PHARMACEUTICAL REMOVAL

Synergy between Biological and Chemical Processes for Wastewater Treatment

Henrik Arnoud de Wilt

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Thesis committee

Promotor
Prof. Dr H.H.M. Rijnaarts
Professor of Environmental Technology
Wageningen University & Research

Co-promotor
Dr A.A.M. Langenhoff
Associate Professor, Sub-department of Environmental Technology
Wageningen University & Research

Other members
Prof. Dr L.E.M. Vet, Wageningen University & Research
Prof. Dr F. Omil, University of Santiago de Compostela, Spain
Dr C.H.M. Hofman-Caris, KWR Watercycle Research Institute, Nieuwegein
Dr P.M.F. van der Maas, Waterlaboratorium Noord, Glimmen

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Thesis

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Моим самым близким и любимым Данюхе, Давидушке и Машеньке

Voor mijn naaste en geliefde Daniël, Davíd en Masha

Contents

Summary

Pharmaceuticals are used worldwide at increasing consumption rates which are not expected to decrease on short time horizons. Large quantities of administrated pharmaceuticals are excreted unaltered or as metabolites via faeces or urine and end-up via the toilet and sewer system at the wastewater treatment plant.

Conventional wastewater treatment plants employing activated sludge treatment are designed to remove bulk constituents, like organic matter, nitrogen and phosphorous, which are present at concentrations of milligrams per litre. These treatment plants were not designed for removing the broad spectrum of pharmaceuticals comprising thousands of highly complex molecules present in wastewater. These compounds, found at low concentrations of nanograms to micrograms per litre, are therefore generally only poorly removed. The biodegradable pharmaceuticals that are removed are rather transformed than mineralized. As a matter of fact, countless pharmaceuticals or their human metabolites and transformation products are discharged into the environment.

The presence of these insidious compounds in the environment are one of the main challenges humanity is facing as they jeopardise the aquatic environment and human health. Adverse effects on the aquatic ecosystem like the feminization of fish are well reported and threaten the entire ecosystem. Intake waters for drinking water production were found to contain high pharmaceutical concentrations emitted by wastewater effluents posing risks to the public health in case drinking water is poorly treated. The presence of pharmaceuticals hampers the reuse of wastewater effluents whereas worldwide water scarcity problems could partially be solved by effluent reuse. Hence, there is a strong motivation to remove pharmaceuticals before they enter the aquatic environment.

Being a main hub in the water cycle, wastewater treatment plants are the most optimal place to raise barriers against pharmaceuticals before they are discharged. Various biological and chemical processes have been studied for their capacities to remove pharmaceuticals of which some have been locally implemented. Nevertheless, these processes show either insufficient pharmaceutical removal or are considered costly and unsustainable. Thus, there is a lack of cost-effective pharmaceutical

treatment processes. Therefore, this dissertation investigates different processes for the cost-effective removal of pharmaceuticals to be applied in wastewater treatment, with a focus on the synergy between biological and chemical treatment processes for an enhanced pharmaceutical removal (Chapter 1).

The low costs associated to biological treatment increases its implementation potential and favours the research on biological processes. Among the various parameters affecting biological processes, redox conditions are considered one of the most important parameters. In batch and column experiments employing constructed wetland sediments aerobic, sulfate reducing and methanogenic redox conditions were most favourable for pharmaceutical removal (Chapter 2). In contrast, micro-aerophilic and nitrate reducing conditions were less effective. Biodegradation and sorption contributed to the observed removal and both were influenced by the applied redox conditions. Saturation of sorption sites for propranolol, i.e. the compound with the highest sorption coefficient among the investigated pharmaceuticals, was found to occur after 300 pore volume changes under most favourable redox conditions. This suggests that in biological filtration applications sorption of pharmaceuticals is of minor importance compared to biodegradation. The persistence of biorecalcitrant pharmaceuticals such as carbamazepine towards biodegradation and sorption under all redox conditions stresses the shortcoming of biological treatment and indicates the need for additional treatment processes.

Mild UV-LED TiO_2 photocatalysis combined with subsequent biological treatment demonstrated improved pharmaceutical removal over single photocatalysis or biological treatment (Chapter 3). Mild photocatalytic treatment removed three out of the nine studied pharmaceuticals. Interestingly, the biodegradation of four pharmaceuticals enhanced after mild photocatalytic pre-treatment, even though only one of them was targeted by photocatalysis. In addition, biodegradation of diclofenac, which persisted during single process biological treatment, was observed after mild photocatalytic pre-treatment. Based on the literature it is postulated that intermediates formed during mild photocatalysis enhanced the biodegradation of pharmaceuticals by the activation of enzymatic systems responsible for initial attack of organic molecules.

The presence of ozone scavengers in wastewater effluent like organic carbon, reduce the effectiveness of ozonation for the oxidation of pharmaceuticals. Elevated total organic carbon (TOC) concentrations, such as the 17.3 mg TOC/L present in the investigated effluent, consequently demand high absolute ozone inputs for pharmaceutical removal, thus reducing the cost-effectiveness of ozonation. A three step bio-ozone-bio process (BO_3B) consisting of two identical trickling filters utilizing sand as biomass carriers and an ozone reactor was therefore designed (Chapter 4). Various hydraulic retention times (HRTs) and ozone doses were investigated aiming at a cost-effective pharmaceutical removal from wastewater effluent. At an HRT of 1.5 hours a 38% TOC removal was found over the first biological reactor, thereby proportionally reducing the ozone demand in the subsequent ozone reactor. Enhanced pharmaceutical removal was observed over the first biological reactor compared to conventional wastewater treatment, despite of the short HRT and low amount of biomass in the BO_3B system. Even pharmaceuticals known to be moderately biodegradable such as sulfamethoxazole were effectively biodegraded. At ozone doses down to 0.2 g O_3/g TOC an effective removal of biorecalcitrant pharmaceuticals such as carbamazepine was found. The 17% TOC removal over the last biological reactor demonstrated the removal of transformation products formed during ozonation.

Simultaneous nutrient recovery and pharmaceutical removal was found in algal photobioreactors running on anaerobically treated black water or urine (Chapter 5). Algae, known to take up nitrogen and phosphorus from these highly concentrated source separated wastewater streams, demonstrated to contribute to the pharmaceutical removal observed in the algal photobioreactors. Control experiments revealed that algae, bacteria and light contributed to the pharmaceutical removal. Pharmaceuticals susceptible to photolysis such as diclofenac were photodegraded, whereas paracetamol and metoprolol were removed by a combination of biodegradation and photodegradation. The sorption of pharmaceuticals to algal biomass was limited which favours the use of harvested algal biomass as fertilizer in agriculture over for instance direct urine application.

The outcomes of this dissertation provide insight into the limitations of single biological and chemical processes for pharmaceutical removal and illuminate the importance of combinations of complementary processes to overcome single process

disadvantages (Chapter 6). Site-specific conditions such as wastewater composition dictate which combination is locally most favourable as there is no universal cost-effective combination of processes. Implementation should therefore rather focus on tailor-made combinations as there is a wide arsenal of biological and chemical treatment processes available. To further improve the cost-effectiveness of combined processes future research should focus on underlying removal mechanisms including the enzymatic pathways of pharmaceutical degradation and transformation product formation and removal.

For the studied removal processes in this dissertation an outlook on further research and upscaling is presented. The BO_3B process and the algal treatment system are ready to be tested on a pilot scale. Especially the upscaling potential of the BO_3B system is high as on lab-scale it was successfully tested for almost a year on real wastewater. During this period high pharmaceutical removal efficiencies were achieved in a cost-effective manner. Upscaling should mainly focus on the operation of the biological reactors as they make the BO_3B process cost-effective. The algal treatment system is currently tested on pilot scale. A focus on the removal of a broad suite of micropollutants is recommended for pilot scale testing. The combination of photocatalysis and biodegradation requires further lab-scale research as TiO_2 immobilization is needed to make the system more sustainable and the system was only tested on a clean matrix.

Obtained knowledge of the past decades stresses the importance of effect based removal strategies as most studies, including this dissertation, primarily rely on chemical parameters such as individual pharmaceutical concentrations, overlooking the effects of pharmaceuticals on for instance the aquatic environment. Source separation based sanitation systems enabling cost-effective pharmaceutical removal and interventions at earlier stages in the chain from pharmaceutical manufacturing to drinking water production reducing the need for end-of-pipe solutions are highly recommended solutions to reduce pharmaceutical emission into the environment. Moreover, stricter legislation regarding the regulation of pharmaceutical concentrations in the effluent is recommended.

The results of this dissertation provide further understanding of combining biological and chemical treatment processes for pharmaceutical removal and is

thereby one of the many steps to be taken to prevent the wastewater related emissions of pharmaceuticals and other contaminants jeopardizing the environment and the public health.

Chapter 1

General introduction

1.1 Pharmaceuticals in the water cycle

1.1.1 Occurrence

Human pharmaceuticals comprise a wide group of various organic compounds intended to treat human illness and diseases. They are mostly synthetically manufactured and are therefore considered xenobiotic. Pharmaceuticals are being used worldwide, though usage of type and quantity differs per country [138]. Around 3000 different medical substances are used in the European Union [85]. In 2007 the estimated human consumption of pharmaceuticals in the Netherlands totalled 1.273 metric tonnes, which equals approximately 78 gram per capita per year. By then a consumption increase of 17% was expected by 2020 due to aging of the population, while for 2050 an increase of 37% was predicted [283]. However, more recent data shows an higher realised increase in Dutch pharmaceutical consumption. Expressed as daily standard doses the consumption of pharmaceuticals in the Netherlands grew per year by 2.6%, 2.9%, 2.5%, 1.1% and 6.4% in 2015, 2014, 2013, 2012 and 2011 respectively [249, 250].

Consumed pharmaceuticals are mainly excreted via urine and faeces [60]. After excretion, human pharmaceuticals end up in wastewater and are transported to wastewater treatment plants (WWTPs) [85, 129, 139]. Conventional WWTPs are commonly designed for the removal of bulk organic matter, nitrogen and phosphorus but not for pharmaceutical removal. Although some pharmaceuticals are removed during wastewater treatment, numerous pharmaceuticals are ineffectively removed and are discharged with the WWTP effluent [65, 287]. Hence, wide ranges of numerous pharmaceuticals at ng/L up to low µg/L concentrations have been measured in wastewater effluents and in surface waters [196] Moreover, low concentrations up to 0.1 µg/L of pharmaceuticals are also detected in various drinking water sources [286, 288]. Pharmaceuticals were only detected in the water cycle over the last decades as only recently analytical techniques allowed the detection of these compounds in aqueous environments at low concentrations [262].

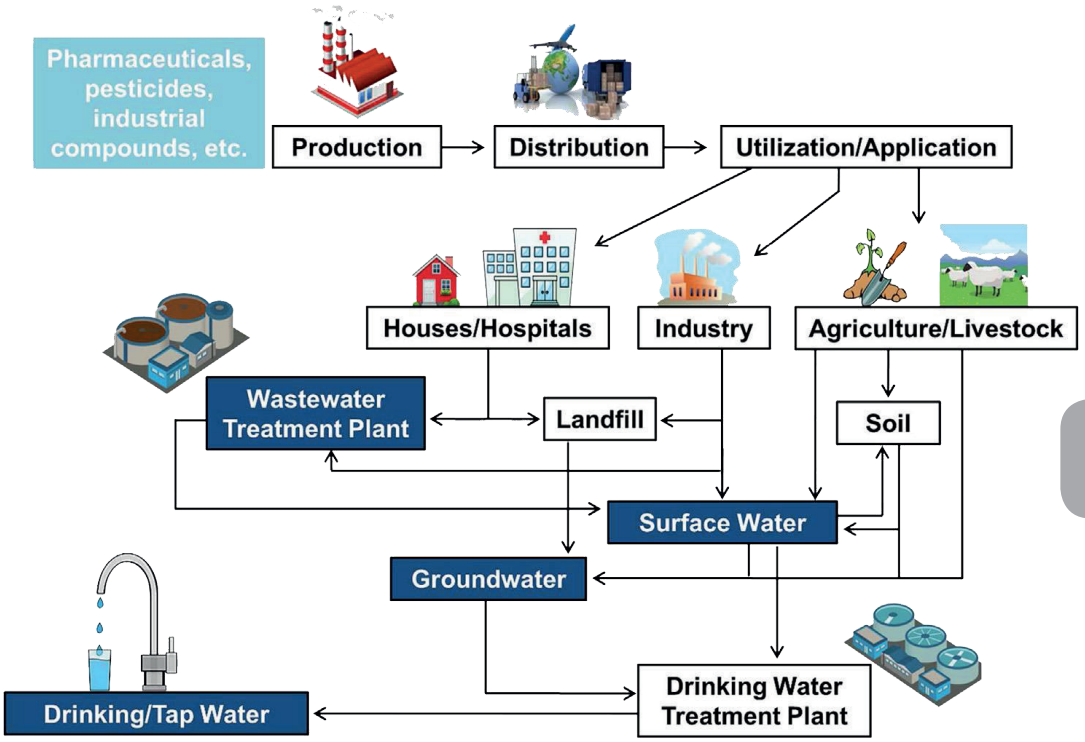


Figure 1.1 Pathways of pharmaceuticals and other micropollutants from production to drinking water (Figure from [22]).

1.1.2 Fate

Effluents of WWTPs treating merely wastewater from households are identified as the main source of pharmaceuticals entering the environment [128]. Besides administration and excretion of pharmaceuticals, unused and expired pharmaceuticals are also disposed down household drains. In a German survey 18% of the respondents said they flushed outdated pharmaceuticals down the drain [95]. In a U.S. study only 23% of the respondents reported to return expired pharmaceuticals to the pharmacy, whereas more than 50% had flushed pharmaceuticals through the toilet [236]. In addition to households, hospitals, agriculture and livestock and industries are also recognised as sources of pharmaceuticals entering the environment (Figure 1.1). Hospital wastewaters contain high concentrations of pharmaceuticals [94]. However, due to the relatively small size

of this stream it is typically diluted by a factor 100 in the sewer system before it enters the WWTPs [137]. In addition, some hospitals have their own tailor made wastewater treatment plant aiming at low pharmaceutical emissions to the sewer system [204]. Both the dilution of wastewater in the sewer system and treatment by some individual hospitals explains why less than 15% of the total pharmaceutical load fed to WWTPs is estimated to originate from hospitals [147].

Pharmaceutical use in agriculture and livestock mainly concerns the use of antibiotics. Sim et al. [240] compared WWTPs treating domestic, hospital, livestock and industrial wastewater in Korea and found highest total pharmaceutical concentrations in the influents of livestock WWTPs. Although livestock WWTPs had the highest pharmaceutical influent concentrations, municipal WWTPs were found the principal source of pharmaceuticals to the aquatic environment as their disposed loads were highest. Nevertheless, these findings might be different for other countries. Pharmaceutical producing industries in Europe and North America are assumed to have minimal pharmaceutical emissions into the environment due to good manufacturing practice regulations [51, 304]. However, no data is available to verify this assumption [139]. In the effluent of an Indian WWTP serving pharmaceutical manufacturers elevated concentrations of the antibiotic ciprofloxacin up to 31 mg/L and high concentrations of numerous other pharmaceuticals have been detected. These concentrations exceed toxic levels to various organisms by multiple orders of magnitude [146]. Similar findings were reported for a manufacturer of the antibiotic oxytetracycline in China which was found in WWTP effluent at concentrations up to 19 mg/L [153].

1.1.3 Characteristics

Pharmaceuticals cover a broad range of compounds with chemically complex structures and many different chemical and physical properties (Table 1.1). Pharmaceuticals are typically polar or charged compounds and thereby hydrophilic [139, 260]. Many of them contain acidic groups which favours speciation of the compound [77]. Polar compounds mostly dissolve well in water and are therefore easily dispersed in aquatic environments. The spread of hydrophobic pharmaceuticals

in aquatic environments is relatively limited and much slower as they tend to accumulate in the fatty tissues of organisms [114].

Metabolic processes in the human body can alter the pharmaceutical into metabolites before excretion (Figure 1.2). After excretion further transformation of the pharmaceutical or its metabolites can take place in wastewater treatment plants and the environment resulting in countless transformation products. Some of the products formed during specific treatment are known to exhibit higher toxicity levels compared to the original compounds [118].

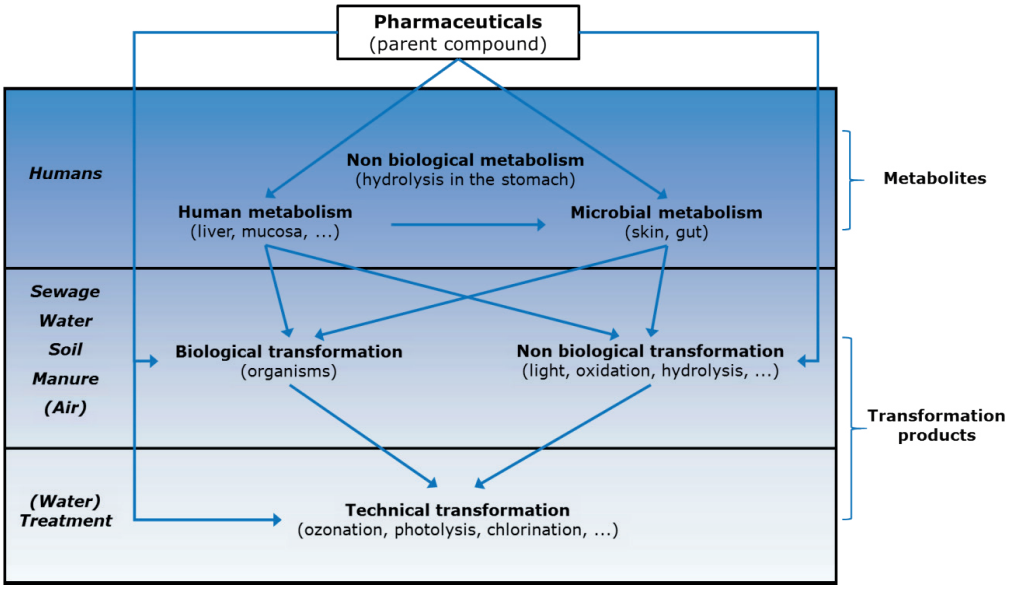
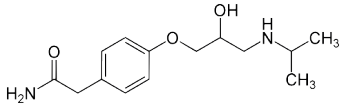
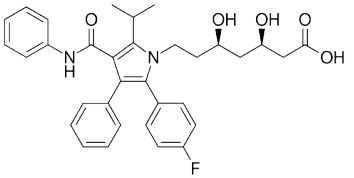
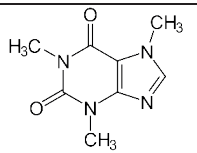
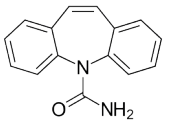
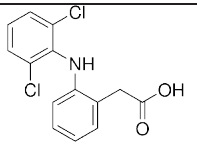
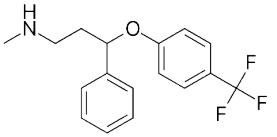
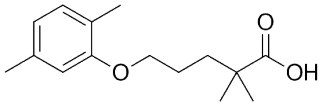
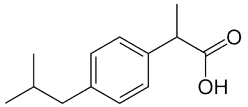
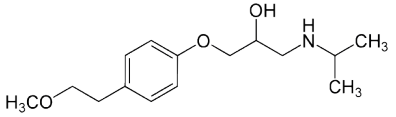
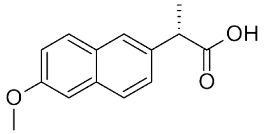
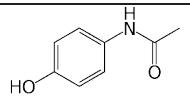
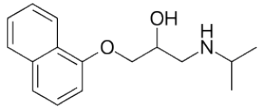
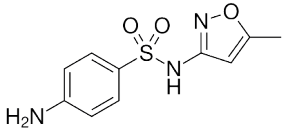
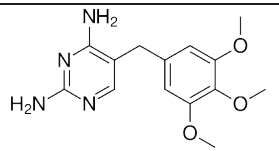


Figure 1.2 Transformation pathways of pharmaceuticals (adapted from [139]).

Table 1.1 Pharmaceuticals studied in this dissertation; their therapeutic function, chemical structure and physico-chemical properties.

Pharmaceutical	**Therapeutic function**	**Chemical structure**	**pKa**[a] [15, 191]	**Log K_{ow}**[b] [15, 191]	**Water solubility** (mg/mL) [121, 191]	**Removal during wastewater treatment** [164]
Atenolol	Beta-blocker		9.4	0.2	955	Moderate
Atorvastatin	Lipid regulator		4.3	4.2	0.1	
Caffeine	Stimulant		10.4	-0.1	21.6	High
Carbamazepine	Anti-epileptic		13.9	2.5	0.01	Low
Diclofenac	Anti-inflammatory		4.2	4.5	0.002	Low
Fluoxetine	Anti-depressant		10.1	4.1	33	

Gemfibrozil	Lipid Regulator		4.7	4.8	0.008	High
Ibuprofen	Anti-inflammatory		4.9	4.0	0.01	High
Metoprolol	Beta-blocker		9.6	1.9	47	Low
Naproxen	Anti-inflammatory		4.2	3.2	0.016	High
Paracetamol	Pain killer		9.4	0.5	0.1	
Propranolol	Beta-blocker		9.4	3.5	1.72	
Sulfamethoxazole	Antibiotic		5.7	0.9	0.01	Moderate
Trimethoprim	Antibiotic		7.1	0.9	0.4	Moderate

a - pKa: dissociation constant; b - Log K_{ow}: octanol-water partitioning coefficient.

1.2 Effects of pharmaceuticals in the water cycle

1.2.1 Environmental effects

Improved analytics of the last two decades allowed the detection of pharmaceuticals in various matrices and enabled investigations into the fate and ecological effects of these compounds [196, 262, 283]. Though the fate of pharmaceuticals, i.e. pathways of pharmaceuticals through the environment, is now relatively well known, the actual ecological effects in the environment are still poorly understood [85, 139, 190, 315]. Effects of numerous individual pharmaceuticals on specific test organisms like bacteria, algae, daphnia and other aquatic organisms are well studied, but limited studies report the effects of pharmaceutical mixtures in the environment [287]. The number of pharmaceuticals emitted into the environment is world-wide still increasing and this is not expected to change in the near future.

Due to the enormous variety in types of pharmaceuticals used and emitted, exposure to organisms in the environment concerns predominantly mixtures rather than to individual compounds. Although concentrations of individual compounds in water and soils are often non-toxic, exposure to these mixtures could result in a synergistic toxicity, thus leading to high risks [233]. Brian et al. [34] studied the effects on fish of exposure to a mixture of estrogenic compounds. They found estrogenic compounds to act together in an additive manner imposing fish toxicity at concentrations of which the individual compounds did not induce a response. Similarly, Cleuvers [50] demonstrated elevated toxicity for a mixture of anti-inflammatory drugs over the toxicity of the individual compounds which could be explained by the principle of concentration-addition.

Also for mixtures of compounds with different modes of toxic action, additive toxic effects were found compared to the individual compounds [5]. Thus, the presence of numerous individual pharmaceuticals in wastewater could result in risks for the aquatic environment near the effluent discharge areas of the WWTPs [77, 141, 325]. The variety among the broad array of individual pharmaceuticals results in that different modes of toxic action and multiple toxicological end-points need to be accounted for to determine actual toxicities [85]. For instance, Guler and Ford [98] discovered behavioural changes resulting in increased predation risks for the marine amphipod *Echinogammarus marinus* when exposed to the

anti-depressant fluoxetine at environmentally relevant concentrations. Decreased activity of the benthic invertebrate *Gammarus pulex* was found at ibuprofen and fluoxetine concentrations of 10-100 ng/L [63]. Kidney, liver and gill cell alterations were observed in rainbow trout (*Oncorhynchus mykiss*) exposed to 1 µg/L concentrations of diclofenac [274].

Acute toxicity tests with standard testing organisms like daphnia, algae and bacteria revealed that the lowest observed effect concentrations (LOEC) are about two orders of magnitude higher than concentrations found in WWTP effluents. However, for other specific organisms LOEC of individual compounds were found at typical WWTP effluent concentrations, e.g. diclofenac for fish, and propranolol and fluoxetine for zooplankton and benthic organisms [85].

All above mentioned toxicity studies were conducted at laboratory scale. Translating results of laboratory toxicity tests to the real aquatic environment is complicated since there seems not to be a single assessment factor which can be applied for all aquatic species and for all pharmaceuticals. Moreover, laboratory tests mostly focus on acute toxic effects rather than on chronic toxicity [57]. In their review on ecotoxicology of pharmaceuticals Fent, et al. [85] state that only little is known about long term effects and chronic toxicity to aquatic organisms. Moreover, limited studies report the actual impact of pharmaceuticals on the aquatic environment as the presence of other potential ecological stress compounds (such as pesticides, personal care products, industrial additives, etc.) complicates parsing out the actual contribution of pharmaceuticals.

Various authors therefore handle a more pragmatic approach and study the ecological effects of WWTP effluent discharge into the aquatic environment without unravelling the contributions of individual compounds. Field studies on river systems receiving WWTP effluents have shown endocrine-disruptive effects resulting in intersex rainbow darters (*Etheostoma caeruleum*) [259, 270]. Moreover, discharge of various anti-depressants by WWTPs resulted in the bioaccumulation of these compounds in the brain, liver, muscle and gonads of various fish species in the Niagara River and are hypothesized to affect the biological diversity [17].

The abovementioned lab-scale and field studies demonstrate that the current understanding of the actual acute and chronic effects of pharmaceuticals mixtures on the environment is incomprehensive and inconclusive. Further

research is required to elucidate especially the potentially harmful long term ecological effects of pharmaceutical mixtures on the aquatic environment at environmentally relevant concentrations, i.e. to assess LOECs of pharmaceutical mixtures for aquatic biota networks in the environment.

1.2.2 Effects on human health

Pharmaceuticals have been detected in various sources of drinking water and thereby pose a potential risk to human health [32]. Touraud et al. [273] concluded in 2011 that there was no consensus among scientists on what risks pharmaceuticals actually pose to human health as their reviewed studies often reported uncertainties and the toxicity of mixture effects on human health is difficult to quantify. Even in very recent studies, utilizing an extensive environmental monitoring dataset with a large number of pharmaceuticals for the human health risk assessment of pharmaceutical mixtures in drinking water, no complete risk overview can be given [113]. Moreover, there are no institutionalized risk assessment methods for the presence of pharmaceuticals in drinking water [273]. Therefore methods using indirect exposure, based on predicted environmental concentrations, and human pharmacology and toxicology data of pharmaceuticals have been applied to assess the toxicological effects of drinking water or fish consumption on human health.

For various individual or groups of pharmaceuticals no appreciable human health risks have been found [25, 58, 59, 122, 226, 252]. Thus, to date there is no direct science based reason for concern as there are no clear indications of adverse effects on human health. However, unlike ecological and aquatic organism studies, studies on the effects of low levels of wide ranges of chemicals on human health are hampered by the impossibility to do controlled exposure experiments with populations. Therefore, it cannot yet be excluded that adverse human health effects exist due to a continuous exposure to low levels of a wide range of chemicals. A recent meta-analysis showed that between 1973 and 2011 the sperm count of Western men expressed as sperm concentration and total sperm count reduced by 52% and 59%, respectively [152]. The authors state that these findings are plausibly associated to multiple environmental influences such as exposure to chemicals. The implications of reduced sperm count go beyond fertility and reproduction concerns as they also pose serious public health, societal and

economical related problems. For this and other reasons, drinking water companies often imply the precautionary principle or a so called "clean source" strategy and policy: they give high value to the image of their water products which is related to the quality of the water resources they use and the perception of drinking water consumers [113, 242]. Thus, drinking water companies strive for use of water resources that are free from unnatural organic components, such as pesticides, pharmaceuticals and other compounds, and this is a strong "society-driven" motivation for considering measures to remove pharmaceuticals from WWTP effluents.

