutical stock solution to obtain concentrations of 500 µg/L (6 mM methanol). For experimental and analytical reasons the spiking concentration is above the environmental relevant concentrations, as was also performed in other research (Jewell *et al*., 2016; de Wilt *et al*., 2018). Prior to light exposure, the batches were equilibrated for 30 min in the dark. For intensive

Table 1 Chemical structure and therapeutic function of the studied pharmaceuticals

Atenolol Beta-blocker	**Atorvastatin** Lipid regulator	**Caffeine** Stimulant
Carbamazepine Anti-epileptic	**Diclofenac** Anti-inflammatory	**Fluoxetine** Anti-depressant
Gemfibrozil Lipid Regulator	**Ibuprofen** Anti-inflammatory	**Naproxen** Anti-inflammatory

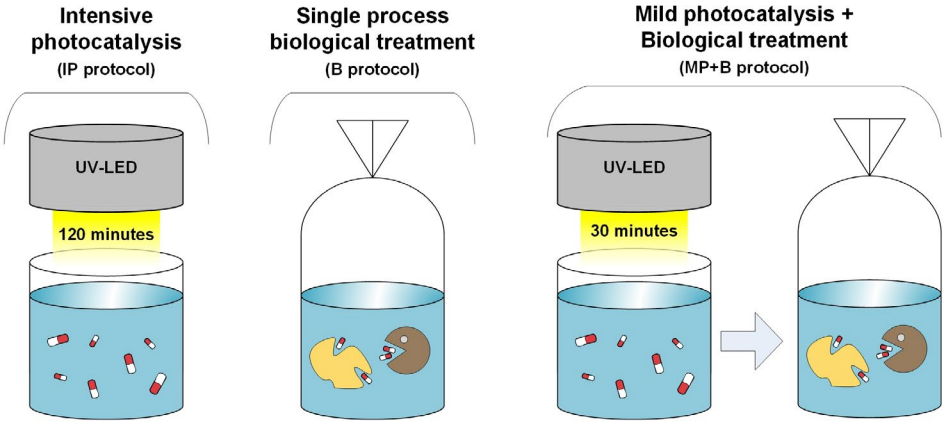

Fig. 1. Experimental protocols, (1) intensive photocatalytic treatment (IP), (2) single process biological treatment (B) and (3) mild photocatalytic pretreatment followed by biological treatment (MP + B).

photocatalytic experiments 2 mL of samples were taken directly before the lamps were activated and after 15, 30, 45, 60 and 120 min of illumination. In the separately performed mild photocatalytic experiments, samples were taken directly before illumination and after 15 and 30 min of illumination. To separate the water from the TiO_2, the contents of the beakers after 30 min of light exposure in the mild photocatalytic experiments were centrifuged (3500 rpm, 30 min) and filtered (0.45 µm, Supor-450 membrane filter, Pall Life Sciences, Canada). Thereafter, samples for pharmaceutical analysis were taken and this solution was further used in biological experiments. Dark control experiments without illumination were performed for 120 min to assess nonphotocatalytic pharmaceutical removal.

Biological experiments

Aerobic batch experiments were performed in 200 mL amber flasks, closed with cotton-wool stoppers. Three types of batches were prepared: 1) B protocol; 2) MP + B protocol; and 3) abiotic controls. The MP + B batches

were filled with the solution obtained after the mild photocatalytic experiments. The B and abiotic control batches were filled with demineralized water and spiked with 50 µL of pharmaceutical stock solution to obtain initial concentrations of 500 µg/L. All batches were amended with macro-nutrients, trace elements and pH buffer as described by de Wilt *et al*. (2018). Biomass obtained from four locations around Waterloo, Canada (secondary sludge of Elmira WWTP, river sediment of Heidelberg creek, sand of polishing filter of Galt WWTP and Rotating Biological Contactor sludge of WWTP Foxboro) were mixed based on equal VSS ratios and used to inoculate batches until final concentrations of 49.4 g TSS/L and 5.5 g VSS/L. TSS and VSS were determined according to standard methods (American Public Health *et al*., 1998). Batch experiments were performed in triplicate and incubated at room temperature on a shaker plate. Abiotic controls were amended with 0.5 mM NaN_3 to suppress biological activity, closed with a rubber stopper and incubated for one day. It has been reported for batch experiments that the sorption equilibrium for pharmaceuticals was reached 30 min and 12 hours after spiking of 2.4 g VSS/L of secondary sludge (Ternes *et al*., 2004) and after spiking of 250 g soil/L (Martínez-Hernández *et al*., 2016), respectively. Therefore, abiotic batches were incubated for 1 day to assess the sorption behaviour of pharmaceuticals in this study. Samples (5 mL) of the biotic batches were taken on day 0, 1, 3, 7, 14 and 21, directly frozen and stored at –10°C prior to pharmaceutical analysis. Replicate batches were tested for outliers according to ANCOVA statistical model (significance = 0.05) in which we considered time as a covariate. The testing criterion was the difference in pharmaceutical removal (C/C_0) between replicates. The same ANCOVA model was used to test for differences in removal (C/C_0) over time in the biological experiments of the B and MP + B protocols.

Pharmaceutical analysis

Samples from the AOP experiments were directly centrifuged for 45 min at 3500 rpm to separate the liquid phase and TiO_2. After thawing the samples from the biological experiments were centrifuged for 10 min at 3500 rpm. Thereafter, the supernatants were extracted by solid-phase extraction (SPE) and analysed by LC-MS/MS according to the procedure described by Arlos *et al*. (2016), but only 2 mL of sample instead of 4 mL was used for SPE in this study.

Results and discussion

This study examined the removal of a selection of pharmaceuticals through photocatalysis and biodegradation separately and in an integrated photocatalysis-biodegradation system. The rate and extent of removal of each compound in each process was examined and, where appropriate, the mechanisms of removal were elucidated.

Photocatalysis

Intensive photocatalysis (IP protocol)

The removal of the target compounds through intensive photocatalysis that involved extended periods of illumination was examined to establish a baseline of performance for this technology. Initially, the results of the illuminated batches and dark controls were compared to assess the contribution of photocatalysis to pharmaceutical removal. The results of the control experiments revealed that non-photocatalytic removal processes such as adsorption to TiO_2 contributed little (<25%) to the pharmaceutical removal in our AOP experiments, as has been reported in the literature (Arlos *et al*., 2016). In contrast, significant removals (>80%) of atorvastatin, atenolol and fluoxetine were observed in intensive photocatalytic (IP) experiments after 2 hours of illumination (Fig. 2a). For these compounds, it was concluded that photo-catalysis was a significant removal mechanism. The other tested compounds were less susceptible to photocatalysis and their observed removal after 2 hours of illumination was less than 40%.

Photocatalytic degradation of organic compounds has been described by Langmuir-Hinshelwood kinetics (Sánchez *et al*., 1997). A simplified first-order kinectic model can be used for low compound concentrations (<mg/L) and denoted as $\ln(\frac{C}{C_0}) = -kt$ in which C/C_0 (µg/L/µg/L) is the actual pharmaceutical concentration divided by the initial pharmaceutical concentration, k (min^{-1}) is the apparent first-order reaction rate constant and t (min) is time. The pharmaceutical concentrations in this study were in the µg/L range, therefore, the dilute system condition was believed to apply and first-order reaction rate constants were calculated according to the abovementioned equation. For atorvastatin, atenolol and fluoxetine the calculated rate constants ($R^2 > 0.99$) were 41.5×10^{-3}, 29.0×10^{-3} and 15.4×10^{-3} min^{-1}, respectively. These results agreed with the findings of the experimental work and the literature reviewed by Arlos *et al*. (2016) on the photocatalytic degradation of pharmaceuticals. Their reported rate constants for atorvastatin, atenolol and fluoxetine ranged between 13.4–68.8 × 10^{-3}, 7.4–14.5 × 10^{-3} and 8.4–40.8 × 10^{-3} min^{-1}, respectively. Thus, atenolol was removed at a higher rate in our study. The rate constants of the other pharmaceuticals did not exceed 4.8×10^{-3} min^{-1} ($R^2 < 0.88$). Similarly, Arlos *et al*. (2016) found low rate constants (<8.7×10^{-3} min^{-1}) to no degradation for