The situation is different for the presence of a specific group of pharmaceuticals, namely antibiotics, in wastewater and in water resource systems used for drinking water that are affected by WWTP effluents. For these compounds environmental exposure raises human and animal health concerns due to the proven development of clinically relevant antibiotic-resistant bacteria [41, 48]. The extent of natural bacterial populations that have acquired resistance to these antibiotics (often used as life-saving medicines) is increasing [31, 168]. This may cause yet also unknown human health risks via exposure of humans to these antibiotic-resistant bacteria through the natural environment, via crops and live-stock food products or via poorly disinfected drinking water.

1.2.3 Need for measures

The abovementioned concerns regarding widespread and continuous emissions of mixtures of pharmaceuticals at low concentrations through WWTP effluents to the aquatic environment is suspected to cause –largely unknown- acute and/or chronic toxicity effects in aquatic populations. As mentioned above, to date there are no direct science based reasons concerning acute risks to human health regarding pharmaceutical emissions. Nevertheless, there are multiple reasons as mentioned above which stress the need to remove these insidious compounds quickly after they enter the water cycle. Thus, from an environmental and human health related perspective to reduce the possible risks pharmaceuticals pose, there are strong motivations to conduct further research on ways to reduce pharmaceutical emissions, i.e. by prevention and technological removal measures like the further treatment of WWTP effluents.

1.3 Legislation

From a legislative perspective first steps have been taken concerning the presence of pharmaceuticals in the water cycle. To date, the most progressive policies have been implemented in Switzerland. In the early 2000s the issue of micropollutants entered the Swiss political agenda [184]. Actions were taken from 2009 onwards when the Swiss Federal Office for the Environment proposed revisions to the nationwide Swiss Waters Protection Ordinance with respect to micropollutants (including pharmaceuticals). In 2016 a revised Waters Protection Act passed both Swiss parliamentary chambers and came into force. Thereafter the implementation of the revised Waters Protection Ordinance was started, which is currently in progress. In brief, the Waters Protection Ordinance foresees the upgrade of large size WWTPs (>100 000 population equivalents (PE)) and moderate size WWTPs (10 000 - 100 000 PE) which discharge into streams with minute dilution or those being used for drinking water extraction. In practice this implies that approximately 50% of the Swiss wastewater will undergo advanced post-treatment as about 100 out of 800 WWTPs will be upgraded [232]. With the upgrade, WWTPs have to meet an 80% removal efficiency of 5 compounds from a list of 12 micropollutants (11 pharmaceuticals and 1 biocide). Individual Cantons are free in their choice for the 5 compounds they monitor and every 5 years the list of 12 compounds is subject to change. The upgrade concerns the investment costs of about 1.2 billion CHF and additional operational costs of 130 million CHF per year. As the polluter pays principle is practiced [184] the Swiss inhabitants are charged an additional 9 CHF per person per year for the next 25 years [232].

Although to date there are no discharge regulation for pharmaceuticals in the European Union, an European wide water policy started already in the year 2000 [22]. This "Water Framework Directive" and its regulations concern the prioritization of potentially harmful substances (amongst other pharmaceuticals) and has already been extended with several directives and amendments. In the Directive 2013/39/EU for the first time a pharmaceutical (diclofenac) and a synthetic hormone (17-alpha-ethinylestradiol) were added to the so called 'watch list'. This watch list comprises of potentially hazardous compounds for which it is recommended that monitoring and treatment solutions should receive extra attention with the objective to protect the aquatic environment and the human

health. Recently three antibiotics (azithromycin, clarithromycin and erythromycin) were added to the watch list via an amendment (Decision 2015/495/EU).

To the best of my knowledge no policies and regulation have been implemented in other countries. Although there is a raise in scientific attention for micropollutants in various countries, this is commonly not reflected in implemented policies. For instance the current environmental quality standard for surface water in China do not mention pharmaceuticals [185]. Similarly Singapore, Japan New Zealand and Australia have no specific legislation for the discharge of pharmaceuticals to the environment even though their water acts aim at reducing risks for human health [203]. Likewise, the primary objective of the United States Federal Clean Water Act is "to restore and maintain the chemical, physical and biological integrity of U.S. waters", but the Act doesn't include legislation on the monitoring or discharge of pharmaceuticals, and the Safe Drinking Water Act does neither.

1.4 Pharmaceutical removal

1.4.1 Current (biological) treatment

WWTPs have a crucial role in the emissions of pharmaceuticals to surface waters [14, 125]. Moreover, they are one of the few main hubs in the entire water cycle. Hence, WWTPs are an ideal spot to prevent pharmaceutical emissions into the environment. Despite pharmaceutical removal is currently not a design parameter for WWTPs, various researchers have reported the removal of pharmaceuticals in WWTPs, indicating the potential of biological treatment [124, 190, 238]. In general, high variations in pharmaceutical elimination efficiencies are found among different WWTPs which are typically linked to the WWTP design [47]. This suggests that pharmaceutical treatment can possibly improve when pharmaceutical removal would be regarded as a design parameter. Various WWTP characteristics and design parameters have therefore been studied to gain a better understanding of the underlying pharmaceutical removal mechanisms. Removal rates seem to depend on treatment plant configurations and parameters like biological nitrogen removal, diversity of the microbial community, hydraulic retention time, sludge retention time and the use of biofilm carriers [47, 49, 81, 82, 123, 124, 241]. Moreover, redox conditions are also found to affect the

pharmaceutical removal [247]. Most pharmaceuticals are best degraded under aerobic conditions while others might need anoxic or anaerobic redox conditions [36, 83, 105]. Besides the design of a WWTP the characteristics of individual pharmaceuticals largely influences their fate during water treatment [312]. Within a given WWTP some pharmaceuticals are generally marginally removed (<5%) while others show removal percentages of over 95% [37, 287]. For instance, aspirin, estradiol, ibuprofen, and paracetamol are known to be effectively biodegraded during wastewater treatment, while crotamiton, diazepam and diclofenac are typically not eliminated [125]. In general, biodegradation and sorption are the mechanisms controlling pharmaceutical removal during biological wastewater treatment [267]. The biodegradability and sorption behaviour of an individual pharmaceutical largely depends on its chemical structure and physico-chemical properties. The great variety in observed removal mechanisms can therefore be explained by the broad array of chemical structures and physico-chemical properties of the pharmaceuticals (Table 1.1).

1.4.2 Room for improvement within biological treatment

The influence of the compound characteristics and the effect of the type of treatment plant configuration suggests that a variety of treatment conditions and processes with different modes of action might be needed for realising effective pharmaceutical removal. WWTPs could be upgraded or extended with additional treatment steps to reduce pharmaceutical emissions. On the one hand, this may be achieved by improving or extending current biological process. I hypothesize that this can be achieved by for instance the implementation of more diverse redox gradients over a treatment plant, oligotrophic zones for specific pharmaceutical degrading biomass, retention of specific biomass, non-continuous feeding (e.g. feed-famine regimes), longer hydraulic retention times, use of other biomass than activated sludge (e.g. fungi or algae). On the other hand, non-biological treatment processes can be applied for a better pharmaceutical removal.

1.4.3 Chemical treatment

Chemical treatment processes like advanced oxidation processes (AOPs) making use of photolysis, photocatalysis, ozone, hydrogen peroxide or Fenton

process, or sorptive processes like activated carbon (AC) addition or filtration can effectively eliminate pharmaceuticals [1, 164, 262]. Most chemical treatment processes originate from drinking water treatment, however they have also been successfully applied in wastewater treatment as a post-treatment step [109, 197, 264, 293, 303, 329]. AOPs rely on the direct or indirect oxidation of a compound [225]. Oxidative processes like ozonation typically break down compounds into smaller fragments, but do not completely mineralize compounds into CO_2 and H_2O [293]. Moreover, in a complex matrix like wastewater ozone will not only react with the target compounds (e.g. pharmaceuticals) but also with other matrix constituents. This reduces the process efficiency and results in the formation of countless by-products which possibly expose a higher toxicity than the pharmaceuticals treated [208]. Ozonation is therefore often combined with a subsequent AC filtration step [90, 134, 218]. Though AOPs can effectively remove pharmaceuticals they require high energy and/or chemical inputs. Sorptive processes like AC filtration rely on the interaction of a sorbate (e.g. a pharmaceutical) with a sorbent like AC. These systems do not transform sorbates but bind them onto the AC sorbent [198]. High pharmaceutical removal efficiencies can be obtained by AC filtration, however removal efficiencies decrease over time due to saturation of the AC and not all pharmaceuticals are effectively removed [96]. Once the sorbent is saturated it should be replaced or regenerated as non-regularly regenerated filters demonstrate low to nil pharmaceutical removal [244]. Disposal of saturated sorbent only shifts the problem, e.g. to landfilling, whereas regeneration requires high energy inputs. In summary, a possible solution to prevent pharmaceuticals entering the environment could be the upgrade of WWTPs supplemented with chemical treatment. Until now these processes have demonstrated higher removal efficiencies compared to biological treatment [164, 222, 287]. Although chemical treatment processes are promising for pharmaceutical removal the wide scale implementation is hampered by some major issues. They require high energy, chemical or material inputs which all translate into high operational costs and are not environmentally sustainable. Moreover, these processes often result in the formation numerous unknown by-products which possibly jeopardise the receiving aquatic environment to a greater extent than the compounds removed.

1.4.4 Combined treatment

Biological and chemical treatment processes for pharmaceutical removal both have their downsides and limitations, e.g. incomplete pharmaceutical removal, high energy and chemical inputs, high operational costs, environmental unsustainability. A combination of complementary treatment processes, abating the limitations of individual processes, could therefore be a versatile alternative. Scott and Ollis [235] reviewed the literature on combined biological and chemical treatment processes for the removal of organic contaminants from water and concluded that combined process can have advantages over single processes. When economical, physical and technological strengths and limitations of individual processes are recognized, the synergy of complementary processes can be used to design a cost-effective combination of processes.

In theory, avoiding the limitations and using the strengths of two different types of processes and to create synergy could be the combination of biological treatment and ozonation. The first is a cheap process but incapable of effectively removing all individual pharmaceuticals, e.g. clofibric acid and bezafibrate are known to be well removed, whereas diclofenac and carbamazepine are reported to be very poorly removed [124]. On the contrary, ozonation has high operational cost and results in by-product formation, for instance it hardly removes clofibric acid and bezafibrate, but effectively oxidises diclofenac and carbamazepine [264]. In addition, biological treatment can possibly be used to remove the harmful ozonation by-products as they are typically biodegradable [243].

In practice, various combinations of processes have already been studied and applied for the removal of organic contaminants from water. Though these studies often report enhanced removal efficiencies compared to single processes, they merely focus on a single compound often in model (simple) matrices rather than on a mixture of compounds in a complex matrix like wastewater [46, 80, 93, 107, 201, 297, 313]. Concerning the removal of a broad array of pharmaceuticals from wastewater, Hofman-Caris, et al. [109] found that anion exchange followed by UV/H_2O_2 treatment was a versatile combination of two chemical treatment processes. Biological activated carbon filtration, a combination of biological treatment and activated carbon filtration, was proven to effectively remove pharmaceuticals and reduce toxicity from WWTP effluents [217]. Ozonation followed by biological activated carbon filtration of WWTP effluent showed even

higher pharmaceutical removal [218]. Sand-filtration followed by ozonation of a secondary clarified effluent effectively removed estrogenicity, whereas sand-filtration only or sand-filtration followed by a moving-bed bioreactor were less effective [99]. Oller, et al. [198] reviewed the combination of AOPs and biological treatment for wastewaters containing pharmaceuticals. They concluded that photocatalysis, ozonation and ultrasound oxidation were well studied and typically result in an increased biodegradability and a decreased toxicity. Moreover, they concluded that the potential of combinations of AOPs and biological treatment is still under exploited since not many cost-effective combinations of chemical and biological treatment are available.

1.5 Knowledge gaps and research opportunities

The need to prevent pharmaceutical emissions into the environment for the abovementioned reasons related to ecological risks and human health, stresses the necessity for versatile treatment processes to eliminate pharmaceuticals from wastewater. As the research on pharmaceutical removal only started some decades ago [263] combined with the wide variety in possible treatment processes [164], there are still numerous knowledge gaps on the fundamental and practical aspects of pharmaceutical removal. These knowledge gaps concern pressing questions on single treatment processes as well as on the combination of treatment processes.

Taking into consideration that current WWTPs merely use biological treatment processes, this suggests that if biological pharmaceutical removal can be improved it would potentially be the most sustainable and cost-effective solution. First, within biological treatment, redox conditions are regarded as one of the key parameters in biological conversions [306]. Though the most prevalent redox conditions in biological processes at WWTPs (aerobic and anaerobic) are well studied [36, 83, 247], there is limited information on how specific redox conditions influence biodegradation and sorption behaviour of pharmaceuticals, which are regarded as the most important removal mechanisms during biological treatment.

Second, the type and presence of organisms utilized in biological treatment is of great importance. Typical biological processes at WWTPs make use of activated sludge, which is in essence a diverse microbial population consisting of mainly bacteria which are loosely bound in a settable floc. As raw wastewater

contains multiple orders of magnitude more easy biodegradable compounds than pharmaceuticals, it is likely that organisms degrading complex substrates, such as pharmaceuticals, are easily outcompeted. Providing oligotrophic environments with minimal substrate competition is therefore hypothesized to be an effective strategy to enrich for pharmaceutical degrading microorganisms. Moreover, pharmaceuticals are typically present in the ng/L to µg/L range, these low substrate levels only support minimal microbial growth, therefore retention of organisms is of great importance to successfully operate biological treatment processes.

In addition to the commonly used microbial populations in wastewater treatment, also other organisms can be employed for the typical objectives of a WWTP. One of the other organisms capable of wastewater treatment are green microalgae. These phototropic organisms are therefore utilized in some wastewater treatment configurations [3, 61, 100, 110]. Algal treatment is well studied for the effective removal and recovery of nutrients from highly concentrated wastewater streams like black water or urine [276, 285]. Little is known however, whether algae are capable of removing the high concentrations of pharmaceuticals which are also present in those streams [36, 157]. Moreover, as the recovered nutrients present in the algal biomass are potentially used as fertilizer in agriculture it is of great importance to known whether pharmaceuticals sorb onto the algal biomass to prevent their introduction into the food chain.

As long as biological treatment does not form an adequate barrier against the majority of the pharmaceuticals present in sewage, further research is also needed on other treatment processes or combinations of them. As described above, combinations of complementary processes are found to have beneficial effects over single processes and therefore have a great potential to reduce pharmaceutical loads into the environment. Nonetheless, there is still a lack of sustainable and cost-effective combined treatment options which motivates the further investigation into these treatment processes.

The work on algal treatment processes for wastewater treatment typically utilize photobioreactors to cultivate the algae [276, 285]. These reactors are illuminated to support algae growth, however the influence of illumination in photobioreactors on pharmaceutical removal is not well studied whereas it is known that photolysis in the natural environment can degrade pharmaceuticals

[140, 143]. As various removal mechanisms like algal degradation, microbial degradation, sorption to biomass and photolysis occur concurrently in photobioreactors, this provides an unique opportunity to study the synergy between biological and chemical treatment processes.

Photocatalysis and biodegradation are known complementary processes for the removal of various organic contaminants such as quinolone, pyridine and pesticides from water [297, 311, 322, 323]. For a limited number of individual pharmaceuticals this combination has been studied [93, 201, 307], but for pharmaceutical mixtures the effectiveness of the combined treatment remains unknown. Moreover, photocatalytic processes commonly require considerable amounts of energy inputs and are thereby not very sustainable. Hence, it is of great importance to improve the resource efficiency, e.g. in an energy efficient combination with biological treatment, before this treatment process can find its way to large scale application in wastewater treatment.

Ozonation and biological treatment is a potentially versatile combination of treatment processes for the removal of pharmaceuticals. Ozonation followed by biological treatment is well established in drinking water treatment [212, 293], but to a lesser extend in wastewater treatment [111, 329]. On the one hand, this is mainly due to the current absence of pharmaceutical removal requirements during wastewater treatment, whereas drinking water production requirements are much more strict. On the other hand, the composition of wastewater is highly different than drinking water sources like surface- or groundwater. Compared to matrices of drinking water sources, wastewater matrices (influent and effluent) typically consist of higher concentrations of a wide array of matrix constituents like organic matter, nutrients, trace elements and other chemicals such as pharmaceuticals, personal care products and pesticides. As ozonation targets multiple matrix constituents others than pharmaceuticals, post-treatment by ozonation is not very resource-efficient for the specific removal of pharmaceuticals. Most of the studies that investigated ozonation as tertiary WWTP step reported relatively low amounts of matrix constituents [111, 117, 329]. Matrices however, are known to be different at each individual treatment plant (e.g. due to different 'clients') and can also vary per geographical region (e.g. due to local water hardness and background natural organic matter). Getting a more comprehensive and local (Dutch) understanding of the versatility of this treatment is therefore of scientific

interest and can help decision makers in implementation of treatment processes. A well-studied aspect of ozonation in drinking water production is the formation of ozonation by-products. These are of concern as they potentially increase toxicity [118]. For ozonation in wastewater treatment toxicity aspects are studied to a lesser extent. Thus, besides increasing the resource efficiency, trapping or transforming the possibly harmful by-products is of great importance. Both issues motivate further research on the combination of ozonation and biological treatment and challenge researchers to come up with innovative ideas how to overcome the issues.

In summary, there are still many knowledge gaps on how to enhance pharmaceutical removal by single and/or combined treatment processes. Inherently, this shows the potential of research opportunities to improve single processes and to search for synergetic combinations of processes.

1.6 Objective of this dissertation

To prevent future pharmaceutical emissions into the environment, adequate measures for the removal of pharmaceuticals from WWTP effluents need to be developed. Development of wide scale implementation of such measures is hampered by the fact that known biological and chemical removal processes result in incomplete removal, and that insufficient insight exists in the functioning and effectiveness of processes used in the synergetic use of combined treatment processes. Therefore, the objective of this dissertation is to contribute to a better understanding and the further development of treatment processes for the removal of pharmaceuticals from wastewater.

1.7 Scope and outline of this dissertation

This dissertation focuses on the research opportunities on single and combined processes for pharmaceutical removal. The experimental work of this dissertation aims at a better understanding of specific aspects of biological treatment processes, like the effect of redox conditions and the type of involved microorganisms, and the interaction between biological and chemical removal processes. Moreover, combinations of treatment processes were developed for a better pharmaceutical removal. Among the broad spectrum of pharmaceuticals a

limited amount was selected for the experimental work of this dissertation. Selection criteria were; consumption, occurrence in wastewater, physico-chemical properties and removal efficiencies during biological treatment. The selected pharmaceuticals are among the most sold in the Netherlands [283] and commonly found in wastewater [287]. Furthermore, they cover a wide range of physico-chemical properties and their reported removal efficiencies in WWTPs range from high to low removal [164].

The influence of specific redox conditions on the biological removal of pharmaceuticals is elucidated in Chapter 2 with a focus on the influence of redox conditions on biodegradation and sorption behaviour.

In Chapter 3 we describe the combination of a mild photocatalytic treatment process and a subsequent biological treatment process for a resource-efficient pharmaceutical treatment.

In Chapter 4 we present a three-step biological-ozone-biological process combining the strengths of biological treatment and ozonation with the aim to minimize the energy input and hydraulic retention time, while effectively removing pharmaceuticals and their associated toxicity.

In Chapter 5 we focus on the removal of pharmaceuticals by algae in alternative sanitation systems. We investigated the effectiveness of an algal photobioreactor for the simultaneous removal of nutrients and pharmaceuticals.

Finally, in Chapter 6 findings and outcomes of the presented work in this dissertation are synthesized and reflected. An outlook on single and combined treatment processes for pharmaceutical removal is given, including the identification of current knowledge gaps and recommendations for further research and the scale up of the processes studied in this dissertation. Attention is paid to the developments in analytical chemistry, including metabolite and transformation product identification, and the need for effect based removal strategies. Moreover, developments in other fields such as restrictive discharge legislation, the role and awareness of other actors in the pharmaceutical chain and the benefits of alternative sanitation systems are discussed as environmental problem solving generally requires a multidisciplinary approach. All together, the synergy between biological and chemical treatment processes and developments in legislation, awareness of other actors and benefits of alternative sanitation systems can be a boost towards lower pharmaceutical emissions into the environment.

Chapter 2

Sorption and biodegradation of pharmaceuticals under different redox conditions

A modified version of this chapter has been published as

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* Equally contributing authors

Abstract

This study explored the removal of six pharmaceuticals in lab-scale experiments with sediments under four redox conditions, namely aerobic, nitrate reducing, sulfate reducing, and methanogenic conditions using batch and column set-ups. Redox conditions were found to influence pharmaceutical removal by sorption and biodegradation. The most optimal pharmaceutical removal was observed at the outer ranges of the redox spectrum, i.e. either aerobic or deeply anaerobic (sulfate reducing and methanogenic conditions), whereas nitrate reducing conditions were found least effective for pharmaceuticals biodegradation and sorption. For instance, sorption coefficient Kd values for metoprolol in column experiments were 90, 65, 42 and 11 L/kg for sulfate reducing, methanogenic, aerobic and nitrate reducing conditions, respectively. For the same conditions Kd values for propranolol were 101, 94, 55 and 55 L/kg, respectively. As expected, biodegradation efficiencies were highest under aerobic conditions, showing >99% removal of caffeine and naproxen, but no removal for propranolol and carbamazepine. The adaptive capacity of sediment was demonstrated by pre-exposure to pharmaceuticals leading to improved pharmaceutical biodegradation. The results of this study indicate the necessity to combine diverse redox conditions, including aerobic conditions, for maximizing pharmaceutical removal by sorption and biodegradation. Furthermore, our findings stress the need for additional treatment measures as recalcitrant pharmaceuticals are not effectively removed under any redox condition.

Keywords

Pharmaceuticals; Redox conditions; Sorption; Biodegradation; Sediment

2.1 Introduction

Pharmaceuticals were developed to target specific human physiological pathways [180]. After consumption, residual pharmaceuticals or/and their metabolites are excreted from human bodies into sewage systems. Conventional wastewater treatment plants (WWTPs) are not specifically designed for removing pharmaceuticals [230]. Therefore, pharmaceuticals that are not completely removed are discharged to the aquatic environment and may even reach drinking water intakes [40]. In this context, efficient post-treatment technologies for removing pharmaceuticals are needed and emerging.

Biological technologies are robust and attractive as post-treatment processes. However, processes involved in biotechnological systems are more complex and require a proper understanding to come to a robust design and operation. In most biological technologies, both sorption and biodegradation play an important role in removing organic contaminants. It is well known that organic matter, temperature and pH affect sorption of organic contaminants [183], however the effect of redox conditions (electron acceptors) on sorption behaviour on organic contaminants is less known. On the contrary for biodegradation, specific electron acceptors select for specific microbial communities with different targeted functions, and thereby strongly influence the biological removal of organic contaminants [84]. For example, transformation of sulfamethoxazole was reported to strongly depend on the occurrence of nitrate reducing conditions and be sensitive to the concentration of nitrate [21].

Although redox conditions are identified as one of the controlling factors for biodegrading pharmaceuticals, the reported dependencies of removal processes on redox conditions vary significantly for specific pharmaceuticals for various reasons. Firstly, most of the previous works only study the removal efficiencies of pharmaceuticals under oxic and anoxic conditions without identifying the dominant terminal electron acceptor [309, 330]. Secondly, results reported for pharmaceutical biodegradation in terms of redox effects are often contradictory. For example, sulfamethoxazole (SMX) was proven to be more rapidly eliminated under anoxic conditions than under aerobic conditions in bank filtration in the work of Heberer et al. [106], while Baumgarten et al. [24] concluded that SMX was more rapidly removed under aerobic conditions compared to anoxic conditions. Conkle et al. [53] concluded that degradation of carbamazepine (CBZ) was

enhanced under aerobic conditions as compared to anaerobic conditions in sediment collected from three types of wetlands; in contrast, Hai et al. [101] reported that CBZ showed degradation only in an anoxic environment instead of under oxic conditions in a membrane bioreactor. Furthermore, the various studies that report the effects of redox conditions on pharmaceutical removal are difficult to compare as they use different reactor setups, different concentrations and different compounds.

Thus, there is a significant knowledge gap on comparative effects of redox conditions on removal of pharmaceuticals in biotechnological systems. To get a more comprehensive understanding of the influence of selected redox conditions on specific pharmaceutical removal via sorption and biodegradation, it is necessary to investigate this in defined experimental setups varying the applied redox conditions. Therefore, the objective of this study is to elucidate the influence of redox conditions on removal mechanisms of six pharmaceuticals applying four specific conditions of the wide redox spectrum. Batch and column systems were used for controlled biological tests under aerobic, nitrate reducing, sulfate reducing, and methanogenic conditions. The results of this study give insight into understanding the influence of redox conditions on pharmaceutical removal in biotechnological systems.

2.2 Materials and Methods

2.2.1 Chemicals and regents

Pharmaceuticals metoprolol (MET), caffeine (CAF), propranolol (PRO), carbamazepine (CBZ), naproxen (NAP), ibuprofen (IBP) were purchased from Sigma-Aldrich (U.S.). Details of the pharmaceutical stock solution, other chemicals used and physio-chemical properties of pharmaceuticals are given in Text S2.1 and Table S2.1.

2.2.2 Experimental setup

2.2.2.1 Sediment

Sediment of constructed wetlands (CWs) at WWTPs Hapert and Land van Cuijk (both in the Netherlands) was collected as a solid phase of the batch and column systems. In addition, the sediments contain microorganisms that serve as

a natural inoculant of the biologically active laboratory systems. CWs at both facilities have received WWTP effluent for several years. Sediment dry matter (DM) and organic matter (OM) content were determined gravimetrically after drying at 105 °C following combustion at 550 °C.

The aerobic column was inoculated with upper layer sediment (0-5 cm) with an OM content of 6.2 g OM/kg DM. Sediment at a depth of 10-20 cm below the surface level with an OM content of 16.2 g OM/kg DM was collected to inoculate the anaerobic columns. A mixture of upper, deeper layer, and rhizosphere sediment was used for batch experiments containing 19 g OM/kg DM. Concentrations of the six pharmaceuticals varied from 0 to 777 ng/g in CW sediment [104].

2.2.2.2 Batch experiments

Four different media were used to enrich dominant bacteria in different redox conditions. Media were prepared according to previous works for aerobic (Table S2.2), nitrate reducing [79], sulfate reducing [145], and methanogenic conditions [112]. The ionic strength of media was calculated by OLI Studio Analyzer 9.2 software. In each batch bottle, 120 mL medium was mixed with 15 g wet sediment as inoculum. Batches were spiked with a mixture of six pharmaceuticals at 1 mg/L each. For analytical reasons the spiking concentration is far above the environmental relevant concentrations of pharmaceuticals, as also been applied in previous works [2, 119]. The gas phase of each bottle was filled with either atmospheric air for aerobic conditions or CO_2/N_2 (20/80, v/v) for anaerobic conditions. Among the four redox conditions, no extra carbon source was added except for the pharmaceuticals. Abiotic controls contained 1.3 g/L of sodium azide in aerobic batch bottles and 0.3 g/L of mercury chloride in anaerobic bottles to supress microbial activity.

The aerobic batch experiment lasted for six weeks and the anaerobic batch lasted for three months. Samples of week 0 were collected the day after spiking to ensure a homogeneous distribution of pharmaceuticals. In order to determine the role of microbial adaptation, mixed pharmaceuticals were re-spiked three times in the aerobic batches (biotic and abiotic) for enrichment after week 6. Batch bottles were incubated on a shaker (120 rpm) at 20°C. Batch bottles were incubated in the dark during the experiment to prevent photolysis. Compared to

the spiked pharmaceutical levels, the initial pharmaceutical concentrations in the sediment were minute and thus desorption of the initial pharmaceuticals to the liquid phase is negligible. Sorption coefficient Kd was calculated from the abiotic controls as the ratio of the pharmaceuticals concentration in the sediment phase and in the water phase at equilibrium. The concentrations of sorbed pharmaceuticals were calculated from the measured water phase concentrations based on mass balance.

2.2.2.3 Column experiments

Column experiments were conducted in four continuous-fed upflow soil columns (Figure 2.1). Identical to the batch experiments, aerobic, nitrate reducing, sulfate reducing and methanogenic conditions were tested. Cylindrical glass columns (0.23 L) were packed with sediment containing 292 g DM and 230 g DM for respectively aerobic and anaerobic experiments. Sediment was retained in the columns by sintered glass filters (pore size 40-100 µm). The packed sediment was circularly homogenised biweekly, while keeping sediment layers at their depths in the columns. This is needed to prevent bypass flows through the columns. Columns were fed with medium at a flow rate of 9.8±0.4 mL/h, resulting in a hydraulic retention time of 8.2±0.3 h. For each redox condition, specific media were prepared (Table S2.2), which contained about 100 µg/L pharmaceuticals during the experiment of 110 days. Prior to the experiment columns were run two weeks on media without pharmaceuticals to allow redox conditioning. Columns and media were kept in the dark during the experiment to prevent photolysis.

Influent and effluent grab samples were collected for chemical analysis within 24 hours from each other per time point. Pharmaceutical removal was calculated based on effluent concentrations per time point over the averaged influent concentration of the entire experiment to deal with fluctuations in the influent concentration. Sorption parameter values (specific sorption coefficient Kd) were determined for sorptive pharmaceuticals by calculating the retardation factor from the time point when the effluent pharmaceutical concentration reached fifty percent of the influent concentration.

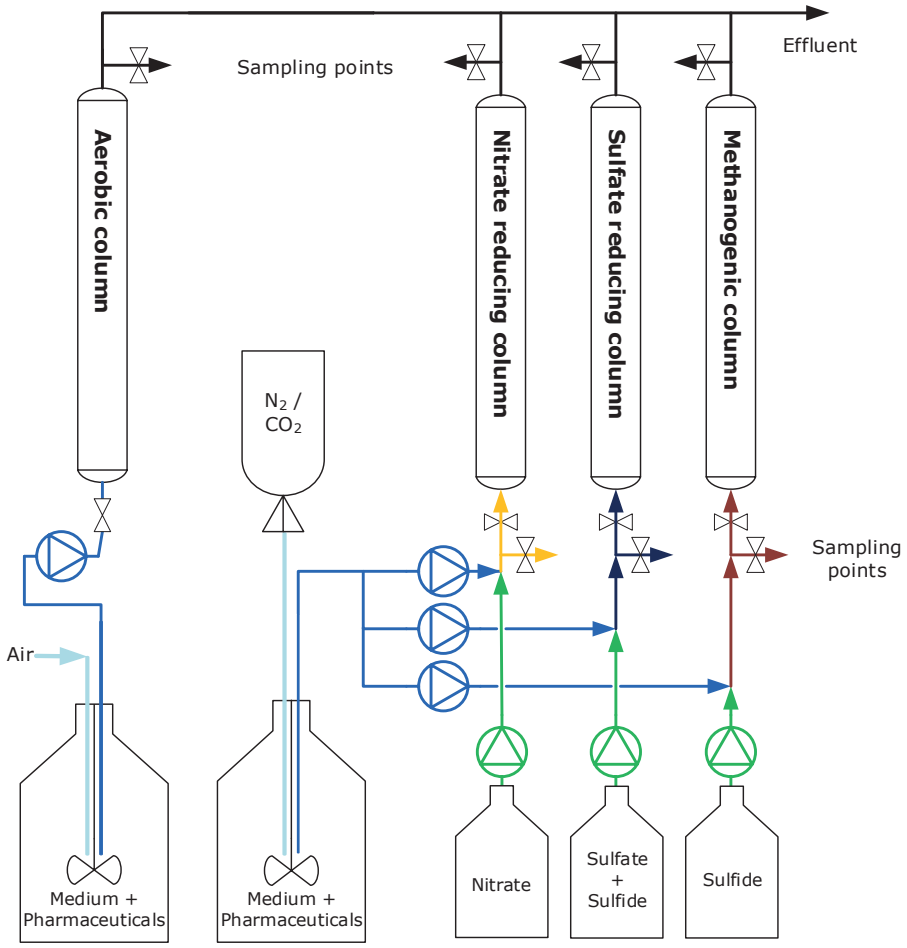


Figure 2.1 Experimental set-up of the column experiments.

2.2.3 Sample collection and analysis

Liquid samples were taken from batch media and column influents and effluents for pharmaceutical measurements. Samples collected from batch bottles were centrifuged at 10 000 rpm for 10 min and stored at -20 °C prior to analysis. Before analysis liquid samples of column experiments were pre-treated by solid phase extraction (SPE) to allow the detection of low concentrations in column effluents. Oasis HLB SPE cartridges (6 cc/60 mg, Waters, U.S.) were pre-

conditioned with 5 mL methanol and equilibrated with 5 mL buffered deionized water (10 µL buffer/mL, pH=10, Merck, Germany). Cartridges were loaded with 3 mL and 9 mL for influent and effluent samples, respectively. Loaded cartridges were washed with 5 mL buffered deionized water and eluted with 10 mL 25% NH_4OH: methanol (8/92, v/v). Eluates were evaporated till dryness under a gentle nitrogen flow. Samples were reconstituted in 1 mL 3.6% methanol. Prior to SPE, 10,11-dihydrocarbamazepine was spiked to samples as an internal standard.

Pharmaceutical analysis was conducted on a liquid chromatograph with a diode array detector as described by He et al. [103]. Quantification was based on the internal standard and external calibration standards. SPE recoveries for all compounds in all samples were within 85-115% and thereby valid for quantification.

Concentrations of nitrite, nitrate, and sulfate were measured by ion chromatography. Before measurement, samples were filtered using a 0.45 µm cellulose filter (VWR, U.S.) and diluted five times with MilliQ water. A Dionex ICS-2100 IC system (Thermo, U.S.) was used for analysis. The system was equipped with an anion exchange column (Dionex, IonPac AS19, 4×250 mm), where the anions were separated using a hydroxide gradient. The eluent was made automatically using the eluent generator configured with a KOH cartridge (Dionex P/N 058900) and deionized water as the carrier. Detection was done by a DS6 Heated conductivity cell.

Oxygen and methane from the headspace of batches were analysed on a gas chromatograph. The instrument (GC-2010, Shimadzu, Japan) contained a parallel combination column: Porabond Q (50 m x 0.53 mm; 10 µm) and Molsieve 5A (25 m x 0.53 mm; 50 µm). The carrier gas was helium and operated at 0.95 bar. Column temperature was 80°C, detector temperature was 150°C, and injection temperature was 120°C. Pressure in the batch was measured by a digital pressure meter (GMH 3151, Greisinger) for calculating gas volume. In the aerobic column dissolved oxygen was measured by optical oxygen meter Fibox 3 trace (PreSens, Germany) fixed near the outlet of the column. For methanogenic column experiments, dissolved methane in liquid effluent samples was turned into gaseous methane as described by Zhang et al. [319] and analysed by GC as described above.

2.3 Results

2.3.1 Redox conditions

Consumption of electron acceptors and accumulation of respiration products were observed in all batches and columns (Figure S2.1). Under aerobic conditions, O_2 consumption was observed. Nitrate and sulfate consumption were detected under nitrate and sulfate reducing conditions, respectively. Production of CH_4 was observed under methanogenic conditions. These results indicated that the desired redox conditions were achieved.

2.3.2 Removal of pharmaceuticals

2.3.2.1 Batch systems

Outcomes from abiotic controls are used to characterize the sorption capacity of the used sediment for targeted pharmaceuticals. In general, a high sorption capacity of CW sediment was found for NAP, CAF, PRO, and MET while CBZ and IBP were much less sorbed (Table 2.1). Sorption of pharmaceuticals under aerobic conditions were not used to compare with anaerobic conditions, as the sorption did not achieve equilibrium under aerobic conditions while it did under anaerobic condition due to the longer cultivation. When comparing pharmaceutical sorption under different anaerobic redox conditions, nitrate reducing conditions showed lower sorption capacity for MET, NAP, and CAF compared to sulfate reducing and methanogenic conditions.

Biodegradation in batches was determined by comparing biotic and abiotic removal efficiencies. Under aerobic conditions, MET, CAF, NAP and IBP were almost completely removed within 6 weeks, which was mainly contributed to biodegradation as sorption only contributed to less than 30% (Table 2.1). In comparison, PRO and CBZ were poorly biodegraded considering their similar biotic and abiotic removal. Under anaerobic conditions, only CAF was readily biodegraded under nitrate and sulfate reducing conditions. Removal of CAF under methanogenic conditions was mainly caused by sorption (Table 2.1). Similarly, the observed removal of PRO, NAP, and MET was a result of their high sorption rather than biodegradation. Under all anaerobic conditions IBP and CBZ were poorly removed in the biotic and abiotic batches.

To determine the role of microbial adaptation, the aerobic inoculum in batches was enriched to select for pharmaceutical removal by re-spiking pharmaceuticals. During the first six weeks, NAP and IBP were approximately 60-70% removed within one week and complete removal was reached at week 3 (Figure 2.2). After re-spiking, complete removal of NAP and IBP was reached within one week. This was mainly contributed to biodegradation as sorption almost achieved saturation after re-spiking (Figure S2.2). Therefore, microbial adaptation by pre-exposure did accelerate the biodegradation of NAP and IBP. Half-lives of NAP and IBP were 1.0 and 1.8 days, respectively; half removal of MET and CAF reached less than 1 day (Figure S2.3).

Table 2.1 Biotic and abiotic removal (%) of pharmaceuticals in batches under different redox conditions: 6 weeks cultivation under aerobic conditions; 3 months cultivation under anaerobic redox conditions.

Redox condition	Type	PRO	MET	CAF	NAP	IBP	CBZ
Aerobic	Biotic	81	95	100	100	96	43
	Abiotic	55	29	27	15	25	22
Nitrate reducing	Biotic	55	-9[a]	94	14	-9	-14
	Abiotic	77	-32	27	26	25	28
Sulfate reducing	Biotic	n.d.[b]	56	95	94	-1	-16
	Abiotic	n.d.	60	48	93	-5	24
Methanogenic	Biotic	66	52	95	94	5	3
	Abiotic	67	32	85	87	-3	22

a - Negative removal might be caused by analytical deviation; b - Not determined as the PRO concentration under sulfate reducing conditions was accidently very low (0.05 mg/L), which is close to the detection limit.

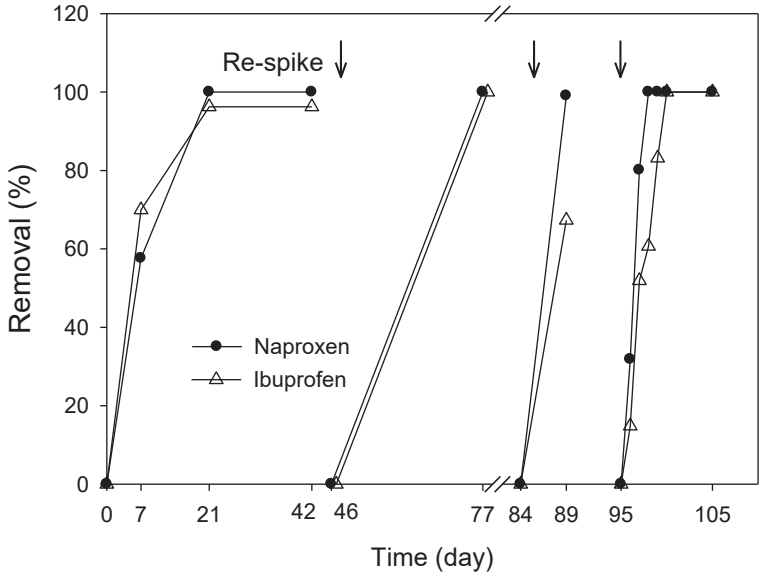


Figure 2.2 Removal of naproxen and ibuprofen under aerobic condition with re-spike of pharmaceuticals at day 46, 84, and 95.

2.3.2.2 Column systems

Different removal patterns were observed for individual pharmaceuticals in columns among the tested redox conditions (Figure 2.3). Main removal mechanisms were interpreted based on the observed removal patterns. Sorption was indicated to be a dominating removal process when initial pharmaceutical removal was followed by breakthrough of that compound. Biodegradation was identified by the presence of a lag phase, significant removal and absence of subsequent breakthrough behaviour. IBP and CBZ for which no significant sorption or biodegradation was observed in any of the columns were classified as persistent (Figure 2.3).

Significant removal (>95%) of PRO and MET followed by compound breakthrough was apparent under all redox conditions (Figure 2.3). Therefore, sorption was identified to be their dominant removal mechanism. Sorption of PRO and MET appears to be affected by the applied redox conditions. Methanogenic and sulfate reducing conditions indicated better sorption compared to aerobic and nitrate reducing conditions for PRO and MET. Especially the removal of MET under nitrate reducing conditions was limited compared to the other anaerobic redox conditions whereas the sediment was identical.

CAF appeared initially to be removed by sorption in each column (65-99%) (Figure 2.3). However, the sorption capacity of the sediment towards CAF was low compared to PRO and MET as breakthrough behaviour was observed after day 4 in all columns. Under all redox conditions, biodegradation of CAF took place after a lag phase: under aerobic and nitrate reducing conditions the lag phase lasted 27 days, while only after 67 days an increase in removal was found in the sulfate reducing and methanogenic columns.

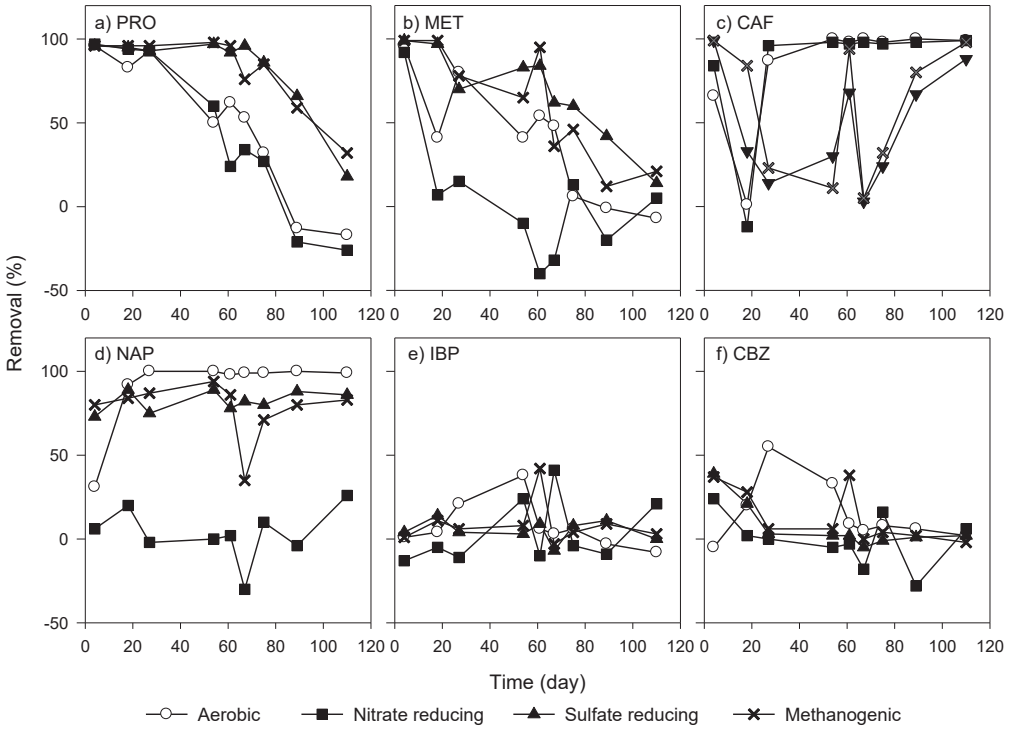


Figure 2.3 Removal of pharmaceuticals under different redox conditions in continuous flow-through columns: a) PRO; b) MET; c) CAF; d) NAP; e) IBP; f) CBZ. Negative removals are due to temporal fluctuations in influent concentrations.

The removal of NAP showed significant differences among the tested redox conditions. Complete NAP removal (>99%) was achieved in the aerobic column after a lag phase of 27 days. The presence of the lag phase followed by the continuously high removal indicates that biodegradation was the main removal mechanisms for NAP under aerobic conditions. Insignificant NAP removal (<25%) was observed under nitrate reducing conditions (Figure 2.3). In the sulfate reducing and methanogenic columns, a constant NAP removal throughout the experiment of around 80% was found. This phenomenon could not be attributed to sorption, as there was neither breakthrough of NAP nor a constant complete NAP removal. Chemical NAP removal by the reactor medium was also excluded as demonstrated in a separate test. Therefore most likely biodegradation is the main removal mechanism for NAP under sulfate reducing and methanogenic conditions. Why the removal did not improve over time, as would be expected due to the growth of more specialized NAP degrading microorganisms in a biological system, is not fully understood.

2.4 Discussion

2.4.1 Sorption of pharmaceuticals

In both batches and columns, stronger sorption was indicated for PRO, MET and CAF while CBZ and IBP showed no significant sorption. NAP was highly sorbed in batches under sulfate reducing and methanogenic conditions but not in columns. Values of pharmaceutical sorption coefficient Kd were calculated for batch systems under all anaerobic conditions. In columns the breakthrough behaviour of PRO, MET and CAF allowed the calculation of the retardation factor and Kd. All these Kd values were compared to literature data on sorption behaviour of target pharmaceuticals in soils and sediments (Figure 2.4). Kd values found in this study are in accordance with reported literature values, except for MET in columns. Like coefficients from literature, we also demonstrate that PRO, CAF and MET sorbed more readily than NAP, CBZ, and IBP (median, Figure 2.4).

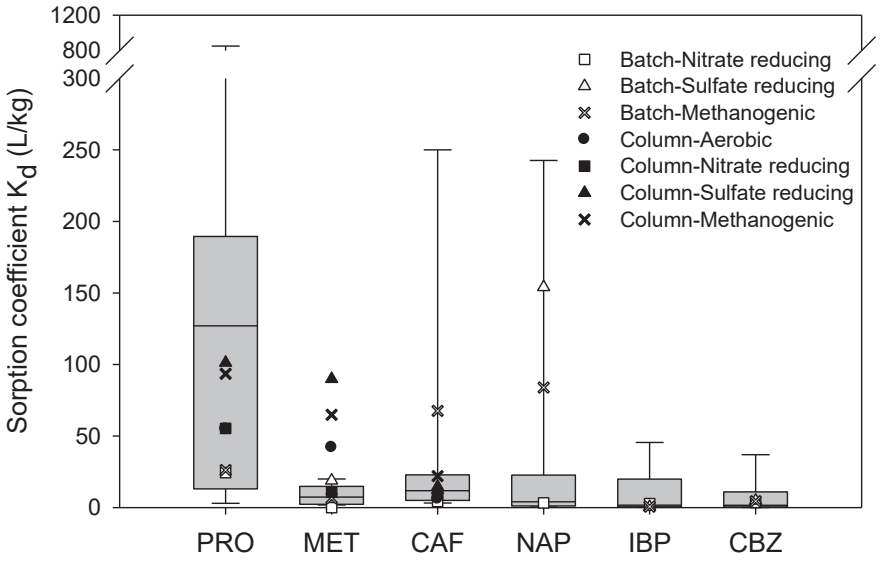


Figure 2.4 Comparison of sorption coefficients Kd between this study and literature [23, 53, 68, 70, 159, 166, 170, 219, 231, 296, 308, 310, 320, 324]. For literature values of PRO, MET, CAF, NAP, IBP and CBZ, n= 12, 7, 6, 15, 19, and 8, respectively. The box plot shows the values found in literature in maximum, third quartile, median, first quartile, and minimum. The squares, triangles, crosses and circles represent the values for the different redox conditions in batch (no filling) and column (filled).

Sorption of pharmaceuticals was reported to depend on their physico-chemical properties, such as hydrophobicity and molecular charge [35]. In this study, we found that pharmaceutical sorption by the sediment is more related to the molecular charge of pharmaceuticals than their hydrophobicity. To characterize the hydrophobicity, the apparent partitioning coefficient Log D_{ow} (Table S2.1) was applied to modify the octanol-water partition coefficients Log K_{ow} with pH [149]. However, pharmaceutical sorption did not correlate with Log D_{ow} (Figure S2.4). Based on pKa values (Table S2.1) and the pH range in our study (pH = 6.8-7.5), CBZ has no charge, IBP and NAP are negatively charged, while the other three pharmaceuticals are positively charged. Most sediment matrix components are negatively charged [166, 310]. This likely explains why the cationic pharmaceuticals (CAF, PRO, MET) showed a stronger tendency to sorb compared to anionic (IBP) and neutral species (CBZ). The high sorption of anionic NAP in batches might be caused by the OM present in the sediment. Similar results were

found by Martínez-Hernández, et al. [166]. These authors observed that sorption onto the inorganic surface of sediment was the predominant sorption mechanism for all positively and negatively charged pharmaceuticals examined with the exception of NAP, which was partitioned to OM instead. The higher OM content in batches in this study might explain its higher sorption compared to the columns.

The variety among the reported Kd values per pharmaceutical in literature (Figure 2.4) are hypothesized to be related to the different experimental conditions, e.g. different soils and sediments [2]. By the unique experimental design of this study, we showed a strong influence of the redox conditions on the experimentally determined Kd values (Figure 2.4). Anaerobic column experiments were inoculated with the same inoculum. Similarly, all anaerobic batches were inoculated with the same sediment. However, different pharmaceutical sorption coefficients were found under different anaerobic redox conditions in both batches and columns (Figure 2.4). Sorption of NAP in batches was found to be lowest under nitrate reducing conditions and highest under sulfate reducing conditions. A similar phenomenon was found for PRO sorption in columns and MET sorption in both systems that followed the order nitrate reducing < methanogenic < sulfate reducing conditions. In both systems, sorption of CAF followed the order nitrate reducing < sulfate reducing < methanogenic conditions.

The sorption differences under various anaerobic redox conditions could not be explained by the characteristics of the media we applied, such as ionic strength. Among all pharmaceuticals investigated in batches, only CAF sorption showed to be inversely correlated to ionic strength with 149.5, 108.1 and 63.8 mmol/L under nitrate reducing, sulfate reducing and methanogenic conditions, respectively. However, the correlation identified for CAF in batches was not observed in columns, where ionic strength are similar under three anaerobic conditions (50.4, 69.3, and 42.6 mmol/L under nitrate reducing, sulfate reducing and methanogenic conditions, respectively). Overall, among all factors influencing sorption behaviour, redox conditions and molecular charge are found to dictate sorption behaviour in this study.

Sorption coefficients Kd of MET and CAF under different redox conditions correlated between batches and columns (Figure 2.5), which indicates a consistency among the two experimental systems. However, the Kd variations between pharmaceuticals seem to be influenced by the experimental system, as

the sorption coefficients of individual pharmaceuticals were not distributed along the 1:1 line (Figure 2.5). Batch experiments are commonly conducted to predict the sorption behaviour of target compounds in continuous systems, however our results demonstrate a high system dependency of the determined Kd values, which hampers the direct translation of Kd from batch to continuous systems. Thus, our data show that a variability in experimentally determined Kd values per pharmaceutical can be introduced by differences in redox conditions and in experimental system. As the effect of redox conditions and experimental system on sorption behaviour of pharmaceuticals is rarely described before, further studies need to be conducted to understand the underlying mechanisms.

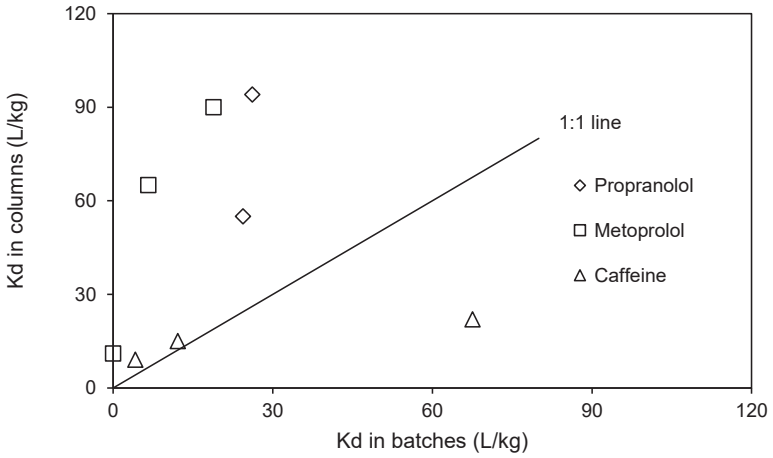


Figure 2.5 Linear relationship of sorption coefficients between batch and column experiments under different anaerobic redox conditions.

Sorption coefficients Kd in our column study show that breakthrough is expected in sediment based applications. For the most sorptive pharmaceutical, PRO in this study, saturation of sediment sorption sites occurred after approximately 300 pore volumes under sulfate reducing conditions. Ramil et al. [213] demonstrated that sorption isotherms of PRO and MET to river and stream sediments are linear between 2-200 µg/L. For linear sorption behaviour retardation factors are independent on the pharmaceutical concentration. Thus, reflecting on sediment based applications treating pharmaceuticals in WWTP effluents that are generally in the same concentration range, the sorption capacity of sorptive

pharmaceuticals like PRO will reach saturation after approximately 300 pore volumes.

2.4.2 Biodegradation of pharmaceuticals

Aerobic batch and column experiments both demonstrated an effective CAF and NAP biodegradation. Their significant biodegradation is consistent with their biodegradation in soil columns reported by Kim et al. [130]. Additionally, in batches IBP and MET were biodegraded, whereas in columns no significant IBP removal was observed and MET was removed by sorption. The poor removal of IBP is contrary to what other authors reported [144], most probably due to insufficient oxygen throughout the entire aerobic column. The applied hydraulic retention time of 8 hours is in a similar range of 3-18 hours for which IBP removal is reported [81], and seems therefore long enough. Zwiener and Frimmel [330] concluded that IBP is primarily degraded under aerobic conditions and poorly in the absence of oxygen in biofilm reactors. Throughout our experiment, no dissolved oxygen (DO) was found in the aerobic column effluent as it was completely consumed in the column. Hence, it is assumed that competition for DO resulted in micro-aerophilic conditions with a too low DO concentration to support aerobic IBP biodegradation. MET was biodegraded and sorbed in batches whereas sorption was the only removal mechanisms in columns. Similar to IBP removal in column, a low DO concentration resulting in micro-aerophilic conditions might be a limiting factor in MET biodegradation in column which was also observed by Radke and Maier [211].