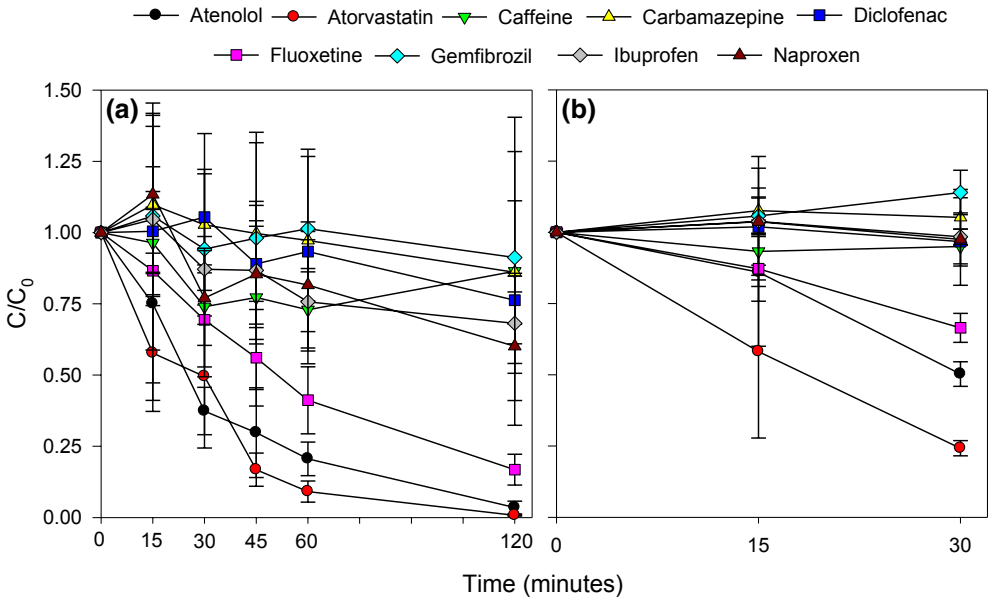

Fig. 2. Pharmaceutical removal (C/C_0) during photocatalysis of (a) IP protocol and (b) MP + B protocol. Error bars represent standard deviations between triplicates.

naproxen, ibuprofen and carbamazepine, which can be explained by the presence of methanol that even at low concentrations acts as a hydroxyl radical scavenger.

Mild photocatalysis (MP + B protocol)

The performance of mild photocatalytic treatment was assessed in the first stage of the experiment that tested the integrated mild photocatalysis-biodegradation protocol (MP + B). For the MP + B photocatalytic experiments, the illumination time was chosen based on the outcomes of the IP experiments. The illumination time was selected to provide (1) substantial removal of photocatalytic degradable compounds and (2) sufficient remaining compound concentrations to study biodegradation. Therefore, 30 min illumination was selected for the resource-efficient mild photocatalytic experiments as substantial removal (>50%) was achieved at 75% less energy input than the IP tests. Further, the remaining pharmaceutical concentrations were sufficiently high after 30 min of illumination to allow for the assessment of pharmaceutical removal in the subsequent biological experiments. During the mild photocatalysis substantial removals of atorvastatin (75%), atenolol (50%) and fluoxetine (35%) were observed, while the other pharmaceuticals showed low removal (<5%) (Fig. 2b). The photocatalysis results in the MP + B test were in good accordance with the findings of the IP protocol, as the same compounds were removed (atorvastatin, atenolol and fluoxetine) at similar removal rates (<20% difference between the two tests). The mild photocatalysis tests confirmed the photocatalytic mechanism for the removal of selected pharmaceuticals and the appropriateness of the first-order kinetic model for describing the rate of removal. This indicates the robustness of the photocatalysis process for pharmaceutical removal.

Biological treatment

Single process biological treatment (B protocol)

The B protocol was employed to assess the biodegradability of the target pharmaceuticals (Fig. 3). The ANCOVA testing revealed a significant outlier in the B protocol results when the pharmaceutical removals (C/C_0) in the individual replicates were compared and therefore the outlier bottle results were not included in the trend analysis. From Fig. 3, it can be seen that the removal rates observed in the biodegradation protocol were consistent with first-order kinetics that have been previously reported for low-concentration pharmaceutical in biological processes (Joss *et al*., 2006). Further, significant removal (>80%) of seven pharmaceuticals was observed in the first 21 days of the B protocol testing. Diclofenac and carbamazepine were recalcitrant towards biodegradation as after 21 days they were reduced by less than 50% and 40%, respectively. These results were in agreement with previous reports of their fate during biological treatment (Verlicchi *et al*., 2012; Alvarino *et al*., 2014). The removal of individual pharmaceuticals through sorption and biodegradation mechanisms was assessed by comparing their responses in the abiotic (Table 2) and biotic (Fig. 3) batches.

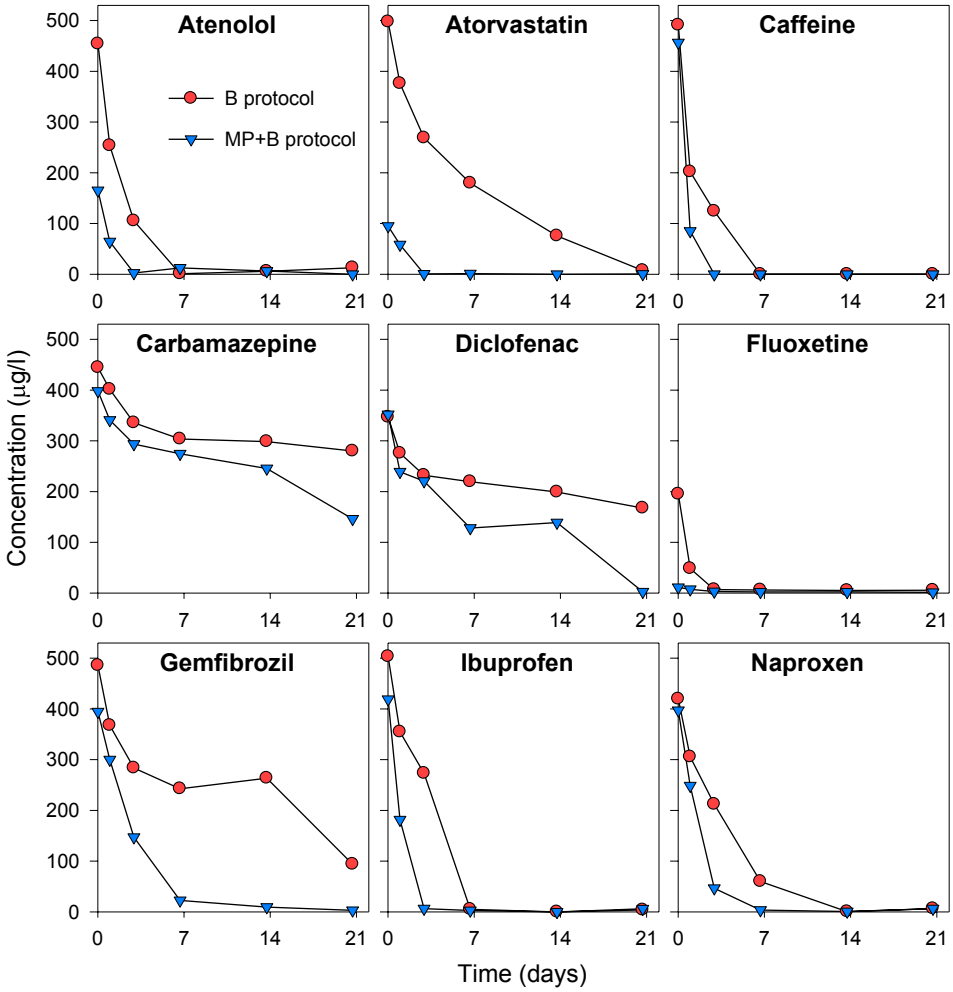

Fig. 3. Pharmaceutical concentrations in biological treatment experiments, B protocol (red spheres) and MP + B protocol (blue triangles).