Anaerobic batch and column experiments both demonstrated an effective CAF biodegradation. To our knowledge, there is a lack of studies on CAF biodegradation under anaerobic conditions in sediment. Among the anaerobic conditions, NAP biodegradation was observed in the sulfate reducing and methanogenic columns. In sulfate reducing and methanogenic batches NAP biodegradation could not be confirmed, as NAP removal was mainly ascribed to sorption. In accordance with the literature, PRO and CBZ were not effectively biodegraded under any redox condition in both batch and column experiments [83, 207, 211].

In our study using well-defined redox conditions, batch and column experiments independently showed that biodegradation of pharmaceuticals was

influenced by the applied redox conditions (Table 2.2). Most optimal pharmaceutical biodegradation was observed at the outer ranges of the redox spectrum, i.e. either aerobic or deeply anaerobic (sulfate reducing and methanogenic conditions), with fastest removal by biodegradation under aerobic conditions. In batch experiments, NAP, MET and IBP were readily biodegradable under aerobic conditions while they were recalcitrant under all anaerobic conditions. A similar conclusion was identified for IBP biodegradation by Conkle et al. [53]. Half-lives of IBP were 7-19 days or >7 months under aerobic or anaerobic conditions, respectively.

Intermediate redox conditions like the micro-aerophilic and nitrate reducing conditions, which are often found in groundwater systems [26, 72, 169], appear not suitable for maximized biodegradation of a wide range of pharmaceuticals. According to previous research on anaerobic biodegradation of fuel hydrocarbons which also contain aromatic rings like pharmaceuticals, nitrate and sulfate appeared to be the most preferred electron acceptors for degrading toluene, ethylbenzene and xylenes [254]. Nevertheless, in our study NAP was poorly biodegraded under nitrate reducing conditions in the column, while it was effectively biodegraded under the other two anaerobic redox conditions.

Table 2.2 Biodegradation of pharmaceuticals under the tested redox conditions in batch and column. Biodegradation observed (+), no biodegradation observed (-).

Pharmaceutical	Aerobic		Nitrate reducing		Sulfate reducing		Methanogenic	
	Batch	Column	Batch	Column	Batch	Column	Batch	Column
PRO	-	-	-	-	-	-	-	-
MET	+	-	-	-	-	-	-	-
CAF	+	+	+	+	+	+	-	+
NAP	+	+	-	-	-	+	-	+
IBP	+	-	-	-	-	-	-	-
CBZ	-	-	-	-	-	-	-	-

Biodegradation of pharmaceuticals is hypothesized to be limited in the natural environment as oxygen is typically absent in soils and sediments [195]. Thus, maximizing biodegradation of the complete suite of pharmaceuticals present in surface and wastewater should include aerobic conditions possibly combined with deeply anaerobic redox conditions (i.e. to save WWTP aeration costs), and is inadequate under micro-aerophilic and nitrate reducing conditions. In engineered

sediment based systems such as CWs, we therefore recommend to include aerobic conditions and deeply anaerobic conditions.

Pre-exposure to pharmaceuticals can improve the biodegradation capacity of sediments towards pharmaceuticals. Batch experiments in this work showed a better removal for IBP, NAP, CAF and MET, compared to half-life times reported in literature [53, 161, 320]. The improved removal can be explained by the exposure of the sediments to pharmaceuticals prior to the laboratory scale experiments. Sediments used in this study were collected from CWs receiving secondary effluents of WWTPs and were in operation for more than 15 years. We detected the target pharmaceuticals in the CW influents in the range of 59-6492 ng/L (Table S2.3) demonstrating that the sediments have been exposed to pharmaceuticals. Adaptation of microorganisms to compounds can significantly improve biodegradation rates [245]. Especially for microbes capable of degrading micropollutants, pre-exposure to the compounds can enhance their biodegradation ability [42, 172]. Enrichment experiments by re-spiking pharmaceuticals in batch further improved the biodegradation rates of IBP and NAP. Thus, the significance of microbial growth by pre-exposure to pharmaceutical resulting in enhanced biodegradation was further confirmed.

Pharmaceuticals demonstrating low to no removal by sorption and biodegradation under all redox conditions even after re-exposure, like CBZ, require additional treatment. In natural environments photo-oxidation has been reported to target pharmaceuticals in surface waters [140]. However, this is restricted to a limited number of pharmaceuticals [143] and photo-oxidation is typically not relevant in soil-sediment systems. Hence, in addition to sorption and biodegradation, other technologies such as advanced oxidation processes are needed in WWTPs to remove recalcitrant pharmaceuticals from treated effluents before they enter the natural environment.

Conclusions

In conclusion, our results show that pharmaceutical removal is influenced by the applied redox conditions via both sorption and biodegradation. Therefore this study provides insights into the importance of redox conditions in developing and designing biological techniques for pharmaceutical treatment. Sorption behaviour among different pharmaceuticals is influenced by the applied redox

conditions and the molecular charge of pharmaceuticals. Hydrophobicity of pharmaceuticals and ionic strength did not have an effect on pharmaceutical sorption in this study. Highest sorption coefficient Kd values are found under sulfate reducing and methanogenic conditions. Additionally, our study indicate a high variability in experimentally determined Kd values per pharmaceutical in different experimental system, i.e. batch and column systems. Most optimal pharmaceutical biodegradation is observed at the outer ranges of the redox spectrum, i.e. either aerobic or deeply anaerobic (sulfate reducing and methanogenic conditions), instead of the intermediate redox conditions like micro-aerophilic and nitrate reducing conditions. Fastest pharmaceutical biodegradation is observed under aerobic conditions. From practical perspective, saturation of the sediment sorption capacity in sediment based applications treating pharmaceuticals is expected within approximately 300 pore volumes for the most sorptive compounds in this study.

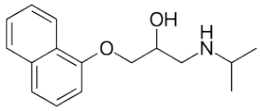
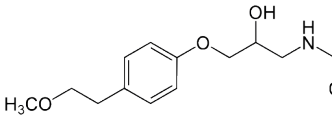
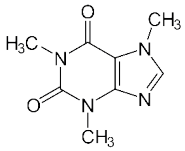
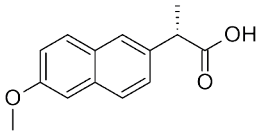
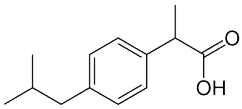
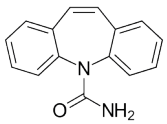
To increase removal efficiencies for a number pharmaceuticals, several strategies are recommended: adapt and enrich biomass by pre-exposure to pharmaceuticals in bioactive soil, sediment or other porous media filters; combine aerobic conditions with deeply anaerobic redox conditions in biotechnological systems; add advanced oxidation processes in WWTPs for removal of recalcitrant pharmaceuticals.

Acknowledgement

We thank Yang Jiang and Linda Verweij for their support during the experiments. Support provided by China Scholarship Council for the research of Yujie He at Wageningen University is kindly acknowledged.

Supplementary Information

Table S2.1 Physico-chemical properties of target pharmaceutically active compounds

Pharmaceutical	Chemical structure	pKa[a]	Log K_{ow}[b]	Log K_{oc}[c]	Log D_{ow}[d]
Propranolol		9.42	3.48	3.45	1.06
Metoprolol		9.67	1.88	2.10	-0.79
Caffeine		10.4	-0.07	1	-3.47
Naproxen		4.15	3.18	2.54	0.33
Ibuprofen		4.91	3.97	2.60	1.88
Carbamazepine		13.9	2.45	3.588	-4.45

a - pKa: dissociation constant; b - Log K_{ow}: octanol-water partition coefficient; c - Log D_{oc}: soil organic carbon-water partitioning coefficient; d - Log D_{ow} is the pH dependent Log K_{ow}, calculated at pH=7. Data from [71, 278].

Table S2.2 Medium composition for all columns and aerobic batches, adjusted from [162].

	Reactor	Compounds	Concentration in medium (mg/L)
Macro nutrients	Aerobic batches and all columns	NH_4Cl	1020
		$CaCl_2.2H_2O$	48
		$MgSO_4.7H_2O$	54
pH buffer	Aerobic batches and all columns	Na_2HPO_4	433
		NaH_2PO_4	234
Trace elements	Aerobic batches and all columns	$FeCl_2.4H_2O$	1.2
		$CoCl_2.6H_2O$	1.2
		$MnCl_2.4H_2O$	0.3
		$CuCl_2.2H_2O$	0.018
		$ZnCl_2$	0.03
		HBO_3	0.03
		$(NH_4)6Mo_7O_{24}.4H_2O$	0.05
		$Na_2SeO_3.5H_2O$	0.06
		$NiCl_2.6H_2O$	0.03
		EDTA (tripex 2)	0.6
		HCl 36%	0.0006
		Resazurin	0.3
Redox specific compounds	Nitrate reducing columns	$NaNO_3$	850
	Sulfate reducing columns	$NaSO_4$	1190
		$Na_2S.9H_2O$	120
	Methanogenic columns	$Na_2S.9H_2O$	120

Note: aerobic column medium was 60-80% oxygen saturated, other column media were flushed with N_2 and did not contain oxygen.

Table S2.3 Concentration of pharmaceuticals (ng/L) in the influent of the constructed wetland where the sediment inoculum was collected (average ± deviation, n=2).

Pharmaceutical	PRO	MET	CAF	NAP	IBP	CBZ
Concentration	58±10	1426±35	118±22	180±17	6492±261	204±7

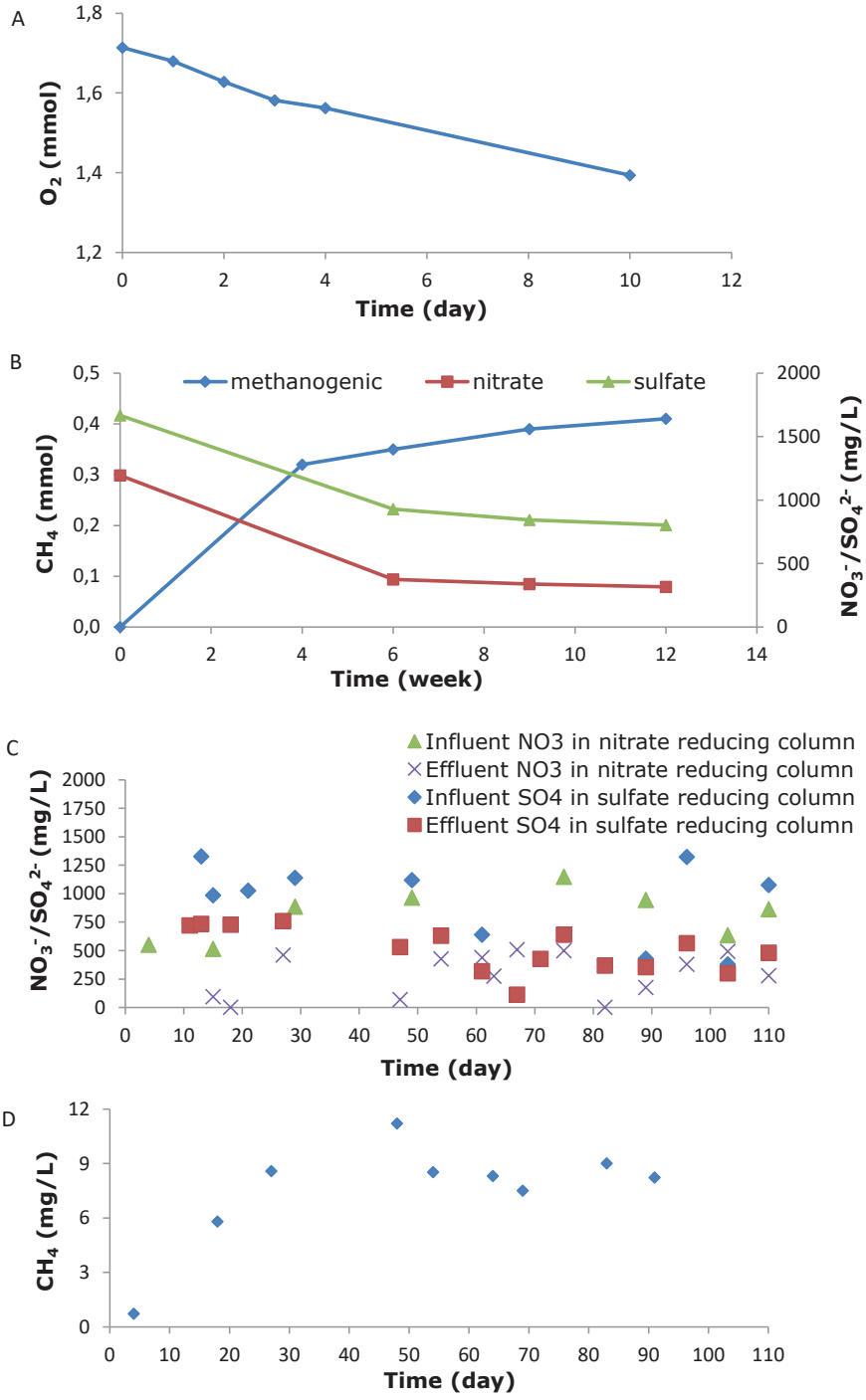


Figure S2.1 Concentrations of electron acceptors and reaction products. A) O_2 concentration in aerobic batch experiment; B) CH_4, NO_3^-, and SO_4^{2-} concentrations in anaerobic batch experiment; C) influent and effluent NO_3^- and SO_4^{2-} concentrations in nitrate and sulfate columns; D) effluent CH_4 concentration in the methanogenic column.

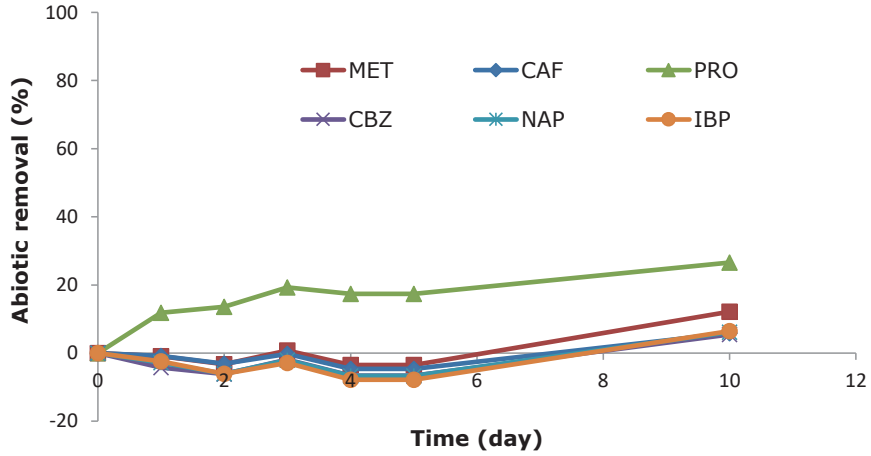


Figure S2.2 Abiotic removal of pharmaceuticals after re-spiking under aerobic conditions.

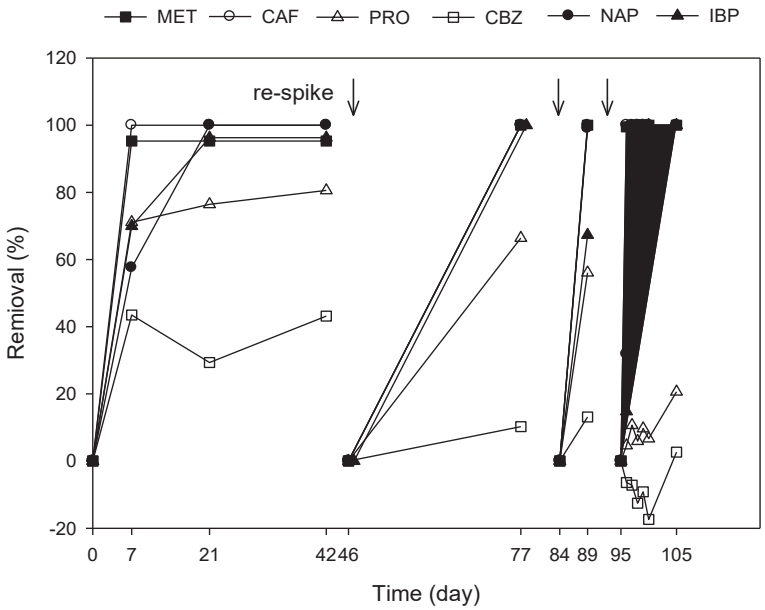


Figure S2.3 Removal of pharmaceuticals under aerobic conditions. Pharmaceuticals were re-spiked three times at day 46, 84, and 95.

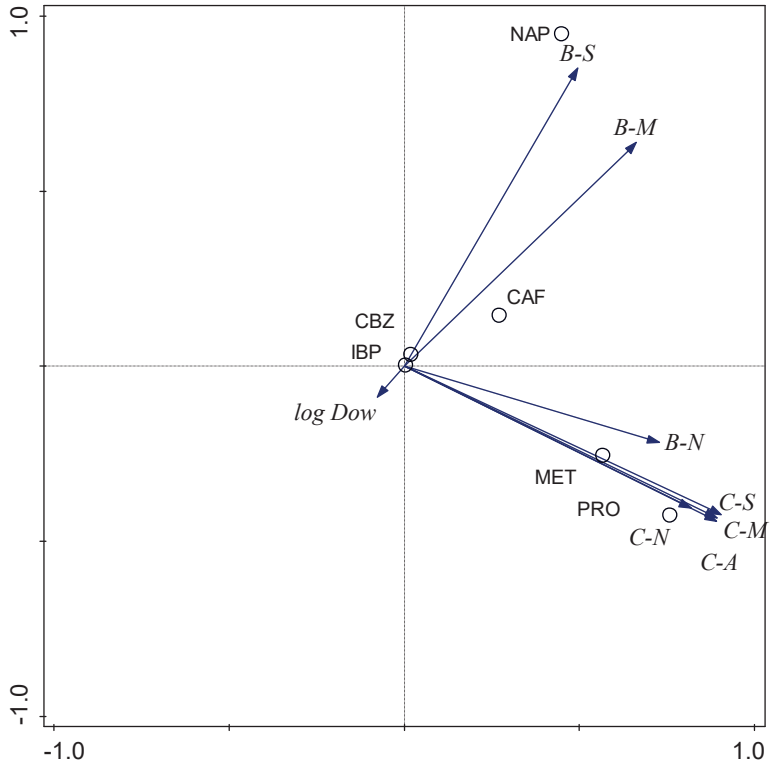


Figure S2.4 Principle component analysis of the relationship between Log D_{ow} of pharmaceuticals and their Kd under the applied redox conditions. B-N, B-S, and B-M represent nitrate reducing, sulfate reducing, and methanogenic conditions in batches; C-A, C-N, C-S, and C-M represent aerobic, nitrate reducing, sulfate reducing, and methanogenic conditions in columns. The eigenvalues of the first and second canonical axis are 0.56 and 0.39. The intersection angles between arrows represent their correlations, in which a more acute intersection angle means stronger correlations. The results show that Lod D_{ow} of pharmaceuticals is not correlated with the Kd value under any applied redox conditions.

Chapter 3

Mild photocatalytic pre-treatment: improved biological degradation of degradable and otherwise recalcitrant pharmaceuticals

A modified version of this chapter has been submitted as

Arnoud de Wilt, Maricor Arlos, Mark Servos, Huub Rijnaarts, Alette Langenhoff and Wayne Parker. Mild photocatalytic pre-treatment: improved biological degradation of degradable and otherwise recalcitrant pharmaceuticals.

Abstract

Pharmaceuticals in the environment are of great concern as they jeopardise the aquatic environment and pose potential risks to human health. This stresses the need for technologies to remove pharmaceuticals before they enter the environment. Single processes for pharmaceutical removal are often found ineffective and resource intensive. In this study the combination of photocatalysis and biodegradation was investigated for the removal of nine selected pharmaceuticals. We designed a combined process consisting of a resource efficient mild photocatalysis and a subsequent biological treatment which was compared to the single processes of intensive photocatalysis and biological treatment. During the UV-TiO_2 based mild and intensive photocatalysis atorvastatin, atenolol and fluoxetine were effectively removed. The biological treatment after mild photocatalytic pre-treatment removed diclofenac effectively while it persisted during the single biological treatment. Moreover, the biodegradation of atorvastatin, caffeine, gemfibrozil and ibuprofen was enhanced after mild photocatalytic pre-treatment compared to single biological treatment. The enhanced biodegradation of those pharmaceuticals was most probably triggered by the biodegradation of photocatalytic products. Biodegradation was the predominant removal mechanism and sorption was of minor importance during biological treatment as shown by abiotic controls. These findings show that mild photocatalysis followed by biological treatment is an effective and resource efficient combination for pharmaceutical removal.

Keywords

Photocatalysis; Biodegradation; Pharmaceuticals; Pre-treatment; Combined treatment

3.1 Introduction

The occurrence of pharmaceuticals in the aquatic environment has become a worldwide environmental concern [233]. After administration, 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 [124, 287]. Advanced oxidation processes (AOPs) making use of photocatalysis, ozone, or hydrogen peroxide (the latter often in combination with Fenton's Reagent), can effectively eliminate pharmaceuticals [164]. The main advantage of AOPs is the complete oxidation of organic contaminants in a wide variety of applications [132]. 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 [118]. Furthermore, the various AOP reaction mechanisms are mostly non-specific, 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 [187].

Using the strengths of AOP and biodegradation in a combined technology could possibly result in better overall pharmaceutical removal. Scott and Ollis [235] 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 [235].

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 [80, 115, 313, 314]. 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 [107]. 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 [311]. Photocatalytic pre-treatment that reduced COD by 8-10%, enhanced the subsequent biodegradation of the dye intermediate H-acid [186]. Chun and Yizhong [46] demonstrated the advantage of combining photocatalysis with biodegradation for wastewater containing non-biodegradable 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 [258].

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 pre-treatment by ozonation [93] and photocatalysis [307]. In the study of Gómez-Pacheco, et al. [93] tetracycline in the influent inactivated the microbial population of biological waste water treatment, whereas AOP pre-oxidation resulted in 100% mineralisable TOC and stable biological treatment. Biodegradation and mineralization of the broad-spectrum antibiotic sulfadiazine could be accelerated by 35 and 71%, respectively, when intimately coupled to photocatalysis [201]. 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 [297]. Complete degradation and detoxification of the herbicide atrazine was obtained by photocatalytic pre-treatment followed by biodegradation. The single photocatalytic treatment resulted in complete atrazine removal but inefficiently mineralized and detoxified the transformation products [44].

The main aim of the current study was to gain insight into the influence of AOP pre-treatment on the subsequent biodegradation of pharmaceutical compounds, as there are only few reports describing this in literature. Since AOP

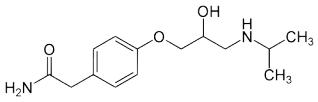
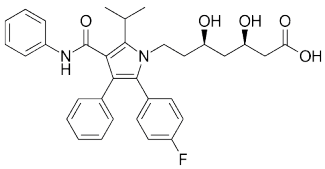
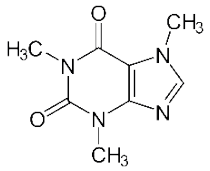
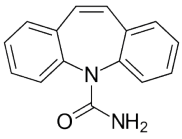
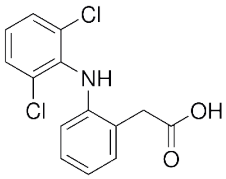
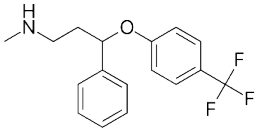
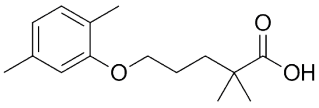
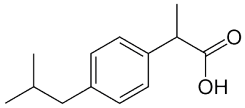
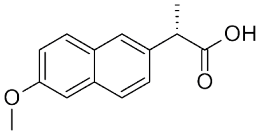
processes are generally energy and/or chemically intensive, we developed a mild photocatalysis method requiring low energy input. The removal of nine commonly detected pharmaceuticals in wastewater was studied by combining this mild photocatalysis method and biodegradation in batch experiments. The pharmaceuticals 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.

3.2 Materials and Methods

3.2.1 Chemicals

A pharmaceutical stock solution that contained 9 compounds (Table 3.1): atenolol, atorvastatin, caffeine, carbamazepine, diclofenac, fluoxetine, gemfibrozil, ibuprofen and naproxen was employed. The stock solution (2 g/L) 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. Suppliers for all other reagents and chemicals used in this study are described in detail elsewhere [14].

Table 3.1 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 

3.2.2 Experimental set-up

Figure 3.1 displays the three experimental protocols that were studied: 1) intensive photocatalytic treatment (IP); 2) single process biological treatment (B); and 3) mild photocatalytic pre-treatment followed by biological treatment (MP+B).

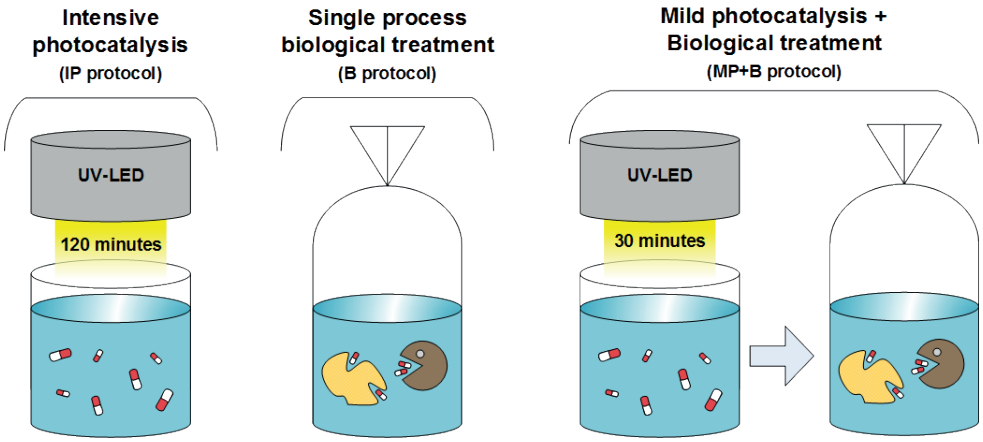


Figure 3.1 Experimental protocols, 1) intensive photocatalytic treatment (IP), 2) single process biological treatment (B) and 3) mild photocatalytic pre-treatment followed by biological treatment (MP+B).

3.2.2.1 Photocatalytic experiments

Batch photocatalytic experiments were performed with the set-up described by Arlos, et al. [15]. 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. The batches were spiked with 150 µL of the pharmaceutical stock solution to obtain concentrations of 500 µg/L (6 mM methanol). Prior to light exposure the batches were equilibrated for 30 minutes in the dark. For intensive photocatalytic experiments 2 mL samples were taken directly before the lamps were activated and after 15, 30, 45, 60 and 120 minutes of illumination. In the separately performed mild photocatalytic experiments, samples were taken directly before illumination and after 15 and 30 minutes of illumination. The contents of the beakers after 30 minutes of light exposure in the mild photocatalytic experiments were centrifuged (3500 rpm, 30 minutes) 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 minutes to assess non-photocatalytic pharmaceutical removal.

3.2.2.2 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 demineralised 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 in Chapter 2. 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) was 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 [8]. Batch experiments were performed in triplicate and incubated at room temperature on a shaker plate. Abiotic controls were initially amended with 0.5 mM NaN_3 to supress biological activity and closed with a rubber stopper. However, oxygen consumption in the abiotic batches could not be fully suppressed, showing that biologic activity was insufficiently inhibited. Additional NaN_3 spikes of 0.5 mM on days 8, 12 and 13, 0.75 mM on day 14 and 1 mM on days 15 and 17 were added and did not completely supress oxygen consumption. Ternes et al. [266] reported that 30 minutes after spiking the sorption equilibrium for pharmaceuticals was reached in batch experiments with 2.4 g VSS/L of secondary sludge. Similarly, Martínez-Hernández et al. [167] achieved sorption equilibrium of pharmaceuticals after 12 hours in batch experiments containing 250 g soil/L. Hence, due to this fast sorption equilibrium only the abiotic results of day 1 were used to assess sorption behaviour 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.

3.2.3 Pharmaceutical analysis

Samples from the AOP experiments were directly centrifuged for 45 minutes at 3500 rpm to separate the liquid phase and TiO_2. After thawing the samples from the biological experiments were centrifuged for 10 minutes 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. [15] but only using 2 mL sample instead of 4 mL was used for SPE in this study.

3.3 Results and Discussion

3.3.1 Photocatalysis

3.3.1.1 Intensive photocatalysis (IP protocol)

Experimental results of the illuminated batches and controls were compared to assess the contribution of photocatalysis to pharmaceutical removal. The results of the control experiments revealed that non-photocatalytic removal processes contributed little (<25%) to the pharmaceutical removal in our AOP experiments, as has been reported in the literature [15]. By contrast significant removals (>80%) of atorvastatin, atenolol and fluoxetine were observed in intensive photocatalytic (IP) experiments after 2 hours (Figure 3.2a). The other tested compounds were less susceptible to photocatalysis and were removed by less than 40%. Photocatalytic degradation of organic compounds has been described by Langmuir-Hinshelwood kinetics [229]. A simplified 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 reaction rate constants were calculated according to the abovementioned equation. For atorvastatin, atenolol and fluoxetine the calculated rate constants (R2 >0.99%) were 41.5, 29.0 and 15.4 x 10^{-3} min^{-1}, respectively. These results agreed with the findings of the experimental work and the literature reviewed by Arlos, et al. [15] on photocatalytic degradation of pharmaceuticals. Their reported rate constants for atorvastatin, atenolol and fluoxetine ranged between 13.4-68.8, 7.4-14.5 and 8.4-40.8 x 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 x 10-3 min^{-1} (R2 <0.88). Similarly, Arlos, et al. [15] found low rate constants (<8.7 x 10-3 min^{-1}) to no degradation for naproxen, ibuprofen and carbamazepine.

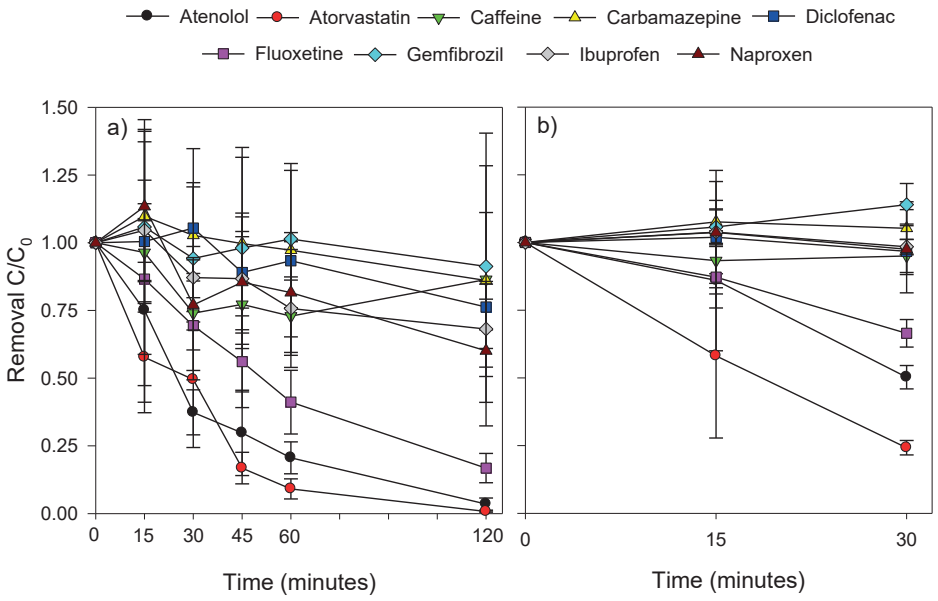


Figure 3.2 Pharmaceutical removal (C/C_0) during photocatalysis of a) IP protocol and b) MP+B protocol. Error bars represent standard deviations between triplicates.

3.3.1.2 Mild photocatalysis (MP+B protocol)

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 minutes 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 minutes of illumination to allow for 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%) (Figure 3.2b). 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). This indicates the robustness of the photocatalysis process for pharmaceutical removal.

3.3.2 Biological treatment

3.3.2.1 Single process biological treatment (B protocol)

The B protocol was employed to assess the biodegradability of the target pharmaceuticals (Figure 3.3). 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 Figure 3.3, it can be seen that 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 [7, 287]. In addition, to parse out the removal mechanisms of individual pharmaceuticals abiotic (Table 3.2) and biotic (Figure 3.3) batches were compared in more detail.

The concentrations of caffeine, ibuprofen, naproxen and atorvastatin were reduced significantly (>99%) within 7, 7, 14 and 21 days, respectively. Partial gemfibrozil removal (80%) was found after 21 days. These findings agree with those of Luo, et al. [164] who reviewed the fate of pharmaceuticals in wastewater treatment and classified caffeine, ibuprofen, gemfibrozil and naproxen as "highly removed (>70%)". The findings for atorvastatin correspond well with those of Golovko et al. [92] where an average of 93% atorvastatin removal was observed on a yearly basis in a Czech WWTP. It was concluded that biodegradation was the prevalent removal mechanism for caffeine, ibuprofen, gemfibrozil, atorvastatin and naproxen in our experiments as during the first day their concentrations in the abiotic batches decreased by only 18-34% (Table 3.2).

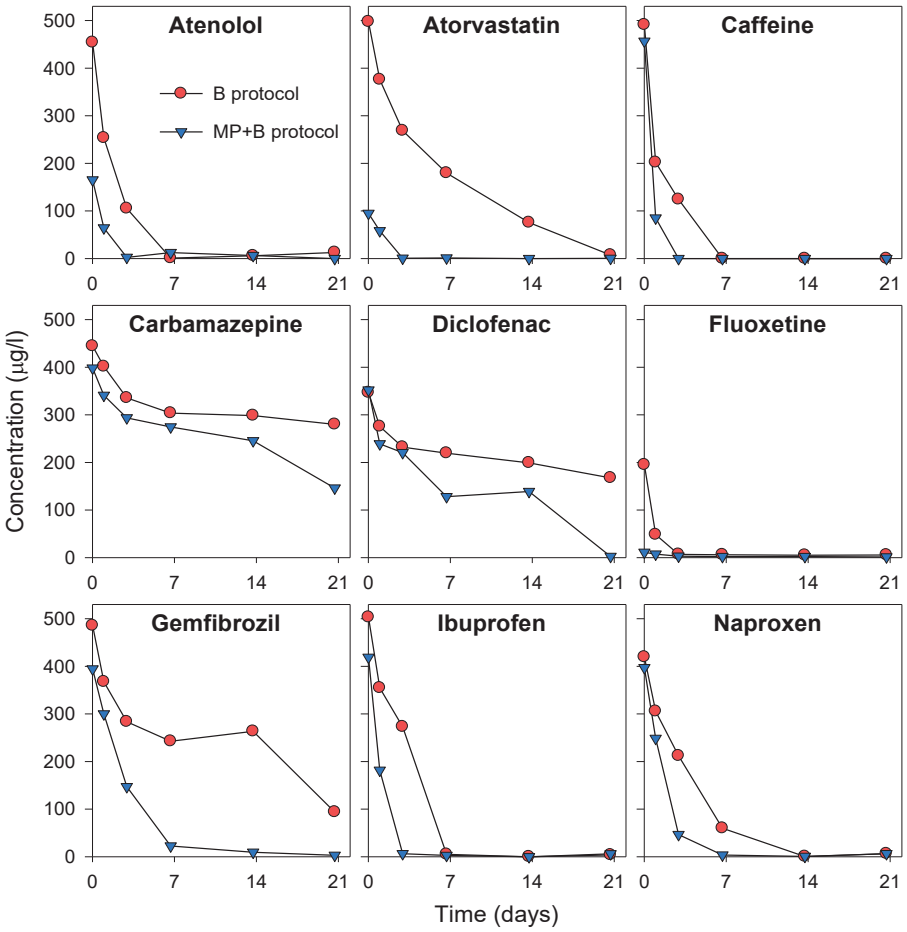


Figure 3.3 Pharmaceutical concentrations in biological treatment experiments, B protocol (red spheres) and MP+B protocol (blue triangles).

Fluoxetine concentrations decreased rapidly in both the biotic and abiotic batches (Table 3.2, Figure 3.3) indicating that sorption was the main removal process. 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 1 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. [206] who concluded that sorption was the only removal mechanism for fluoxetine.

Table 3.2 Pharmaceutical removal (C/C_0) after 1 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%

The atenolol concentrations at day 1 were reduced by 53% in the abiotic batches as compared to 44% in the biotic batches (Table 3.2, Figure 3.3), suggesting 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 [176, 206]. The low sorption coefficients reported in the literature were contrary 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). Kodešová et al. [135] found a positive correlation between clay content and atenolol sorption which was driven by cationic exchange between negatively charged clay particles and positively charged atenolol at neutral pH.

3.3.2.2 Biological treatment after mild photocatalysis (MP+B protocol)

High pharmaceutical removals (>99%) were observed after 21 days in the biological experiments of the MP+B protocol (Figure 3.3). Only carbamazepine was incompletely removed (65%) by the end of the experiments. The initial concentrations of atorvastatin, and atenolol were lower compared to the B protocol due to their photocatalytic degradation during pre-treatment. The low initial fluoxetine concentration was attributed to removal during photocatalysis and rapid sorption to the inoculum as observed in the B protocol. Moreover, approximately 50% of the fluoxetine was lost during the centrifugation and filtration steps that were carried out between the photocatalytic and biodegradation experiments. The predominant 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 faster removal was found for atorvastatin, caffeine, diclofenac, gemfibrozil and ibuprofen in the biological experiments of the MP+B protocol as compared to the B protocol. Results of the ANCOVA are provided in Table S3.1. Out of these pharmaceuticals, only atorvastatin was partially eliminated (75%) during mild photocatalytic pre-treatment. The enhanced atorvastatin removal may have been due to a co-substrate effect as reported by other authors. Yan, et al. [311] found faster quinoline removal during biological treatment after photolytic pre-treatment. They suggested this could result from quinoline and its photolysis products being simultaneously biodegraded and thereby both contributing to biomass growth. Further, enhanced biodegradation induced by photolysis products was demonstrated for sulfadiazine by Pan, et al. [201], 2,4,6-trichlorophenol by Wang, et al. [297] and for pyridine by Zhang, et al. [323]. These authors reported that biodegradation of the main photolysis product generated intracellular electron carriers that initiated the initial mono-oxygenation reaction for biodegradation of target compounds. In sunlit surface water two photolysis products of atorvastatin were found, one as a result of N-dealkylation, the other formed by photonucleophilic aromatic substitution of the F atom by OH [143]. Similar to the 2,4,6-trichlorophenol photolysis the dehalogenation of atorvastatin could indicate the formation of a more readily biodegradable product.

Enhanced biological removal of caffeine, diclofenac, gemfibrozil and ibuprofen was observed after pre-treatment, even though they were not significantly removed during mild photocatalysis. Contrary to the B protocol results in which diclofenac was classified as recalcitrant, 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. Although 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. By contrast, pre-treatment did not improve the removal of naproxen in subsequent biodegradation testing while naproxen was effectively biodegraded in the B protocol.

The molecular structures of the pharmaceuticals (Table 3.1) were compared to assess whether there was a relationship between structure and the biological removal responses observed after pre-treatment. A carboxyl group is present in atorvastatin, diclofenac, gemfibrozil and ibuprofen but also in naproxen. Caffeine is the only pharmaceutical without a phenyl ring, yet not the only one exhibiting enhanced biodegradation. Atorvastatin, caffeine, gemfibrozil and ibuprofen have methyl groups, as does naproxen, whereas diclofenac does not have it. In particular, the response of naproxen after pre-treatment 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 [209, 239, 302, 326], and occur mostly co-metabolically [209, 302]. The reported ibuprofen degradation pathways are hydroxylation, dealkylation followed by hydroxylation, demethylation followed by O-hydroxylation and demethylation followed by dehydrogenation [30]. Hence, the literature reveals a limited overlap in the broad and complex array of transformation pathways for naproxen and ibuprofen. Moreover, co-metabolic processes appear to be important for naproxen removal. These factors could possibly explain why naproxen removal was not enhanced after the mild photocatalytic pre-treatment. Overall, no clear relation was found between the chemical structure and biodegradation responses of the pharmaceuticals after pre-treatment.

Atenolol and carbamazepine removal efficiencies were similar in the biological experiments conducted for the B and MP+B protocols. Atenolol sorption to clay particles was hypothesized to explain the lack of difference observed in removal efficiency between the two protocols, indicating that photocatalytic pre-treatment did not affect atenolol sorption. The similar removal of carbamazepine with or without photocatalytic pre-treatment was attributed to the general persistence of carbamazepine towards biodegradation, as observed in many

previous studies [164, 287]. 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 pre-treatment with a subsequent biological treatment was found to be an effective combination of processes to improve the removal of pharmaceuticals. Scott and Ollis [235] 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 pre-treatment. Furthermore, biodegradable pharmaceuticals like atorvastatin, caffeine, gemfibrozil and ibuprofen were biodegraded at a higher rate compared to biological treatment without pre-treatment. Hence, our study demonstrates that mild photocatalytic pre-treatment and biodegradation are complementary processes and their synergy can result in a better pharmaceutical removal compared to the single processes.

3.4 Conclusions

Sequentially combined mild photocatalysis and biological treatment effectively removed 8 out of 9 studied pharmaceuticals. The biological degradation of 5 pharmaceuticals improved after mild photocatalytic pre-treatment in a subsequent biological treatment of which only atorvastatin was removed during mild photocatalysis. Biodegradation of the atorvastatin photocatalytic degradation products most probably triggered the enhanced atorvastatin biodegradation by initiation of mono-oxygenation reactions. Caffeine, diclofenac, gemfibrozil and ibuprofen were not susceptible to photocatalysis, however their biodegradation efficiency enhanced after mild photocatalytic pre-treatment. During intensive and mild photocatalysis 3 out of 9 pharmaceuticals were removed, atenolol, atorvastatin and fluoxetine. We hypothesize that their photocatalytic products resulted in the enhanced biodegradation of caffeine, diclofenac, gemfibrozil and ibuprofen. Similar observed photocatalytic rate constants of the separately performed intensive and mild photocatalytic experiments indicate the process robustness of photocatalysis for pharmaceuticals removal. 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. Carbamazepine was recalcitrant

towards photocatalysis and biodegradation. 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 an enhanced biodegradation of biodegradable and recalcitrant pharmaceuticals susceptible and non-susceptible to photocatalysis.

Acknowledgement

We would like to thank Leslie Bragg for her support on the analytical work and Robert Liang for providing the experimental setup and the P25 powder. We kindly acknowledge financial support from the Association Canada Studies the Netherlands and the Knowledge-to-Knowledge project within the Partners in Business Water and Soil Canada cluster.

Supplementary Information

Table S3.1 Significance output of the ANCOVA statistical model in which we considered time as a covariate. Results are the significances of the difference in pharmaceutical removal (C/C_0) per compound between the single process biological experiments (B protocol) and the mild photocatalytic pre-treated biological experiments (MP+B protocol). A significance of <0.05 was considered significant (highlighted in green).

Pharmaceutical	Atenolol	Atorvastatin	Caffeine	Carbamazepine	Diclofenac	Fluoxetine	Gemfibrozil	Ibuprofen	Naproxen
Significance	0.219	0.011	0.044	0.250	0.002	n.d.	0.008	0.024	0.184

n.d. – not determined because too few data points available due to rapid sorption

Chapter 4

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

A modified version of this chapter has been submitted as

Arnoud de Wilt, Koen van Gijn, Tom Verhoek, Amber Vergnes, Mirit Hoek, Huub Rijnaarts and Alette Langenhoff. Enhanced pharmaceutical removal from water in a three step bio-ozone-bio process

Abstract

Individual treatment processes like biological treatment or ozonation have their limitations for the removal of pharmaceuticals from secondary clarified effluents with high total organic carbon (TOC) concentrations (i.e. 17 mg/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 hours 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 O_3/g TOC and an HRT of 0.3 hours. At HRTs of 0.47 and 1.46 hours 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 wastewater treatment costs by 15%, whereas the yearly tariff per population equivalent in the Netherlands is estimated to increase by less than 10%. Overall, the BO_3B process is a cost-effective treatment process for the removal of pharmaceuticals from secondary clarified effluents.

Keywords

Biodegradation; Ozonation; Combined treatment; Pharmaceuticals; Wastewater

4.1 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 [283]. After administration pharmaceuticals are excreted and disposed into the sewer [85]. This results in elevated pharmaceutical concentrations in wastewater, as is illustrated by measurements of numerous studies over the past decades [188, 228]. Many pharmaceuticals are persistent in conventional wastewater treatment plants (WWTPs) [222, 287]. Pharmaceutical levels in WWTP effluents jeopardize the aquatic environment [77, 85, 141, 325] and reach drinking water resources [188]. 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 [164]. Ozonation is an effective technique to oxidize pharmaceuticals [116]. Ozone targets electrophilic compounds that contain double bonds, aromatic structures or amine groups which are often found in the chemical structure of pharmaceuticals [193]. Ozonation leads to shorter and more oxidized products which are not further broken down by ozone, but are more susceptible to biodegradation [243]. High removal rates can be obtained by ozonation, but process efficiency reduces when other compounds than the pollutants of interest are present [187]. 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 [6]. However, toxic by-products can be formed during ozonation [118], resulting in the need of a subsequent treatment step after ozonation [222]. 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 [134]. 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. In general, biological treatment requires low energy and chemical inputs. However, complex molecules like pharmaceuticals present at low concentrations are challenging to biodegrade [164]. For many pharmaceuticals insufficient degradation rates result in incomplete removal in biological processes applied at WWTPs [124]. Moreover,

specific micropollutant degrading microorganisms are easily outcompeted by other microorganisms that depend on easily degradable substrates present at higher concentrations [155].

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 [235]. Effective degradation and mineralisation of the degradation products was achieved in a combined ozone-biological process for the widely prescribed antibiotic tetracycline [93]. Biodegradation and mineralisation of the antibiotic sulfadiazine were accelerated by UV-photolysis pre-treatment by 35 and 71%, respectively, [201].

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 to investigate the process removal efficiency. Applied ozone dose and the hydraulic retention time (HRT) of the bioreactors are the studied key-parameters to adjust the process performance. The aim 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.

4.2 Materials and Methods

4.2.1 Feed solution and inoculum

Secondary clarified effluent was obtained from WWTP Bennekom (Bennekom, the Netherlands) as feed solution for the experimental work. WWTP Bennekom has a carrousel configuration, is operated with 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 S4.1 and S4.2. 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).

4.2.2 Chemicals

The pharmaceutical stock solution was prepared in HPLC grade methanol and consisted of caffeine, carbamazepine, diclofenac, gemfibrozil, ibuprofen, metoprolol, naproxen, sulfamethoxazole and trimethoprim. 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.

4.2.3 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 (Figure 4.1). 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 HRTs of the bioreactors were studied during a 5-month experimental period. HRTs of respectively 1.46, 0.47 and 0.3 hours 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.

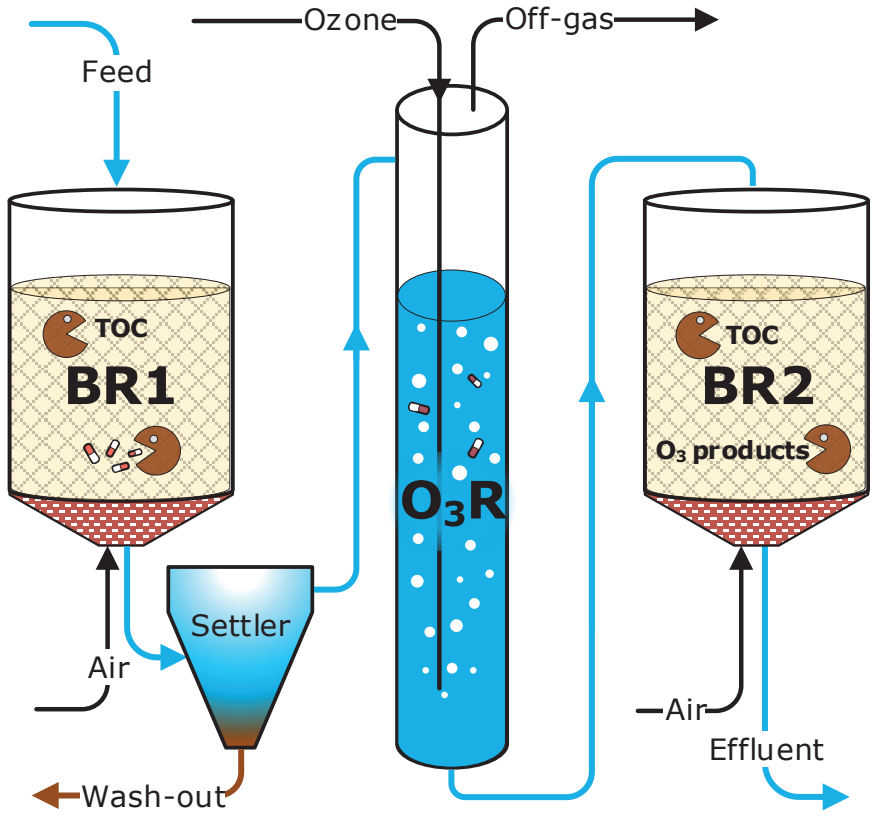


Figure 4.1 Schematic of the experimental set-up. BR1 represents the first biological reactor, O_3R the ozone reactor and BR2 the second biological reactor.

4.2.3.1 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 4.2.1, 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 seconds.

4.2.3.2 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, λ=254) and residual ozone in the liquid effluent by the indigo method [18]. In both streams ozone was not detected during the experiment. The applied ozone dose was expressed as g O_3/g TOC.

4.2.4 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 (Chapter 2). 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 hours.

4.2.5 Analytical methods

4.2.5.1 Pharmaceuticals

Liquid samples for pharmaceutical analysis were taken from the influents and effluents of the reactors and batch experiments. Samples 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 in Chapter 2.

4.2.5.2 TOC

Liquid samples for TOC determination were taken from the influents and effluents of the reactors and batch experiments and analysed on a Shimadzu TNM-L ROHS TOC-L. TOC was calculated as the difference between Total Carbon (TC) and Inorganic Carbon (IC). IC samples were acidified to pH 2 to convert inorganic carbonates into CO_2. CO_2 was converted from the liquid phase to the gas phase by sparging synthetic air (80% N_2, 20% O_2, 0% CO_2) and analysed by a non-dispersive infra-red (NDIR) detector. The carbon in the TC samples was burned at 720°C and converted into gaseous CO_2 where after it was analysed on the NDIR detector.

4.2.5.3 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 [75, 76, 88]. 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 hours. *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 hour exposure. *P. subcapitata* growth inhibition and toxicity towards *V. fischeri* were tested according to the methods described by He, et al. [103] but using $K_2Cr_2O_7$ as positive control to validate the protocol for the *P. subcapitata* assay.

4.3 Results and Discussion

4.3.1 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. Results of the batches amended with a nutrient and trace element solution did not demonstrate a higher TOC or pharmaceutical removal efficiency (data not shown). Pharmaceutical removal patterns and rates were identical between batches with or without the additional nutrients and trace elements. This showed 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.

4.3.2 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 [295]. Hence, Lee et al. [150] 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 hours 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 [117], 2.9-4.2 [193], 4.2-6.0 [111], 2.4-4.8 [329] and 4.7-7.1 mg DOC/L [150]. 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 [298] and 15-26.4 [150] and 23.0 mg DOC/L [265]. 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 0.1-0.5 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 [20]. The average TOC removal over BR2 was 17±3%, which is a result of the increased biodegradability after ozonation [243].

4.3.3 Pharmaceutical removal in the BO_3B process

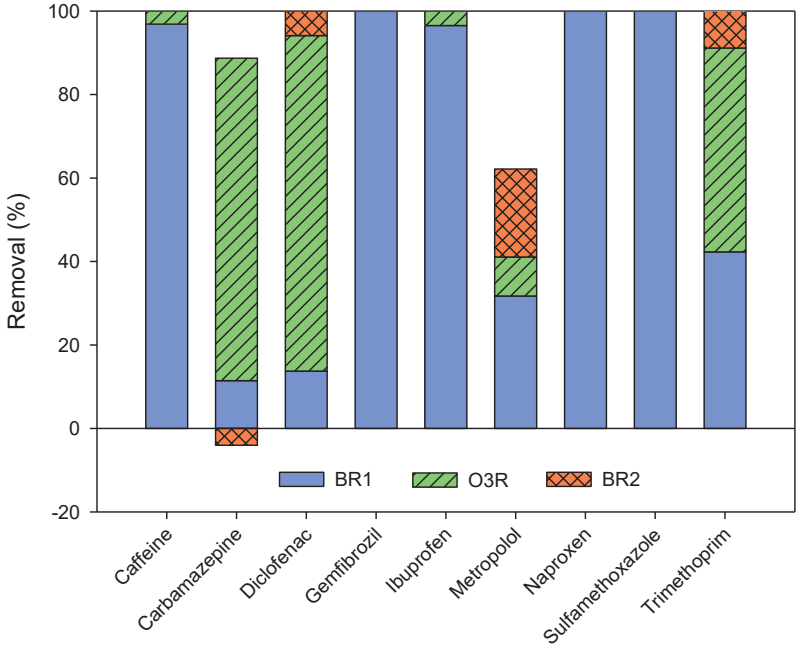


Figure 4.2 Pharmaceutical removal over the BO_3B process at an ozone dose of 0.2 g O_3/g TOC and an HRT of 1.46 hours.

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 hours is depicted in Figure 4.2. 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. Back-transformation of conjugated metabolites to the parent compound is found for carbamazepine during biological treatment [210, 291]. Back-transformation of ozonation products is to our knowledge not described in the literature, thus no firm explanation 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 conventional activated sludge (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% [65, 164, 241, 287]. 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 [65, 164, 241, 287]. Especially the removal of gemfibrozil and sulfamethoxazole in BR1 was high (>99%). Biodegradation is the predominant removal mechanisms in biological treatment [7]. 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 [91, 217]. A better removal in a sand-filter than in CAS systems was found by Reungoat, et al. [217] 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. [91] 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 [7, 91, 171]. 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 hours is in good agreement with their high reported ozonation rate constants (kO_3) of 6.8, 3 and 2.7 x 10^5 M^{-1} s^{-1}, respectively [329]. The limited metoprolol removal during ozonation corresponds well with its moderate kO_3 of 2 x 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. [111]. 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 [243]. For example, the formation of different quinazoline-containing products during carbamazepine ozonation [178]. Therefore considerable amounts of ozonation products will enter BR2.