Table 2 Pharmaceutical removal (C/C_0) after one day in the abiotic experiments

Pharmaceutical	Atenolol	Atorvastatin	Caffeine	Carbamazepine	Diclofenac	Fluoxetine	Gemfibrozil	Ibuprofen	Naproxen
Abiotic removal	53%	31%	18%	9%	25%	84%	24%	23%	34%

This analysis revealed that the concentrations of caffeine, ibuprofen, naproxen and atorvastatin decreased by only 18–34% in the abiotic batches (Table 2) but were reduced significantly (>99%) within 7, 7, 14 and 21 days, respectively. Further, substantial gemfibrozil removal (80%) was found after 21 days. These findings were consistent with those reported for biological systems by Luo *et al*. (2014) and Golovko *et al*. (2014). It was concluded that biodegradation was the prevalent removal mechanism for caffeine, ibuprofen, gemfibrozil, atorvastatin and naproxen.

Fluoxetine concentrations decreased rapidly in both the biotic and abiotic batches (Table 2, Fig. 3), indicating that sorption was the main removal mechanism. In samples taken on day 0, the fluoxetine concentrations were found to be less than the spiking concentrations (>25%) in both the biotic and abiotic batches. After one day, fluoxetine removal in the biotic and abiotic batches was 75 and 84%, respectively, and by day 3 over 99% removal was observed in both batches. These findings correspond well with lab-scale experiments of Pomiès *et al*. (2015) who concluded that sorption was the only removal mechanism for fluoxetine.

The atenolol concentrations at day 1 were reduced by 53% in the abiotic batches as compared to 44% in the biotic batches (Table 2, Fig. 3), suggesting that

sorption was initially the major removal mechanism. Thereafter biodegradation became the predominant removal mechanism as higher removals were observed in the biotic batches. In the literature, biodegradation has been reported to be the predominant removal mechanism of atenolol in wastewater treatment, whereas sorption to activated sludge has been found to be low (Maurer *et al*., 2007; Pomiès *et al*., 2015). The low sorption coefficients reported in the literature were in contrast to the initial atenolol removal observed in the abiotic batches of this study. The differing sorption behaviour might be explained by the inoculum mixture used in this study as the creek sediment likely contained a significant clay fraction (not measured) that enhanced sorption as a removal mechanism in the current study. Kodešová *et al*. (2015) found a positive correlation between clay content and atenolol sorption which was driven by the cationic exchange between negatively charged clay particles and positively charged atenolol at neutral pH.

Biological treatment after mild photocatalysis (MP + B protocol)

The removal of the pharmaceuticals during the biological treatment component of the MP + B protocol to assess the role of the biodegradation mechanism in the combined system. It was found that high removals (>99%) were observed for many of the compounds after 21 days in the biological experiments of the MP + B protocol (Fig. 3). Only carbamazepine was incompletely removed (65%) by the end of the experiments. The initial concentrations of atorvastatin and atenolol (Fig. 3) were lower compared to the B protocol due to their photocatalytic degradation during pretreatment. The low initial fluoxetine concentration was attributed to both removals during photocatalysis and rapid sorption to the inoculum as observed in the B protocol. Approximately 50% of the fluoxetine was lost during the centrifugation and filtration steps that were carried out to remove TiO_2 between the photocatalytic and biodegradation experiments. Analysis of centrifuged and filtered photocatalytically treated samples revealed that this TiO_2 removal step did not result in the loss of the other pharmaceuticals.