4.3.4 Effect of ozone dose

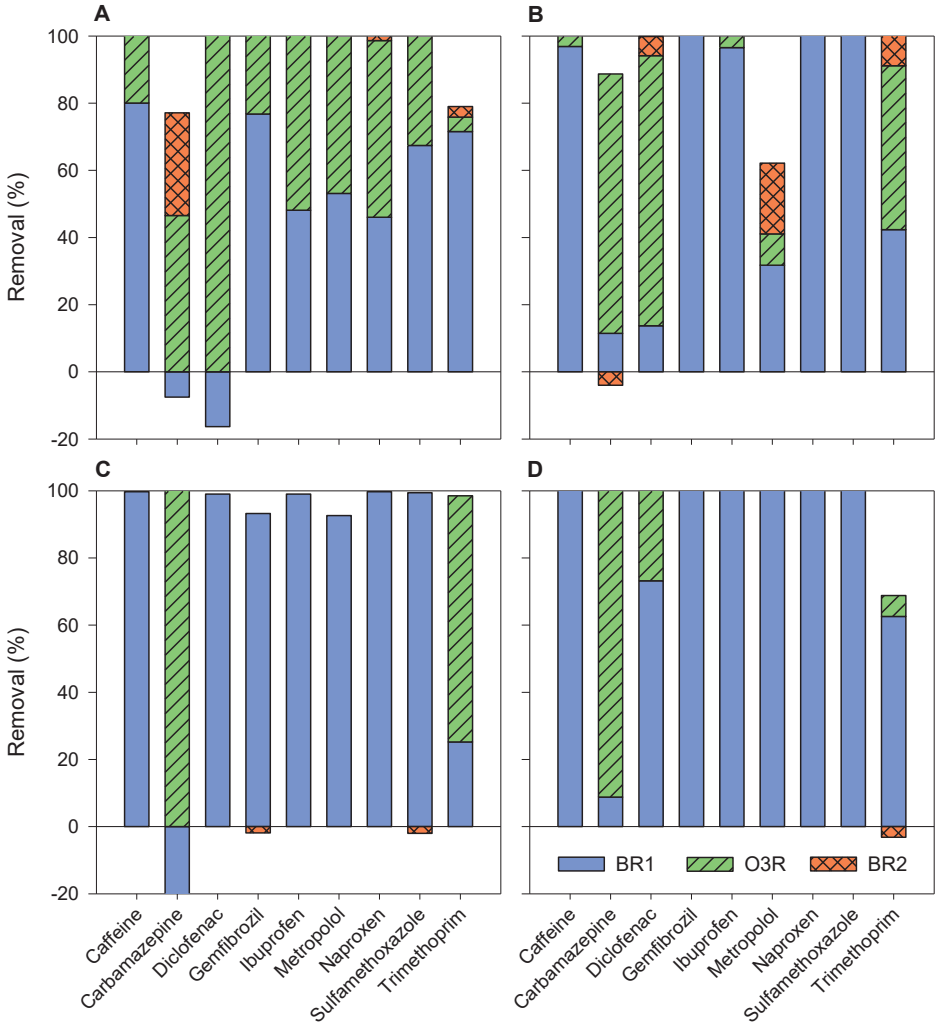


Figure 4.3 Pharmaceutical removal over the BO_3B process at an HRT of 1.46 hours and ozone doses of (A) 0.1, (B) 0.2, (C) 0.4 and (D) 0.5 g O_3/g TOC.

Pharmaceutical removal over the BO_3B process at ozone doses varying from 0.1-0.5 g O_3/g TOC at an HRT of 1.46 hours is depicted in Figure 4.3. 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. [150], 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 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. [216] 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 [65, 164, 241, 287], 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, Zimmermann et al. (2009) who found ~90% and ~60% metoprolol removal at 0.62 and 0.40 g O_3/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 [292]. 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 [294].

4.3.5 Effect of HRT

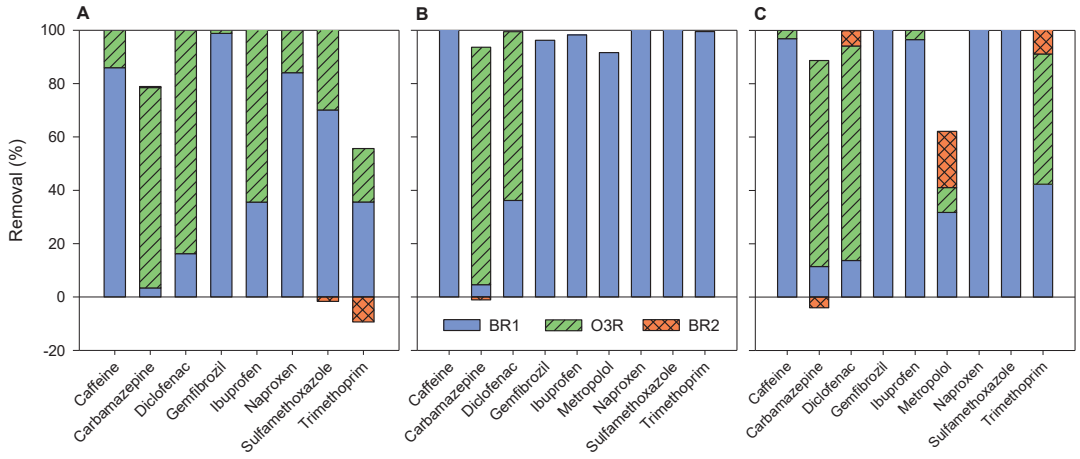


Figure 4.4 Pharmaceutical removal over the BO_3B process at an ozone dose of 0.2 g O_3/g TOC and HRTs of 0.3 (A), 0.47 (B) and 1.46 (C) hours.

Pharmaceutical removal over the BO_3B process at varying HRTs from 0.3-1.46 hours at an ozone dose of 0.2 g O_3/g TOC is depicted in Figure 4.4. 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 hours 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 hours. This agrees well with the results of the ozone dose tests (Figure 4.3) 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 hours compared to the longer HRTs suggests that 0.3 hours is too short for an effective biological treatment. Matamoros, et al. [171] 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 hours, 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 hours of this study, this suggest that the pharmaceutical removal of BR1 was relatively high. Escolà Casas and Bester [78] 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 hours^{-1} at HRTs of 5.7, 9.01, 0.108 and 0.13 hours, respectively. The HRT related reaction rate constants of diclofenac in this study are 0.10, 0.96 and 0.59 hours^{-1} at HRTs of 1.46, 0.47 and 0.3 hours, respectively, and thereby at the higher end compared to the reported rate constants by Escolà Casas and Bester [78]. 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 [78, 171]. 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 an HRT of 0.3 hours, 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% to 19% at HRTs of 1.46, 0.47 and 0.3 hours, respectively. Similarly, Matamoros, et al. [171] found a decrease in TSS and BOD_5 at decreasing HRTs.

4.3.6 Lowest ozone dose and lowest HRT

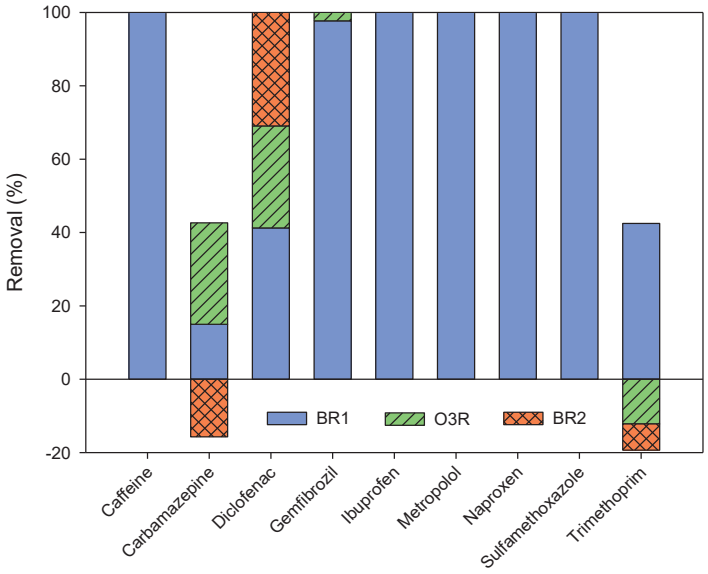


Figure 4.5 Pharmaceutical removal over the BO_3B process at an ozone dose of 0.1 g O_3/g TOC and an HRT of 0.3 hours.

The pharmaceutical removal at an ozone dose of 0.1 g O_3/g TOC and an HRT of 0.3 hours is depicted in Figure 4.5. 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 hours at an ozone dose of 0.2 g O_3/g TOC (Figure 4.4). 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 hours is recommended to achieve a continuous and high pharmaceutical removal. At an HRT of 0.3 hours 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 hours (Figure 4.3). 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.

4.3.7 Toxicity

The pharmaceutical removal during the campaign in which toxicity was tested is depicted in Figure S4.1. 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 (Figure S4.2-S4.4). 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 [118]. However, this can be explained by the high concentrations (>mg/L) applied in that study compared to this study. Kaiser et al. [127] 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 [33]. 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 [248]. Nevertheless, further ecotoxicological investigations are recommended as pharmaceuticals and their transformation products formed during biological and ozone treatment can pose diverse ecotoxicological risks [164].

4.3.8 Costs and energy demand

Depending on the energy price and the system requirements, production costs of ozone are reported to be 0.8 - 1.6 €/kg O_3, which represents 20-40% of the total costs for large scale ozonation installations [265]. The BO_3B process operated at an ozone dose of 0.2 g O_3/g TOC and HRT of 1.46 hours 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 <0.004 €/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. [125], 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 [280]. 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 [280]. 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 [89]. 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 [125]. 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 [280]. 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.

4.4 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 hours 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 hours. At the lowest tested HRT of 0.3 hours 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 hours 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%, whereas the yearly tariff per population equivalent 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.

Acknowledgement

We would like to thank Lilian Prinsen for her practical support.

Supplementary Information

Table S4.1 Concentrations (mg/L) of the ionic constituents from the WWTP Bennekom secondary clarified effluent.

	F^-	Cl^-	NO_2^-	NO_3^-	NH_4^+	SO_2^{4-}	PO_4^{3-}	Br^-
Batch 1	0.14	260.4	n.d.	5.98	0.08	6.65	1.62	n.d.
Batch 2	0.08	60.2	0.53	4.50	4.70	3.32	1.01	n.d.

n.d. – not detected, NO_2^- detection limit is <50 µg/L, Br^- detection limit is 5 µg/L. Anions were analysed on an Ion Chromatograph (Dionex ICS 2100, Dionex IonPac AS17, 4 x 2505 mm). An injection volume of 10 µL was used with a constant flow of 1 mL / minute and the following flow program: 10 minutes at 5 mM KOH, in 15 minutes a linear increase to 30 mM KOH, 3 minutes on 30 mM KOH and back to 5 mM KOH in 2 minutes. Samples were diluted 10 times. Measurements were performed in triplicate, stated values are the average. Ammonium was analysed by Hach Lange kits LCK 303 and 304.

Table S4.2 Average concentrations (mg/L) and standard deviations of the carbon fractions in the feed solution of the BO_3B process over the 5-month experimental period.

Total carbon	Total inorganic carbon	Total organic carbon
48.5±9.1	31.2±6.3	17.3±3.3

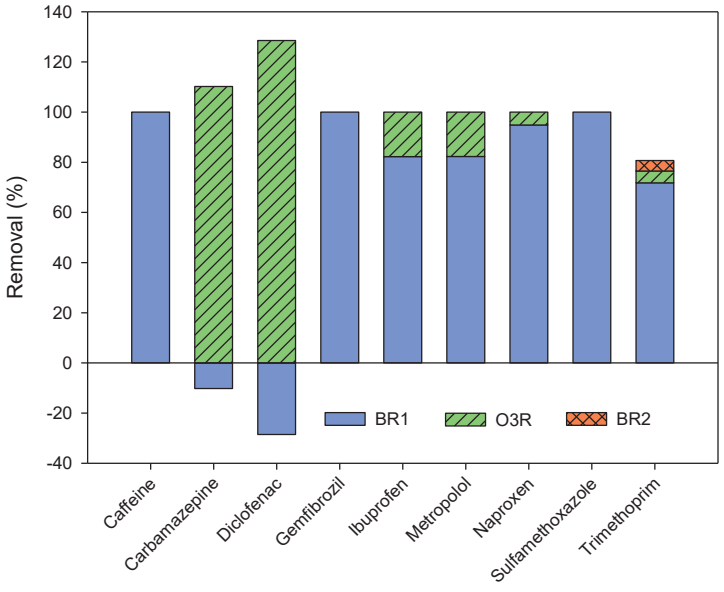


Figure S4.1 Pharmaceutical removal over the BO_3B process during the toxicity tests at an ozone dose of 0.2 g O_3/g TOC and an HRT of 1.46 hours.

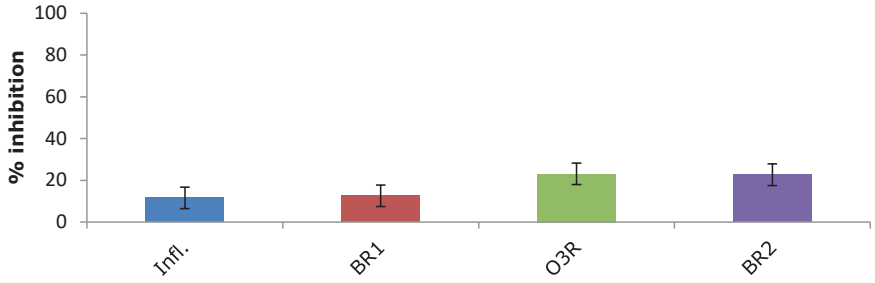


Figure S4.2 Toxicity towards *V. fischeri* of the feed water (Infl.), first biological reactor (BR1), ozone reactor (O_3R) and second biological reactor (BR2). Columns represent the average toxicity (n=6), error bars represent standard deviations.

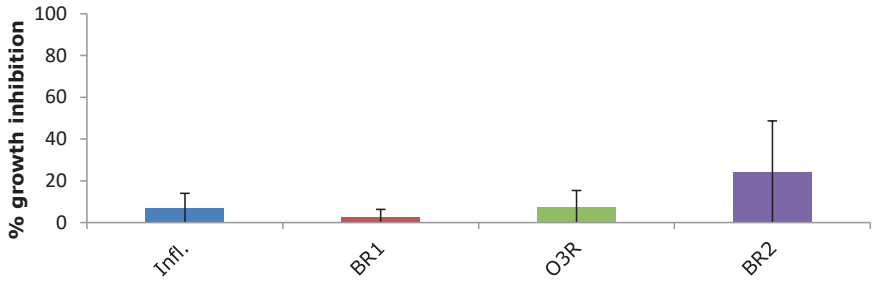


Figure S4.3 Toxicity towards *P. subcapitata* of the feed water (Infl.), first biological reactor (BR1), ozone reactor (O_3R) and second biological reactor (BR2). Columns represent the average toxicity (n=6), error bars represent standard deviations.

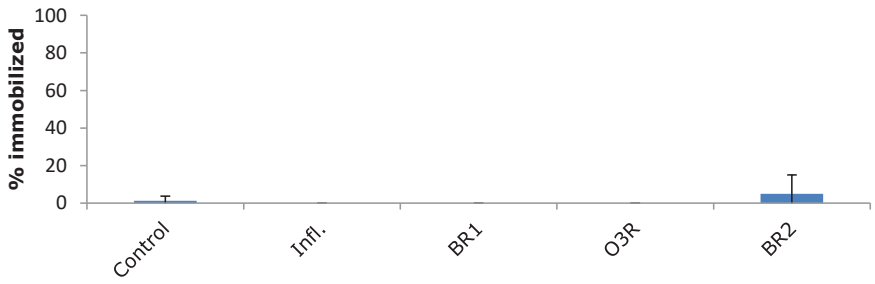


Figure S4.4 Toxicity towards *D. magna* of the feed water (Infl.), first biological reactor (BR1), ozone reactor (O_3R) and second biological reactor (BR2). Columns represent the average toxicity (n=4), error bars represent standard deviations.

Chapter 5

Micropollutant removal in an algal treatment system fed with source separated wastewater streams

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Abstract

Micropollutant removal in an algal treatment system fed with source separated wastewater streams was studied. Batch experiments with the microalgae *Chlorella sorokiniana* grown on urine, anaerobically treated black water and synthetic urine were performed to assess the removal of six spiked pharmaceuticals (diclofenac, ibuprofen, paracetamol, metoprolol, carbamazepine and trimethoprim). Additionally, incorporation of these pharmaceuticals and three estrogens (estrone, 17β-estradiol and ethinylestradiol) into algal biomass was studied. Biodegradation and photolysis led to 60 – 100% removal of diclofenac, ibuprofen, paracetamol and metoprolol. Removal of carbamazepine and trimethoprim was incomplete and did not exceed 30% and 60%, respectively. Sorption to algal biomass accounted for less than 20% of the micropollutant removal. Furthermore, the presence of micropollutants did not inhibit *C. sorokiniana* growth at applied concentrations. Algal treatment systems allow simultaneous removal of micropollutants and recovery of nutrients from source separated wastewater. Nutrient rich algal biomass can be harvested and applied as fertilizer in agriculture, as lower input of micropollutants to soil is achieved when algal biomass is applied as fertilizer instead of urine.

Keywords

Micropollutants; Wastewater; Algae; Source separation; Pharmaceuticals

5.1 Introduction

The global increase of population and consequent shortage of natural resources causes increasing interest in mining of commodities present in waste streams, such as the domestic wastewater, which is a potential source of energy, organic matter and nutrients [199, 289].

The use of algae in wastewater treatment is promising for their high nutrient recovery capacity. Algae have demonstrated simultaneous removal of nitrogen and phosphorus from domestic wastewater down to very low concentrations of 2.2 mg/L and 0.15 mg/L, respectively, by uptake of the nutrients into their cells [28]. High biomass yields can be achieved when growing algae heterotrophically in the presence of ammonia and nitrate [131]. Harvested algal biomass is a potential source for production of fertilizer and bioenergy [38, 179, 205, 224].

Source separated collection of toilet wastewater makes recovery of organic matter and nutrients economically feasible because of their high concentration in a relatively small volume [316]. This volume is less than 30% of the total volume of domestic wastewater when conventional toilets are used, and even less than 5% when vacuum toilets are used due to the reduced volume of flush water [268]. It contains 38% of organic matter, 88% of nitrogen and 68% of phosphorus of the domestic wastewater [136]. Toilet wastewater, comprising of urine, faeces, toilet paper and flush water, can be collected either as a single stream, called black water, or as two separate streams, urine (yellow water) and faeces (brown water) [200]. Most of the organic matter comes from faeces, while nutrients are mainly present in urine [136]. Treatment of black water in an up-flow anaerobic sludge blanket (UASB) reactor allows conversion of organic matter into biogas [317]. Numerous techniques are available to remove nitrogen and phosphorus from the nutrient rich anaerobically treated black water (AnBW) or urine. However, most of these techniques are characterized by nutrient removal rather than by nutrient recovery [261, 284]. Techniques that allow nutrient recovery like struvite or calcium phosphate precipitation often have the drawback that they cannot adequately recover nitrogen and phosphorus simultaneously [179, 269, 317]. According to the stoichiometry of the process only 3% of nitrogen can be recovered from source-separated urine by struvite precipitation while phosphorus can be almost completely recovered [175]. Thus, an algal treatment system might be more suitable, and can be used instead for nutrients recovery from AnBW or urine

(Figure 5.1) as it is one of the few techniques demonstrating simultaneous nitrogen and phosphorus recovery.

Tuantet, et al. [276] showed that 85% of phosphorus and 90% of nitrogen are recovered from urine by incorporation into algal biomass. Vasconcelos Fernandes, et al. [285] also showed high recovery of nitrogen (74%) and phosphorus (98%) from AnBW, when growing *Chlorella sorokiniana* in batch [86]. The algal biomass, enriched with nutrients, can be used as fertilizer [277].

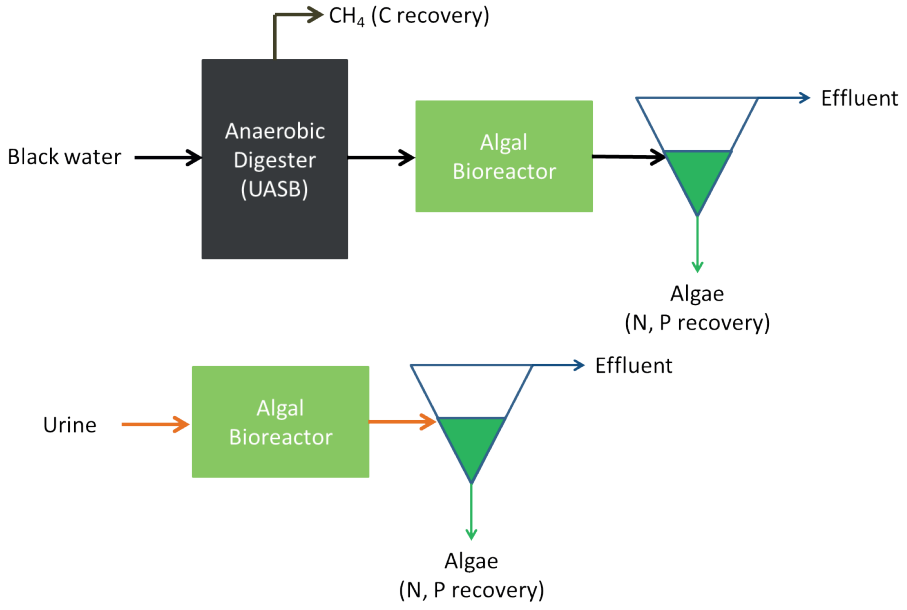

Figure 5.1 Two options of algal treatment system utilization for treatment of source separated wastewater streams.

In addition to carbon and nutrients, black water contains pathogens and organic micropollutants. Lienert, et al. [157] investigated excretory routes of 212 pharmaceuticals, and showed that nearly two thirds of them are excreted with urine, while the rest are excreted with faeces.

Micropollutants are insufficiently removed during anaerobic treatment of black water and end up in the AnBW [62]. Thus, successful treatment of AnBW or urine should provide adequate removal of micropollutants before the treated

effluent is discharged or reused. Shi et al. [237] showed that natural and synthetic estrogens can be effectively removed in algae-based wastewater treatment systems. However, little is known about the removal of pharmaceuticals by algae. The quality of the algal biomass, grown on AnBW or urine, is also a focus for research, because algae can potentially accumulate persistent organic micropollutants [54]. The presence of micropollutants in algal biomass grown on AnBW or urine can hinder the possibilities for biomass utilization as fertilizer and needs to be addressed [158, 301].

The aim of this study is to assess micropollutant removal and sorption to biomass in an algal treatment system with AnBW and urine used as growth media. Six pharmaceuticals (ibuprofen, diclofenac, paracetamol, trimethoprim, metoprolol and carbamazepine) and three estrogens (estrone (E1), estradiol (E2), and ethinylestradiol (EE2)) were studied, with the estrogens determined in algal biomass only. The possibilities for reuse of algal biomass in agriculture are further discussed. Additionally, the inhibiting effect of elevated concentrations of micropollutants on algae growth is presented.

5.2 Materials and Methods

5.2.1 Micropollutants

Ibuprofen, diclofenac, metoprolol, trimethoprim, paracetamol, fenoprofen, diaveridine, dihydrocarbamazepine, trimethoprim-d9, estrone, 17β-estradiol, ethinylestradiol and 17β-estradiol-d3 were purchased from Sigma-Aldrich (Germany). Carbamazepine was purchased from Fagron (the Netherlands). The standard and spiking solutions of the compounds were prepared in methanol. The chosen pharmaceuticals represent different therapeutic groups and are widely used in large quantities in Europe and North America. Most of them are persistent, as they are not readily degradable in municipal wastewater treatment plants, except for paracetamol [124]. The studied compounds also cover a wide range of physical-chemical properties and biodegradability.

5.2.2 Inoculum and media

C. sorokiniana CCAP211/8K was obtained from the Culture Collection of Algae and Protozoa (Oban, UK). Algae were pre-cultivated in an incubator at 35°C in 250 mL shake flasks containing 100 mL M8a medium [133], enriched air with 3% CO_2 (v/v) and continuous average irradiation of 80 µmol m^{-2} s^{-1}. Male urine was collected from waterless urinals at the sub-department of Environmental Technology, Wageningen University, the Netherlands. Urine was diluted six times with demi water to support equal biomass growth in all batches. Urine was stored at 4°C prior to the start of the experiments. AnBW was collected from a UASB reactor operated at 25°C and HRT of 37 days treating black water from 60 households in Sneek, The Netherlands. AnBW was diluted three times to support equal biomass growth in all batches and was stored at 4°C prior to the experiments. Urea-based synthetic urine was prepared to mimic non-hydrolysed urine and consisted of (in 1L) 600 mg urea ($CO(NH_2)_2$), 56 mg K_2SO_4, 110 mg K_2HPO_4, 30 mg $Na_2HPO_4.2H_2O$, 320 mg NaCl, 21.2 mg $CaCl_2.2H_2O$ and 73.8 mg $MgSO_4.7H_2O$. M8a medium was used as a reference medium for biomass growth in urine and AnBW [133]. M8a medium was diluted two times to support equal biomass growth in all batches. Dilutions to support equal biomass growth for all media were based on preliminary experiments. Composition of all media is shown in Table 5.1.

All media were filtered over Whatman® Ashless, Grade 589/1 paper filters with pore size Ø 12-25 µm. Iron and micronutrients were added to the diluted urine, synthetic urine and diluted AnBW to obtain final concentrations of 160 µM EDTA ferric sodium salt, 100 µM $Na_2EDTA.2H_2O$, 0.5 µM H_3BO_3, 33 µM $MnCl_2.4H_2O$, 5.5 µM $ZnSO_4.7H_2O$ and 3.7 µM $CuSO_4.5H_2O$. At the beginning of the experiment all media were buffered with 75 mM Hepes at pH 7.0. During the experiment the pH was manually controlled with 2M HCl or 2M NaOH at pH 7.0.

Table 5.1 Composition of urine, AnBW, synthetic urine and M8a medium.

Medium	Dilution factor	PO_4-P (mg/L)	P_{total} (mg/L)	NH_4-N (mg/L)	N_{total} (mg/L)
Urine	6	24.7	25.2	540	854
AnBW	3	24.5	24.7	235	236
Synthetic Urine	-		21.2		305
M8a medium	2		107.7		257

5.2.3 Experimental set-up

Batch experiments were performed in 500 mL sterilized Erlenmeyer flasks, closed with sterile cotton-wool stoppers. The flasks were filled with 300 mL medium, inoculated with 1.66 x 10^5 cell/mL C*. sorokiniana*, and spiked with a solution of micropollutants in a final concentration of 100-350 µg/L (Table S5.1). Spiking of micropollutants was necessary to obtain sufficient LC-MS response (>100 times higher than the limit of quantification). After spiking, the concentrations of the pharmaceuticals were in the same order of magnitude as the maximal concentrations reported in AnBW and source-separated urine (Table S5.1). Batch experiments were performed at ten different conditions, with each condition performed in triplicate (Table 5.2). Batches without algal inoculum were started one month later to prevent cross-contamination from other batches in the incubator. All batches were run until the algal growth curves reached the stationary phase.

Batches were incubated at 35°C (Infors HT® Multitron incubator) with a continuous average illumination of 68 µmol m^{-2} s^{-1}. The incubator headspace was enriched with 3% CO_2 (v/v) and humidity controlled at 75%. Batches were continuously shaken and randomized daily for equal light distribution. Abiotic batches were set up to test the non-biological micropollutant removal. Biological activity was supressed in the abiotic batches by addition of 0.2 g/L NaN_3. Liquid samples of 3 mL were taken from each replicate at intervals during the 31 day incubation period to monitor biomass growth. Duplicate samples of 5 mL were taken from each replicate for micropollutant analysis on day 0, 1, 3, 10, 17, 23 and 31.