It was hypothesized that the presence of different levels of methanol in the MP + B and B batches might have affected the biodegradation of the pharmaceuticals and therefore methanol removal during photocatalysis was reviewed in the literature. It was found that methanol can be oxidized to CO_2 in TiO_2 based photocatalysis (Chen *et al*., 1999b). However, under similar conditions (i.e. methanol and TiO_2 concentrations) the conversion of methanol to CO_2 was limited and only occurred after 80 min of illumination (Chen *et al*., 1999a). Therefore, it was assumed that methanol concentrations were similar in the MP + B and B protocols and considered not to be a discriminative factor for pharmaceutical degradation between the protocols. The predominant pharmaceutical removal mechanisms were considered to be similar in these tests as in the B protocol; sorption for fluoxetine, sorption and biodegradation for atenolol and biodegradation for the other pharmaceuticals.

Significantly higher rates of removal of atorvastatin, caffeine, diclofenac, gemfibrozil and ibuprofen were observed in the biological experiments of the MP + B protocol as compared to the B protocol (ANCOVA results in Table S1 of the Supplementary Material). Out of these pharmaceuticals, only atorvastatin was partially eliminated (75%) during mild photocatalytic pretreatment. The enhanced atorvastatin removal after photocatalysis in the MP + B protocol may have been due to a co-substrate effect on the biodegradation removal mechanism as reported by other authors. Yan *et al*. (2013) found faster quinoline removal during biological treatment after photolytic pretreatment. They suggested this could result from quinoline and its photolysis products being simultaneously biodegraded and thereby both contributed to biomass growth. Further, enhanced biodegradation that was induced by the presence of photolysis products was demonstrated for sulfadiazine by Pan *et al*. (2014), 2,4,6-trichlorophenol by Wang *et al*. (2015) and for pyridine by Zhang *et al*. (2014). These authors reported that the biodegradation of the main photolysis product generated intracellular electron carriers that initiated the initial mono-oxygenation reaction for the biodegradation of target compounds. In sunlit surface water two photolysis products of atorvastatin were found, one due to N-dealkylation, the other formed by the photonucleophilic aromatic substitution of the F atom by OH (Lam and Mabury, 2005). Similar to the 2,4,6-trichlorophenol photolysis the dehalogenation of atorvastatin could indicate the formation of a more readily biodegradable product. When viewed collectively, the partial degradation of the target atorvastatin in the mild photocatalysis process appeared to enhance the subsequent biodegradation mechanism by introducing co-substrates that enhanced biological activity.

Enhanced biological removal of caffeine, diclofenac, gemfibrozil and ibuprofen was also observed after pretreatment, even though they were not significantly removed during mild photocatalysis. In contrast to the B protocol results in which diclofenac was classified as recalcitrant, the biological removal of diclofenac was found in the MP + B protocol. At day 21 more than 99% removal of diclofenac was observed, whereas only 50% was removed in the B protocol. In addition, caffeine and ibuprofen were removed within 3 days as compared to 7 days in the B protocol, while gemfibrozil removal at day 7

was 95% compared to 50% in the B protocol. Like atorvastatin, it is hypothesized that the enhanced biodegradation of caffeine, diclofenac, gemfibrozil and ibuprofen was triggered by the presence of photocatalytic products. Though none of these pharmaceuticals was effectively removed during mild photocatalysis, the products formed during photocatalytic removal of atenolol, atorvastatin and fluoxetine may have enhanced their biodegradation. The potential for photocatalytic products from nontarget compounds to enhance the biodegradation of target pharmaceuticals indicates the importance of studying mixtures of compounds when assessing the performance of integrated photocatalytic-biodegradation systems and is a key finding of this study.

The molecular structures of the pharmaceuticals (Table 1) were compared to further assess whether there was a relationship between structure and the biological removal mechanisms after pretreatment. A carboxyl group is present in atorvastatin, diclofenac, gemfibrozil and ibuprofen but also in naproxen. Caffeine was the only pharmaceutical without a phenyl ring, yet not the only one exhibiting enhanced biodegradation with photocatalysis. Atorvastatin, caffeine, gemfibrozil and ibuprofen have methyl groups, as does naproxen, whereas diclofenac does not have this group. In particular, the response of naproxen after pretreatment was elucidated as it behaved differently than the other pharmaceuticals that displayed enhanced removal. As ibuprofen removal is well described in the literature and its structure has many commonalities with naproxen, their reported degradation pathways were compared. Naproxen was the only pharmaceutical in this study that contained an ether group. Ether cleavage, hydroxylation and aromatic ring cleavage are known naproxen degradation pathways (Sidelmann *et al*., 2001; Zhong *et al*., 2003; Quintana *et al*., 2005; Wojcieszyńska *et al*., 2014) and occur mostly co-metabolically (Quintana *et al*., 2005; Wojcieszyńska *et al*., 2014). The reported ibuprofen degradation pathways are hydroxylation, dealkylation followed by hydroxylation, demethylation followed by O-hydroxylation and demethylation followed by dehydrogenation (Boix *et al*., 2016). Hence, the literature reveals a limited overlap in the broad and complex array of transformation pathways for naproxen and ibuprofen. Therefore, we hypothesize that the chemical structure can affect the biodegradation responses of the pharmaceuticals after pretreatment, but the mechanisms behind this response remain unclear. In addition, co-metabolic processes appear to be important for naproxen removal, which could possibly explain why naproxen removal was not enhanced after the mild photocatalytic pretreatment.

Atenolol and carbamazepine removal efficiencies were similar in the biological experiments conducted for the B and MP + B protocols. The prominence of sorption to clay particles as a removal mechanism for atenolol was hypothesized to explain the lack of difference observed in its removal efficiency between the two protocols. The similar removal of carbamazepine with or without photocatalytic pretreatment was attributed to the general persistence of carbamazepine towards biodegradation, as observed in many previous studies (Verlicchi *et al*., 2012; Luo *et al*., 2014). We could not test the differences in fluoxetine removal efficiencies as it was substantially removed during filtration prior to the biological experiments of the MP + B protocol.

In summary, mild photocatalytic pretreatment with a subsequent biological treatment was found to be an effective combination of processes to improve the removal of pharmaceuticals. This was a significant outcome of the current work that could be integrated into the design of future wastewater treatment plants. Scott and Ollis (1995) indicated that choosing complementary processes is a key design priority in order to benefit from synergistic effects. In this study, the recalcitrance of diclofenac towards biodegradation was overcome by pretreatment. Furthermore, biodegradable pharmaceuticals like atorvastatin, caffeine, gemfibrozil and ibuprofen were biodegraded at a higher rate compared to biological treatment without pretreatment. Hence, our study demonstrates that mild photocatalytic pretreatment and biodegradation are complementary processes and their synergy can result in better pharmaceutical removal compared to the single processes. Further research is recommended to assess the effectiveness of this combined process on real WWTP effluent as its constituents might affect the process efficiency. Process configurations that could cost-effectively integrate this technology into existing full-scale WWTPs providing secondary or tertiary treatment require additional development.

Conclusions

(1) Sequentially combined mild photocatalysis and biological treatment effectively removed eight out of nine studied pharmaceuticals.

(2) Biodegradation was the predominant removal mechanism in biological experiments for most pharmaceuticals, whereas fluoxetine was removed by sorption to the inoculum and atenolol by both sorption and biodegradation.

(3) Carbamazepine was recalcitrant towards photocatalysis and biodegradation.

(4) The biological degradation of five pharmaceuticals improved after mild photocatalytic pretreatment in subsequent biological treatment of which only atorvastatin was removed during mild photocatalysis.

(5) Biodegradation of the atorvastatin photocatalytic degradation products most probably triggered the enhanced atorvastatin biodegradation by the initiation of monooxygenation reactions.

(6) Caffeine, diclofenac, gemfibrozil and ibuprofen were not susceptible to photocatalysis; however, their biodegradation efficiency enhanced after mild photocatalytic pretreatment.

(7) We hypothesize that the photocatalytic products of atenolol, atorvastatin and fluoxetine resulted in the enhanced biodegradation of caffeine, diclofenac, gemfibrozil and ibuprofen.

(8) Overall, mild photocatalysis followed by biological treatment is an effective and resource-efficient combination, achieving a substantial reduction of energy input for the photocatalysis and enhanced biodegradation of biodegradable and recalcitrant pharmaceuticals susceptible and nonsusceptible to photocatalysis.

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