Table 5.2 Overview of the batch experiments (each variation reproduced in triplicate).

Medium	Algae	MP	NaN_3	Comments
M8a medium	+	-	-	Unlimited algal growth
Urine	+	-	-	Algal growth in urine
AnBW	+	-	-	Algal growth in AnBW
Synthetic urine	+	+	-	MP removal in synthetic urine with algae
Urine	+	+	-	MP removal in urine with algae
AnBW	+	+	-	MP removal in AnBW with algae
Urine	-	+	-	MP removal in urine without algae
AnBW	-	+	-	MP removal in AnBW without algae
Urine	-	+	+	MP removal in urine at abiotic conditions
AnBW	-	+	+	MP removal in AnBW at abiotic conditions

MP - Micropollutants

5.2.4 Biomass growth determination

Chlorophyll-a concentrations were measured with a Phyto-PAM (Heinz Walz GmbH, Germany). Chlorophyll-a concentrations provide an estimate of the amount of algal biomass. As a measure of actual versus optimal algae growth, chlorophyll-a results are presented as relative concentrations compared to M8a (C_x/C_{max_M8a}) regarding M8a as the most optimal growth medium for algae in this experiment.

The optical density at 750 nm (OD_{750}) was used as a non-specific indicator of microbial biomass growth. OD_{750} was determined by spectrometry analyses (Helios UV, Unicam, UK). The measured OD_{750} values of the batches were corrected for initial OD_{750} values of the media.

Dry weight was measured before and after the experiment. Samples were filtered over pre-washed 0.2 µm glass microfiber filters (GF/F filter, Whatman Plc., UK) and dried overnight at 60°C. The filters were cooled to room temperature in a desiccator prior to the weighing. Dry weight produced during the experiment was calculated as the difference between dry weight measured at the last and first day of the experiment.

5.2.5 Nutrient analysis

The concentrations of nitrogen and phosphorus were measured with Hach Lange test kits in the batches at the beginning and end of the experiment (N_{total} – LCK338, NH_4-N – LCK303, NO_2-N – LCK342, NO_3-N – LCK340, P_{total} – LCK350 and PO_4-P – LCK350). Mg^{2+} was measured with Inductively Coupled Plasma Optical

Emission Spectrometer (ICP-OES, Perkin-Elmer Optima 5000 DV) at the end of the experiment.

The samples for nutrients analysis were centrifuged at 3400 rpm for 10 minutes and filtered through <2 µm pore size paper filter. Nitrogen removal is presented as the difference between N_{total} at the beginning and NH_4^+-N at the end of the experiment, as NO_2-N and NO_3-N were not found in the batches at the end of the experiment. Absolute phosphorus removal is presented as the difference between initial and final PO_4-P concentrations. Relative nitrogen and phosphorus removal is presented as C_x/C_0.

5.2.6 Micropollutant analysis

Samples for micropollutant analysis were centrifuged at 3400 rpm for 10 minutes directly after sampling for separation of liquid phase from suspended matter, which represents algal biomass. Liquid samples and solid pellets were stored separately at -20ºC until analysis. Liquid samples were directly injected onto the LC-MS/MS. Solid pellets were mixed in 5 mL of acetonitrile and sonicated during 20 minutes for micropollutant extraction. The extract was separated from the solids by centrifugation. For pharmaceuticals analysis 2 mL of extract was evaporated till dryness in the vacuum chamber under a gentle nitrogen stream. The precipitate was dissolved in methanol, then diluted with milliQ in a ratio 9:1 and injected into the column. For estrogen analysis 1 mL of extract was evaporated till dryness. The precipitate was dissolved in 50 µL of 0.1 M Na_2CO_3 solution and 50 µL of dansyl chloride solution in dried acetone and incubated at 60ºC during 10 minutes according to the modified method of Anari et al. [9]. 0.2 mL of acetonitrile and 0.2 mL of milliQ were added to the extract after incubation before injection to the column.

Analysis of micropollutants was performed by LC-MS/MS consisting of Agilent 1200 HPLC with Agilent 6410 triple quadruple MS/MS operated with Agilent Masshunter software. A Phenomenex Kinetex Phenyl-Hexyl column (100x2.1 mm, 100 Å pore size) was used for separation of pharmaceuticals, and a Phenomenex Gemini Phenyl-Hexyl column (150x3.0 mm, 110 Å pore size) was used for separation of estrogens. The injection volumes, composition of elution solvents, elution gradients and mobile phase flows are presented in Table S5.2. The MS/MS was working in multiple reaction monitoring (MRM) mode. The temperature of

electrospray ionization source was 320°C with nitrogen used as a collision gas. The nebulizer pressure was 50 psi and a capillary voltage 4000 V. The retention times, monitored ions and MS parameters of the studied compounds are presented in Table S5.3.

The internal standards were spiked to liquid samples prior to injection and to solid samples prior to extraction (Table S5.1). The samples for calibration curves (7-point calibration for liquid samples and 8-point calibration for solid samples) were prepared in triplicates separately for liquid and solid samples by dissolution of analytes in milliQ. Calibration samples were treated in the same way as liquid and solid samples. The precision of the method was determined as a relative standard deviation between triplicate measurements and was below 15% for each analysed concentration. For recovery determination analytes were spiked to the liquid samples taken on day 0 and to the solid samples (Table S5.4). Recoveries were determined as the ratio of the measured amount of an analyte in a spiked sample minus the measured background amount of an analyte in a sample divided by the spiked amount of an analyte. A signal-to-noise ratio (S/N) of three was used to estimate limits of detection and signal-to-noise ratio of ten was used for estimate limits of quantification (Table S5.5).

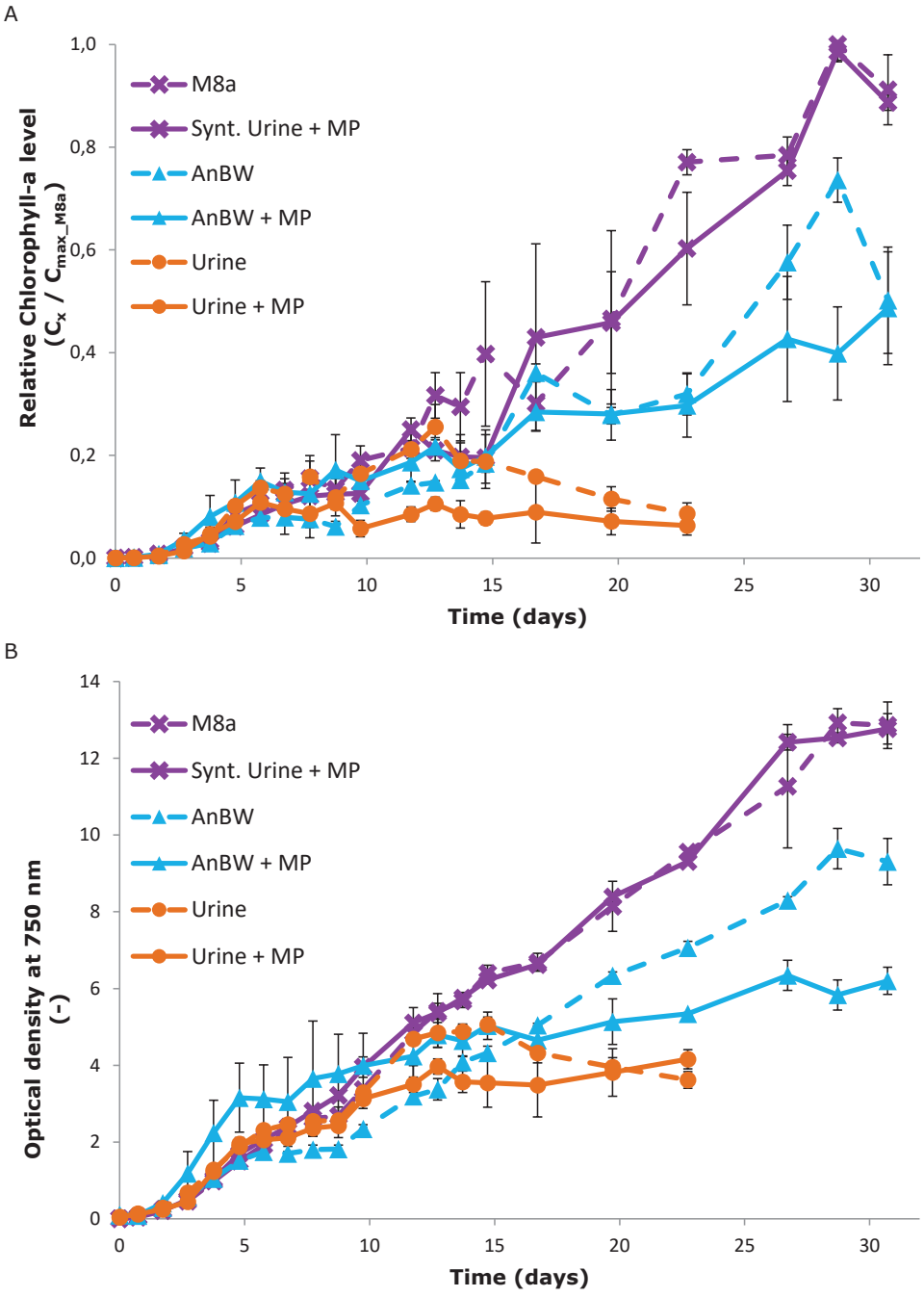

Figure 5.2 Chlorophyll-a levels (A), expressed concentration divided over maximal concentration in M8a medium and optical densities at 750 nm (B). Error bars represent standard deviations between triplicates

5.3 Results and Discussion

5.3.1 Algae growth and nutrient removal

Algal biomass growth, presented as chlorophyll-a (Figure 5.2a), and non-specific biomass growth, presented as OD_{750} (Figure 5.2b), and dry weight (Figure 5.3) were determined in all batches to assess possible toxic effects of spiked micropollutants on algae. During the first week of the experiment all inoculated batches displayed a similar increase in chlorophyll-a and OD_{750}, except for the more pronounced increase in OD_{750} in spiked AnBW. The similarities in biomass growth up to day 14, regardless of the medium, confirmed the absence of acute toxic effects of the spiked micropollutants to *C. sorokiniana*. Experiments were stopped when no further algae growth was expected. Urine batches were incubated for 23 days, all other batches for 31 days. Final chlorophyll-a levels, OD_{750} and dry weights were similar between spiked and non-spiked inoculated AnBW, spiked and non-spiked inoculated urine and synthetic urine and M8a medium, except for OD_{750} in AnBW. The final OD_{750} in the non-spiked AnBW was 33% higher than in the spiked AnBW. However, chlorophyll-a levels and dry weights in these batches were similar. Apparently the spiked micropollutants were not toxic for *C. sorokiniana* at the applied concentrations (~100-350 µg/L) within the timeframe of the experiment.

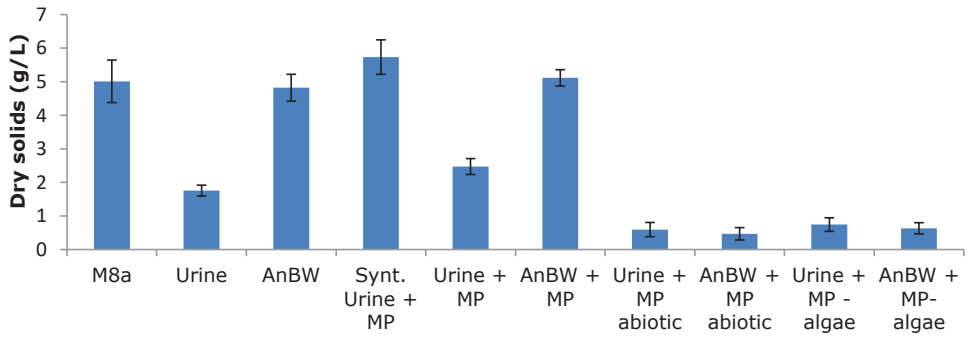

Figure 5.3 Dry weight of the algal biomass grown on the different media. Error bars represent standard deviations between triplicates; MP – Micropollutants.

Chlorophyll-a was not present in abiotic batches and non-inoculated batches, confirming the absence of algal biomass in these batches. Abiotic and non-inoculated batches showed a maximal OD_{750} of 0.5 and dry weight of 0.7 g/L

only, most likely due to the presence of bacteria, fine particles and precipitate formation in the form of struvite, calcium phosphate, or other precipitates during the experiment [279]. Average OD_{750} in non-inoculated and abiotic batches was more than an order of magnitude lower than in inoculated urine. As optical density is proportional to bacterial density throughout the bacterial growth phase no noticeable bacterial growth occurred in the non-inoculated and abiotic batches [189].

Despite no difference observed between growth of algae in spiked and non-spiked batches, differences in biomass growth parameters were found between the media used in the experiments (Figure 5.2, Figure 5.3). Depletion of essential nutrients in the media was investigated as the possible reason of the observed differences. Nitrogen and phosphorus were completely removed in AnBW and synthetic urine at the end of the experiment (Figure 5.4). Complete nitrogen removal is in accordance with other studies on nutrient removal by algae [276, 285].

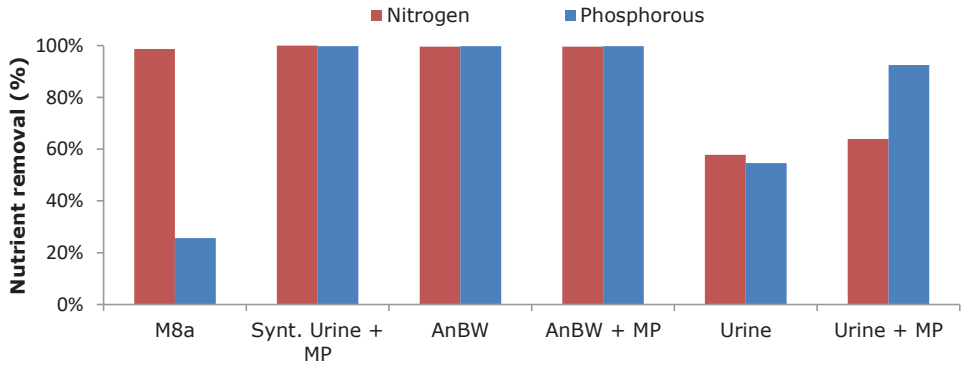

Figure 5.4 Nitrogen (red bars) and phosphorus (blue bars) removal in the inoculated batches at the end of the experiment (23 days for urine batches and 31 days for other batches); MP – Micropollutants.

Molar N:P ratios in AnBW and synthetic urine in this study were 21:1 and 32:1 respectively, which is above the average N:P ratio in algal biomass (16:1), also known as Redfield ratio [214]. However, both nutrients were depleted in AnBW and synthetic urine, whereas only phosphorus depletion was expected based on the Redfield ratio. Tuantet, et al. [276] and Vasconcelos Fernandes, et al. [285] found N:P ratios of 15:1 to 33:1 in *C. sorokiniana* biomass grown on human urine and AnBW, showing similar deviations of actual N:P ratios from the Redfield ratio. The N:P ratios reported by Tuantet, et al. [276] and Vasconcelos Fernandes, et al. [285] evince the possibility of simultaneous nitrogen and phosphorus depletion observed in this experiment .

Growth on M8a medium was nitrogen limited. Batches with M8a medium showed the highest absolute phosphorus removal but lowest removal efficiency (25%). This was expected as the molar N:P ratio in the medium was only 5:1 which is much lower than the Redfield ratio (16:1).

Algal biomass growth on urine was not limited by nitrogen or phosphorus. Both nutrients were still present in urine at the end of the experiment, while the absolute nitrogen removal was two times higher than in the other batches. However, growth of algal biomass on urine was already lower than that on AnBW and synthetic urine on day 14. Tuantet et al. [275] demonstrated that magnesium (Mg^{2+}) deficiency in urine can limit algae growth. Dry weight magnesium content in *Chlorella* sp. ranges between 0.36% and 0.8% [86] indicating the required initial Mg^{2+} concentrations in the medium equal to 6.5 – 20 mg/L based on their biomass densities of 2.5 and 1.8 g/L, respectively. Literature reports Mg^{2+} concentrations of 77 mg/L [279], 119 mg/L [86] and 145 mg/L [163] in fresh urine and 0 to 11.1 mg/L [175], 0.14 to 0.77 mg/L [276] and 1 mg/L [279] in hydrolysed urine. About 60% of the urine was hydrolysed by the beginning of the experiment. Moreover, prior to pH controlling and buffering the pH in the collected urine was 9.1. Hypothesized is that due to the hydrolysis and high pH most of Mg^{2+} precipitated and thus became unavailable for uptake by algal cells. This assumption is confirmed by measurements of magnesium concentrations, giving 16.8±0.6 mg/L Mg^{2+} in AnBW and 0.9±0.4 mg/L Mg^{2+} in urine by the end of the experiment. Therefore Mg^{2+} deficiency limited further algae growth and nutrients removal in the inoculated urine batches.

5.3.2 Micropollutant removal

The removal of spiked pharmaceuticals from the liquid phase in batches with and without algal inoculum and in abiotic batches is presented as the relative concentration (C_x/C_0) (Figure 5.5).

Diclofenac removal (40% to 60%) was observed in all batches. As the removal was similar in all batches, including the abiotic ones, it is most likely attributed to phototransformation. Indeed, direct photolysis plays an important role for removal of diclofenac [140], while biodegradation of diclofenac in wastewater treatment systems is insignificant [321].

Ibuprofen removal was observed in all batches, with a higher removal in AnBW as growth medium. Indirect photodegradation in the presence of photosensitizers, such as dissolved organic matter (DOM) is a possible pathway of ibuprofen removal [160]. Canonica et al. [39] showed that the rate of indirect photolysis can vary depending on the DOM species used as sensitizer, thus, the faster removal of ibuprofen in AnBW could be explained by better photosensitizing properties of DOM species present in AnBW compared to other used media. Paracetamol was rapidly removed in all batches, except for the urine under abiotic conditions. Paracetamol is a readily biodegradable compound, as indicated by a reported biodegradation constant k_{bio} >10^2 L g_{ss}^{-1} day^{-1} [124]. Additionally, De Laurentiis et al. [64] reported that direct photolysis is an important mechanism of paracetamol removal in surface waters. Both mechanisms could have been involved in the paracetamol removal in this study, because the compound was removed in the inoculated batches and abiotic AnBW. However, no sound explanation for the absence of paracetamol removal in abiotic urine batches was found.

Complete removal of metoprolol was achieved in batches with algae grown on synthetic urine and AnBW. Approximately 70% of this compound was removed in the non-inoculated batches with AnBW. Lower removal was observed in the urine batches. However, high standard deviations in the urine batches did not allow to conclude on the extent of metoprolol removal in urine. Biotransformation was the dominant mechanism responsible for the removal of metoprolol, as no removal was observed in the abiotic batches. The removal of carbamazepine did not exceed 10% except for the batch with algae grown on AnBW, where it reached 30%. Low removal of carbamazepine is consistent with the data of other researchers, who

showed that carbamazepine is stable towards biodegradation under aerobic conditions and phototransformation [11, 321].

Trimethoprim removal did not exceed 40%, except for the batch with algae grown on AnBW, where removal reached 60%. It is consistent with findings of Suarez et al. [255], who reported high resistance of trimethoprim to biological removal in aerobic conditions. Removal of trimethoprim in the abiotic batches reached 20% indicating that photolysis, apart from biotransformation, is a possible mechanism of trimethoprim removal in urine and AnBW, as was also demonstrated in previous studies [227].

The observed removal of paracetamol, ibuprofen, metoprolol, carbamazepine and trimethoprim correlates with published aerobic biodegradability of these compounds [124, 256]. Algae did contribute to the removal of metoprolol, as a higher removal was observed in batches inoculated with algae compared to the non-inoculated batches. This is explained by mixotrophic nature of microalgae, including *Chlorella* species, and their ability to degrade micropollutants [257]. However, removal of the other micropollutants was neither enhanced nor decreased in the presence of algae. Therefore biotransformation of these micropollutants was possibly attributed to activity of natural present microorganisms in AnBW and urine.

In addition to biotransformation, photolysis appears to be a major removal mechanism in algal treatment systems. Unlike most other biological treatment processes, the algal treatment systems use illumination for biomass growth, which increases the removal of the light-sensitive micropollutants. For example, removal of diclofenac and metoprolol in a conventional sewage treatment plant (STP) operated at SRTs of 10 days is less than 25%, as reported by Radjenović, et al. [210]. Removal of these compounds in the algal treatment system studied in the present paper is higher, e.g. 40-60% removal of diclofenac and complete removal of metoprolol [210]. Removal of paracetamol (99%), ibuprofen (99%), trimethoprim (40-60%) and carbamazepine (30%) is similar to that reported for STP (99%, 99%, 40% and <30%, respectively) [210, 321]. Hence algal wastewater treatment systems are at least as good as conventional STPs with respect to pharmaceutical removal. Removal of micropollutants and recovery of nutrients could be also expected in microalgal biofilms treating the effluent of STP, as described by Boelee, et al. [28].

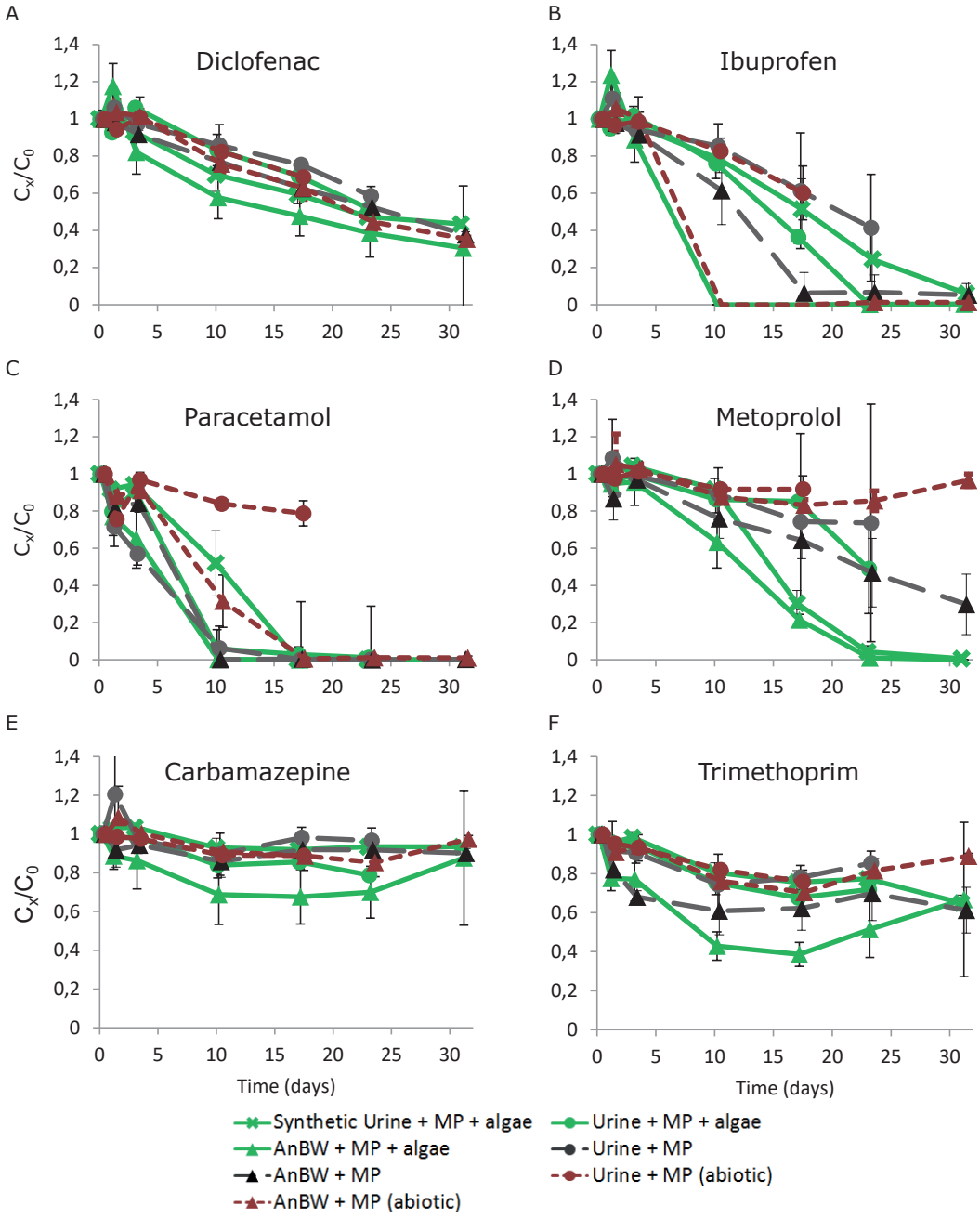

Figure 5.5 Pharmaceuticals removal from liquid phase, expressed as triplicate averaged concentration divided over concentration at the beginning of the experiment (C_x/C_0) Error bars represent standard deviations between triplicates; MP – Micropollutants.

The potential of more intensively illuminate algal treatment systems with respect to higher micropollutant removal is therefore an interesting topic for further research.

The concentrations of spiked micropollutants in suspended matter at the end of the experiment of the batches with AnBW and urine were compared (Table 5.3). Diclofenac, trimethoprim, carbamazepine and ethinylestradiol were adsorbed to the suspended matter in both growth media. Natural estrogens and metoprolol were only adsorbed to the suspended matter in the batches with urine. Ibuprofen and paracetamol were not detected in suspended matter.

According to the data (Table 5.3), only limited sorption of the studied pharmaceuticals to the suspended matter was observed. The results are comparable with the previous studies of Lai et al. [142], who showed that only 6% of estrogens were adsorbed to algal biomass and of Hirooka et al. [108], who observed no accumulation of bisphenol A in algal cells. Concentration limits for pharmaceuticals and hormones in organic fertilizers do not exist yet, which makes it impossible to assess the feasibility of application of algae as fertilizer from a legislative point of view. However, the environmental impact of algae applied as fertilizer can be assessed by the model, developed by Rieß [220] and extended by Hammer and Clemens [102].

Table 5.3 Sorption of micropollutants to suspended matter.

Compound	Concentration, ug/g		% adsorbed (of initial concentration)		% adsorbed (of removed amount)	
	urine	AnBW	urine	AnBW	urine	AnBW
Diclofenac	2.9 ± 1.5	1.4 ± 0.6	7.5	5.5	15.6	9.1
Ibuprofen	<LOD	<LOD	<0.5	<0.6	<0.5	<0.6
Paracetamol	<LOD	<LOD	<0.1	<0.1	<0.1	<0.1
Metoprolol	0.2 ± 0.1	<LOD	0.4	<0.1	0.8	<0.1
Trimethoprim	0.7 ± 0.4	1.2 ± 0.4	1.1	3.7	3.8	7.6
Carbamazepine	0.9 ± 0.4	1.2 ± 0.5	2.6	5.0	12.3	16.7
Estrone	10.2 ± 2.5	<LOD	n.d.	n.d.	n.d.	n.d.
β-estradiol	0.1 ± 0.1	<LOD	n.d.	n.d.	n.d.	n.d.
Ethinylestradiol	2.5 ± 1.4	0.2 ± 0.1	n.d.	n.d.	n.d.	n.d.

n.d. – not detected

The model assumes that a certain input of nutrients to the agricultural soil (N – 170 kg ha^{-1} $year^{-1}$, P – 26.4 kg ha^{-1} $year^{-1}$, K – 132.8 kg ha^{-1} $year^{-1}$) is required for crop production. To prevent over fertilization, the application of organic fertilizers is limited by the nutrient concentration. The nutrient with the lowest fertilizing demand will determine the application rate. According to the elemental composition of biomass (C H1.75±0.02 O0.42±0.04 N0.15±0.02 P0.008±0.003 Mg0.002±0.0003) [276], *C. sorokiniana* contains in weight percentages 9.2% of nitrogen and 1% of phosphorus. Nitrogen is the limiting nutrient with 1.9 ton ha^{-1} $year^{-1}$ of algal biomass required to meet the nitrogen demand. If algal biomass from this study would be applied as fertilizer, the annual input of micropollutants is 0.0055 kg ha^{-1} diclofenac, 0.0017 kg ha^{-1} carbamazepine, 0.01938 kg ha^{-1} estrone and 0.0048 kg ha^{-1} ethinylestradiol. These values are considerably lower than the annual input of diclofenac (2.9 kg ha^{-1}), carbamazepine (26.6 kg ha^{-1}), estrone (0.275 kg ha^{-1}) and comparable to the annual input of ethinylestradiol (0.0025 kg ha^{-1}) predicted from direct application of raw urine as a fertilizer [102]. Thus, agricultural application of algal biomass, grown on urine or AnBW is preferred over direct application of urine to achieve lower inputs of micropollutants to soil.

Biomass grown on AnBW contains lower concentrations of micropollutants than biomass grown in urine, as shown in this study. This is probably due to the low biomass yield in the urine batches as a result of the low Mg^{2+} content in the hydrolysed urine. Unless the urine is freshly collected and used for microalgae growth, addition of Mg^{2+} directly to the microalgae culture would be a relatively simple option. The nutrient ratio in AnBW is favourable for algal growth. Additionally, micropollutants can be removed during the anaerobic treatment process, reducing their loads for the algal treatment system and, potentially, sorption to the algal biomass. For example, trimethoprim is stable under aerobic conditions, while being removed during anaerobic treatment [62].

5.4 Conclusions

Our results show that the tested micropollutants did not inhibit *C. sorokiniana* growth at spiked concentrations (100-350 µg/L). In our batches, ibuprofen and diclofenac were photolytically removed, whereas the combination of photolysis and biodegradation was responsible for the removal of metoprolol and paracetamol. Carbamazepine and trimethoprim were recalcitrant towards both biodegradation and photolysis. Algae enhanced metoprolol removal, but did not enhance nor constrain removal of the other micropollutants. Removal of metoprolol and diclofenac in the studied algal treatment system is higher than in the conventional STP with a SRT of 10 days showing the removal potential of algae based systems. Sorption to the algal biomass accounted for <20% of the removed micropollutants, showing that the use of algal biomass grown on urine or AnBW as fertilizer introduces less micropollutants to soil compared to the direct application of urine. Therefore algal treatment systems have a potential to remove micropollutants while simultaneously closing the cycle of nutrients in a safer way.

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Supplementary Information

Table S5.1 Measured initial concentrations (µg/L) of the studied pharmaceuticals in the batches after spiking.

Compound	Measured initial concentrations (C_0)	Maximal reported concentrations in AnBW	Maximal reported concentrations in urine
Ibuprofen	317 ± 33	456 [62]	794 [271]
Diclofenac	147 ± 9	59.1 [62]	72 [27]
Carbamazepine	117 ± 17	6.2 [62]	29 [271]
Metoprolol	181 ± 62	91.4 [36]	n.f.*
Paracetamol	337 ± 23	602 [62]	n.f.*
Trimethoprim	202 ± 30	2.1 [62]	1300 [27]

n.f. – data not found

Table S5.2 Composition of mobile phases and elution gradient programs for LC separation of the studied micropollutants.

Mobile phases	
Mobile phase A	Pharmaceuticals (positive electrospray ionization): 2.5 l H_2O + 1.5 mL CH_2O_2 + 0.5 mL $C_4HF_7O_2$ + 30 mg $C_2H_2O_4$
	Pharmaceuticals (negative electrospray ionization): 2.5 l H_2O + 0.75 mL CH_2O_2 + 1.5 mL NH_3
	Estrogens (positive electrospray ionization): 2.5 l H_2O + 1 mL CH_2O_2 + 1 mL NH_3
Mobile phase B	Pharmaceuticals (positive electrospray ionization): C_2H_3N + 0.1% CH_2O_2
	Pharmaceuticals (negative electrospray ionization): C_2H_3N
	Estrogens (positive electrospray ionization): C_2H_3N

Gradient elution programs								
Pharmaceuticals (positive electrospray ionization)[a]			Pharmaceuticals (negative electrospray ionization)[b]			Estrogens (positive electrospray ionization)[c]		
Time, min	Mobile phase B, %	Pump, ml/min	Time (min)	Mobile phase B, %	Pump, ml/min	Time (min)	Mobile phase B, %	Pump, ml/min
0.0	2	0.35	0.0	5	0.35	0.0	10	0.5
1.0	2	0.35	1.0	5	0.35	2.0	10	0.5
8.0	100	0.35	7.0	100	0.35	3.0	75	0.5
9.0	100	0.35	9.0	100	0.35	8.0	75	0.5
9.5	2	0.35	9.5	5	0.35	8.5	100	0.5
15.0	2	0.35	15.0	5	0.35	11.0	100	0.5
						11.1	10	0.5
						15.0	10	0.5

a - injection volume 3 µL; b - injection volume 10 µL; c- injection volume 2 µL

Table S5.3 Retention times (RT), monitored ions, MS parameters and internal standards, used for correction of peak areas of the studied micropollutants.

Compound	RT (min)	Precursor ion	Product ion	Fragmentor voltage (V)	Collision energy (eV)	Corresponding internal standard	Ionization mode
Analysed pharmaceuticals							
Ibuprofen	7.34	205.0	161.0	80	0	Fenoprofen	Negative
Diclofenac	7.36	294.0	250.0	80	5	Fenoprofen	Negative
Carbamazepine	6.87	237.2	194.2	155	16	Dihydrocarbmazepine	Positive
Metoprolol	6.21	268.2	191.1	80	15	Diaveridine	Positive
Paracetamol	3.10	152.1	109.9	90	13	Diaveridine	Positive
Trimethoprim	5.96	291.1	261.1	160	25	Trimethoprim-d9	Positive
Estrone[a]	10.17	504.2	171.1	200	40	β-Estradiol-d3	Positive
β-Estradiol[a]	9.46	506.2	171.1	200	40	β-Estradiol-d3	Positive
Ethinylestradiol[a]	9.65	530.2	171.1	200	40	β-Estradiol-d3	Positive
Internal standards							
Fenoprofen	7.23	241.0	197.0	80	0		
Diaveridine	5.89	261.2	245.2	155	16		
Dihydrocarbmazepine	6.91	239.2	194.2	160	22		
Trimethoprim-d9	5.92	300.0	280.0	145	26		
β-Estradiol-d3[a]	9.44	509.2	171.1	200	40		

a - dansylated forms

Table S5.4a Recoveries of the studied micropollutants in liquid samples (in %).

Compound	Medium content						
	Synthetic urine + algae	Urine + algae	AnBW + algae	Urine	AnBW	Urine (abiotic)	AnBW (abiotic)
Ibuprofen	89.8	96.8	96.8	95.0	92.0	94.9	92.6
Diclofenac	113.3	119.8	102.4	111.9	111.9	103.6	116.4
Carbamazepine	94.1	100.2	71.1	108.1	109.6	114.9	115.7
Metoprolol	105.8	81.9	109.1	92.0	80.0	148.4	103.2
Paracetamol	85.7	79.8	75.1	85.8	100.9	86.8	98.0
Trimethoprim	73.9	66.3	67.7	74.4	71.7	92.1	83.7

Table S5.4b Recoveries of the studied micropollutants in solid samples (in %).

Compound	Medium content		
	Synthetic urine + algae	Urine + algae	AnBW + algae
Ibuprofen	110.0	101.2	98.4
Diclofenac	73.6	80.0	80.1
Carbamazepine	126.0	120.0	131.9
Metoprolol	120.2	125.3	112.4
Paracetamol	136.4	127.4	161.6
Trimethoprim	174.4	123.2	137.2
Estrone	113.3	116.0	110.1
B-Estradiol	105.8	129.3	99.7
Ethinylestradiol	103.9	167.3	114.2

Table S5.5 Limits of detection and quantification of the studied micropollutants in liquid samples (in µg/L) and in solid samples (µg/g).

Compound	Limits of detection		Limits of quantification	
	Liquid phase, µg/L	Solid phase, µg/g	Liquid phase, µg/L	Solid phase, µg/g
Ibuprofen	0.25	0.29	0.84	0.96
Diclofenac	0.05	0.01	0.15	0.03
Carbamazepine	0.41	0.13	1.36	0.42
Metoprolol	0.26	0.03	0.87	0.09
Paracetamol	0.48	0.03	1.59	0.10
Trimethoprim	0.35	0.07	1.17	0.24
Estrone	n.d.	0.01	n.d.	0.02
Estradiol	n.d.	0.00	n.d.	0.01
Ethinylestradiol	n.d.	0.00	n.d.	0.01

n.d. – not detected

Chapter 6

General discussion

Synergy between biological and chemical processes; towards lower pharmaceutical emissions

6.1 The need for safe water

Worldwide drinking water supply systems are under increasing pressure as water scarcity, climate change, population growth, demographic changes and urbanization challenges the provision of safe drinking water [305]. Limited accessibility to safe drinking water is on the political agendas globally already for several decades. As a result, the seventh goal of the United Nations Millennium Development Goals (MDG) established in 2000 aims at reducing the proportion of the world's population without sustainable access to safe water by 2015 [281]. Although big steps were made during these 15 years, by 2015 still 2.1 billion people did not have access to "safely managed drinking water services" which is defined as drinking water from an improved water source that is located on premises, available when needed, and free from faecal and priority chemical contamination [305]. Of these people, 1.3 billion had access to basic drinking water services (i.e. improved drinking water availability within a round trip of 30 minutes), the other 844 million people even lacked basic drinking water services. Because of the inaccessibility to clean drinking water the United Nations Sustainable Development Goals established in 2015 included the following targets on water quality; 6.1 "*By 2030, achieve universal and equitable access to safe and affordable drinking water for all*" and 6.3 "*By 2030, improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally*" [282]. The increasing pressure on safe drinking water supply is a worldwide problem, not only affecting developing countries. For instance, droughts over the past 16 years in the southern states of the U.S. caused historically low water levels in the Colorado river leading to water scarcity and thereby a high pressure on the drinking water supply [13].

An important strategy to overcome issues regarding drinking water supply is the reuse of wastewater for water recovery [305]. The reuse of WWTP effluents for drinking water production is already practiced over 50 years in many countries [69]. In most cases this concerns an indirect reuse, applying a natural system such as a groundwater aquifer for additional treatment, retention and dilution with other water streams between the WWTP effluent and drinking water intake. However, over the

past decade the direct reuse of WWTP effluent for drinking water production has also been applied. Especially in the U.S., Southern Africa and Singapore multiple projects have started applying the direct reuse for drinking water production as additional treatment processes are able to eliminate contaminants to drinking water inlet standards [69, 148]. Nevertheless, there is an ongoing debate regarding the public health, treatment of emerging contaminants, monitoring, costs and maintenance aspects of direct reuse [69]. In addition to the reuse for drinking water purposes, reuse of WWTP effluents for other purposes such as irrigation, industrial application, urban uses and environmental purposes like flow restoration have also been extensively studied [202]. Even in countries like the Netherlands where droughts and water scarcity are no major issues the debate of using non-traditional drinking water sources is conducted as costs for drinking water production increase due to environmental issues such as pollution and salinization of aquifers [253].

The presence of numerous micropollutants is one of the main issues when reuse of WWTP effluent for other purposes is considered [305]. Among the broad suite of micropollutants covering pharmaceuticals, pesticides, herbicides, personal care products, hormones and many others contaminants, the focus of this dissertation is only on pharmaceuticals. Chapter 1 describes the effects which pharmaceuticals can have on the environment and human health when discharged to surface waters as currently practiced. Both the (direct) reuse and the discharge to surface waters of WWTP effluents motivate the elimination of pharmaceuticals and other micropollutants at the WWTP and call for immediate action. Different treatment processes for the removal of pharmaceuticals are therefore presented in this dissertation. In this chapter these processes are further discussed with respect to their functioning and how they can contribute to a strategy towards a safer water reuse or discharge. The parameters and processes identified for the research presented in this dissertation are presented in Table 6.1 were.

Table 6.1 Overview of the parameters and processes studied for pharmaceutical removal in the chapters of this dissertation.

Parameter/Process	Chapter 2	Chapter 3	Chapter 4	Chapter 5
Biological treatment				
Redox conditions	X			
Mixed inoculum		X	X	
TOC removal			X	
Real wastewater			X	X
Algae				X
Chemical treatment				
UV-LED TiO_2 photocatalysis		X		
Ozonation			X	
Photolysis				X

6.2 Pharmaceutical removal by biological treatment

Conventional WWTPs are not designed for pharmaceutical elimination and optimization of the current treatment processes or extension with other treatment processes is needed to obtain an improved pharmaceutical removal. WWTPs typically employ biological processes for bulk organic matter, nitrogen and phosphorous removal as they are regarded robust and more cost-effective compared to other treatment processes. Based on these arguments biological treatment for pharmaceutical removal is therefore desired. Multiple factors and parameters influence the biological removal processes in wastewater treatment of which many cannot be adjusted as they relate to the incoming wastewater, e.g. amounts of organic matter, nutrients, trace elements, toxins, etc. Others can be used to steer the process performance, e.g. hydraulic and solid retention times, redox conditions, type of biomass, biomass attachment, substrate dosage and substrate availability in individual treatment steps. The influence of redox conditions, the effect of biodegradable substrates and the application of alternative microorganisms is elucidated in this dissertation and further elaborated and discussed in this section.

6.2.1 Influence of redox conditions

The influence of redox conditions is of great importance for any biological conversion. In Chapter 2 we demonstrated that aerobic conditions exert the most optimal biological removal of pharmaceuticals. However, under deeply anaerobic conditions, i.e. sulfate reducing or methanogenic conditions, we also found high pharmaceutical removal. Intermediate redox conditions like micro-aerophilic and nitrate reducing conditions were less effective. In general aerobic conditions are reported in the literature to be most favourable for the biodegradation of a wide variety of pharmaceuticals [7, 26, 83, 215, 247]. However, some individual pharmaceuticals are better degraded under a specific redox condition. For instance, sulfamethoxazole was better removed under micro-aerophilic conditions compared to fully aerobic and anoxic/aerobic cycling [247]. Moreover, sulfamethoxazole and trimethoprim were better removed under methanogenic conditions compared to aerobic conditions [7] and venlafaxine, diatrizoate and tramadol were better removed under iron reducing, sulfate reducing and methanogenic conditions compared to aerobic conditions [83]. This suggests that a biological process covering a redox gradient from aerobic to methanogenic conditions has advantages over a single redox condition which is therefore recommended for all biological processes aiming at pharmaceutical removal.

6.2.2 Effects of biodegradable substrates

The presence of easily biodegradable substrates in high concentrations is hypothesized to negatively influence the pharmaceutical removal (Chapter 1). In Chapter 4 a relatively high pharmaceutical removal was observed in the investigated biological reactor of the BO_3B process compared to the literature on pharmaceutical removal in WWTPs. In comparison with WWTPs the applied HRT in our system was short and biomass levels were low, which would suggest a low pharmaceutical removal. However, the observed removal was high which is most likely related to the difference in organic matter (OM) concentration and composition of the feed solutions as the BO_3B process was running on secondary clarified effluent. Whereas raw wastewater entering WWTPs contains high amounts of OM (80-260 mg TOC/L) with

a high BOD/TOC ratio (1.2-2.0), the secondary effluent is typically low in OM (<20 mg TOC/L) and less biodegradable (BOD/TOC 0.2-0.5) as OM removal (i.e. BOD removal) is one of the main objectives of wastewater treatment [261]. The negative influence of OM on the pharmaceutical removal is also described in other studies. In column experiments mimicking managed aquifer recharge (MAR) systems the removal of pharmaceuticals enhanced at lower biodegradable organic matter (BOM) concentrations, i.e. biodegradable dissolved organic carbon concentrations of 0.69 mg/L versus 1.55 mg/L [155]. A higher microbial density at increasing ratios of BOM over OM was reported, whereas both the microbial diversity and the metabolic capability of the microorganisms to degrade pharmaceuticals declined. Moreover, at low compared to high OM levels higher microbial diversities and lower microbial densities were found in field and laboratory scale MAR systems, demonstrating that high substrate levels limit the capacity of a microbiome to degrade a broad array of compounds [154]. Additionally, in MAR systems the biological removal of pharmaceuticals could be linked to co-metabolism rather than to direct substrate utilization by comparing the pharmaceutical removal in a pre-exposed microbial community to a unexposed community [4]. The authors found a similar pharmaceutical removal in pre-exposed and unexposed microbial communities, suggesting that microbial adaptation to pharmaceuticals did not occur. Hence, the utilization of pharmaceuticals as primary substrate is unlikely which is further supported by the high concentration difference between pharmaceuticals and other substrates in MAR systems. Instead of adaptation, the capacity for pharmaceutical degradation is dictated by other factors which influence the microbial community such as the composition and concentration of primary substrates [4]. In most environments primary substrates are present in higher concentrations than pharmaceuticals, e.g. raw wastewater, primary clarified effluent, secondary clarified effluent, surface water and groundwater, most likely favouring co-metabolic degradation. Similarly, the work presented in Chapter 3 showed that photocatalytic products enhanced the subsequent biodegradation of atorvastatin, caffeine, diclofenac, gemfibrozil and ibuprofen. Based on the literature it was postulated that biodegradation of photocatalytic products generated intracellular electron carriers

that initiated the initial mono-oxygenation reaction for biodegradation of the pharmaceuticals, suggesting that co-metabolisms was highly important. In contrast, adaptation to pharmaceuticals was found in Chapter 2 as pharmaceutical removal in batch experiments enhanced after re-spiking pharmaceuticals. Pharmaceuticals dissolved in methanol were added multiple times without the addition of other OM sources. As every re-spike contained the same ratio of pharmaceuticals to methanol the observed increase in removal efficiency could be related to adaptation or to an increase of pharmaceutical degrading biomass. Hence, the role of microbial adaptation and co-metabolic degradation in the biological removal of pharmaceuticals remains ambiguous. Likely, the microbial adaptation differs per individual pharmaceutical as was also found for non-pharmaceutical contaminants by Spain and Van Veld [246]. In their study on pre-exposed and unexposed microbial communities adaptation was found towards 2,4-dichlorophenoxyacetic acid and p-nitrophenol, whereas no adaptation was found to trifluralin and p-cresol was well degraded in both communities. Moreover, the contaminant concentration during pre-exposure was found to affect the adaptation, e.g. lower concentrations p-nitrophenol were needed to achieve adaptation than for methyl parathion [245]. These findings on pharmaceuticals and non-pharmaceutical contaminants stress the need for additional research as adaptation can potentially enhance the biodegradation in real applications. Therefore further investigations should focus on the functioning of microbial adaptation and unravel the parameters that steer the adaptation which can be used in application.

6.2.3 Algae and fungi

Biological processes for treatment of various contaminants reported in the literature mainly concern the utilization of bacteria, e.g. wastewater treatment with activated sludge, anaerobic sludge digestion, in-situ soil remediation and sand filtration in drinking water production. However, also other microorganisms such as algae or fungi are known for their capacities to degrade or take up various contaminants [66, 74, 87, 192]. The use of algal wastewater treatment processes is of increasing interest as they provide a clean effluent, and enable resource recovery

in the form of fertilizer, protein-rich feed or biofuel via the harvested algal biomass [55]. Most studies focus on the resource recovery by algae, whereas the removal of pharmaceuticals which are also present in those systems is only limitedly studied. Chapter 5 describes an algal treatment process for the simultaneous removal of nitrogen and phosphorus and pharmaceuticals in an alternative sanitation system. It was found that algae contributed to the removal of pharmaceuticals while also recovering nitrogen and phosphate from the wastewater. For instance, metoprolol removal enhanced in the presence of algae. Sorption and uptake of pharmaceuticals by algae was limited and was hypothesized be safe for the application of algal biomass as fertilizer. The photobioreactor in which the algae were cultivated had a positive effect on the pharmaceutical removal as compounds susceptible to photolysis such as diclofenac were effectively removed by photodegradation. Moreover, the naturally present microbes in the wastewater also contributed to the pharmaceutical removal. Hence, a synergy was found in the combination of algal-, microbial- and photodegradation for the removal of pharmaceuticals. Similarly, Matamoros et al. [174] also concluded that algae contributed to the removal of pharmaceuticals from wastewater as the presence of algae enhanced ibuprofen and caffeine removal. Moreover, they did not found uptake of pharmaceuticals by the algae. In an outdoor pilot scale algal treatment plant an effective pharmaceutical removal and a subsequent reduction in hazard quotient were found when operated in a warm (25±1°C) and cold season (13±1°C) [173]. Nevertheless, this was achieved at HRTs of 4 and 8 days and a reactor of depth of 0.3 cm to allow sunlight penetration into the reactor. Hence, these HRTs and reactor depths exhibit a large surface footprint and are thereby unrealistic design parameters for densely populated countries like the Netherlands where available land is scarce and costly. Moreover, algal treatment systems require light input and moderate to high temperatures for algae growth. Climate conditions in the Netherlands were found unsuitable for WWTP effluent post-treatment by algal biofilm reactors as solar irradiance levels and temperatures were not high enough to support sufficient algal biomass growth [29]. Therefore outdoor solar based algal reactors for resource recovery and pharmaceutical removal are hypothesized not to be a feasible treatment option in the Netherlands. However, for

locations at lower latitudes (e.g. in Spain), for events like festivals or campsites during summer in the Netherlands when irradiation and temperatures are higher or for systems running on artificial light sources like LED-UV systems in temperature controlled greenhouses an algae based process might be a versatile treatment system. The continuous improvements of LED irradiation suggest that LED based algal treatment systems, preferably combined with solar irradiance, can become a cost-effective wastewater treatment process. Further research is recommended to focus on the technical and financial feasibility of LED based algal treatment systems in greenhouses for the cost-effective recovery of nutrients and simultaneous pharmaceutical removal from wastewater. Moreover, research has to include a much wider pallet of pharmaceuticals and toxicity assessment of the effluent as both are currently limitedly understood.

Among the various fungi species used in biological treatment processes, white-rot fungi and their enzymes have been well studied for the removal of pharmaceuticals [56]. These organisms have a ligninolytic enzymatic system allowing them to produce extracellular enzymes which can target various contaminants in a non-specific and radical based manner [45]. Their ability to produce strong oxidants like peroxide effectively targets compounds that persist in other biological treatment processes such as the recalcitrant pharmaceutical carbamazepine [223]. Although white-rot fungi are promising for pharmaceutical removal, issues related to the contamination of the reactors, and thereby the competition with other microorganism suppressing the fungi, have been identified when culturing them under non-sterile conditions using real wastewater [19, 156]. This might be one of the reasons why implementation of fungal based technologies in domestic wastewater treatment is limited.

6.2.4 Opportunities in biological treatment

Multiple biological processes and the parameters influencing these processes have been studied for the removal of pharmaceuticals. Findings of these studies have resulted in a better understanding of biological treatment processes and relevant aspects for the design and operation of them. Nevertheless none of them, nor a combination of them, has been found adequate to achieve a complete removal of the

pharmaceuticals typically found in wastewater [1, 83, 164, 222]. Similarly, in this dissertation (Chapters 2-5) it was demonstrated that biological processes do not form an effective barrier against all pharmaceuticals. Large molecules typically persist during biological treatment, whereas intermediate or small size molecules are easily biodegraded or even mineralized [165]. In combination with additional treatment steps targeting large molecules, biological removal might therefore be a versatile treatment process. Reflecting on the outcomes of this dissertation several recommendations are given regarding the design and operation of biological processes when biological treatment is considered for pharmaceutical removal. Concerning bacterial based processes, it is recommended to incorporate multiple redox stages, including fully aerobic and methanogenic conditions, to create niches for a diverse microbial community targeting a wide spectrum of pharmaceuticals. In addition, pharmaceutical removal can be best accomplished under oligotrophic conditions, i.e. in a post-treatment step where primary substrate levels are low. In case of sub-optimal performance of the secondary treatment and clarification, a two-step process might be desired to remove primary substrates in the first and pharmaceuticals in the second step. When irradiance levels and temperatures allow a cost-effective algae growth, algal treatment systems are recommended as they form a barrier against multiple pharmaceuticals and provide opportunities for resource recovery. However, algal treatment systems have their limitations regarding pharmaceutical removal. For a safe direct reused or discharge effluents of algal treatment systems the non-removed pharmaceuticals should therefore be additionally treated by other treatment processes. Therefore it is recommended for future research focusing on sustainable water treatment to employ algal treatment systems in combination with other processes for the efficient resource-recovery and a safe effluent production.

Research on biological removal processes is ongoing and will continue, nevertheless it is questionable with the obtained knowledge of the past decades whether it can be expected that a biological process will remove the highly complex mixture of structurally diverse pharmaceuticals. Considering that pharmaceuticals are only part of the micropollutants which are currently detected in wastewater the entire

load of harmful compounds at low concentrations is much higher which further complicates an adequate elimination of all of these insidious compounds. Nonetheless, biological processes can be part of a broader treatment system for the cost-effective removal of micropollutants which justifies the further investigation and understanding into biological processes. Research opportunities lie, amongst others, in the further unravelling of metabolic pathways of pharmaceutical degradation. A better understanding of degradation pathways can result in the further optimization of biological processes by steering for specific conditions favouring enzymatic systems involved in pharmaceutical degradation. Moreover, insight in the diversity of involved metabolic pathways can indicate to what extend different enzymatic systems are needed for the degradation of a broad spectrum of pharmaceuticals. This can be useful for the design of a comparted biological treatment, creating optimal conditions for specific metabolic pathways in each compartment to target specific pharmaceuticals. First steps have been made in the field of understanding the metabolic pathways involved in pharmaceutical degradation [97, 182, 299, 300]. Yet, to date, there is still a limited understanding of all enzymatic processes involved in pharmaceutical degradation. Although research suggests that biological pharmaceutical removal follows co-metabolic degradation pathways conclusive enzymatic evidence to confirm this is still lacking [4].

6.3 Pharmaceutical removal by chemical treatment

Chemical treatment processes involving ozonation, photolysis, photocatalysis, hydrogen peroxide, chlorination, Fenton's reagent, electrolysis, ultrasound, ionising radiation, microwaves and pulsed plasma have been applied for drinking water treatment, industrial wastewater treatment as well as in domestic wastewater treatment [52, 264].

In drinking water sources numerous contaminants and matrix constituents are present at very low (ng/L) and low (mg/L) concentrations, respectively [264]. Hence, the production of drinking water free of contaminants and low in matrix constituents favours one or more generic treatment steps. Industrial wastewaters typically contain

a limited number of contaminants present at high concentrations (g/L) in simple matrices which eases a tailor made design of effective treatment processes [165].

In contrast, domestic WWTP effluents comprise a highly diverse mixture of matrix constituents typically present at orders of magnitude higher concentrations compared to contaminants like pharmaceuticals. Moreover, WWTP effluent contaminants cover an array of structurally different compounds. Chemical treatment processes effectively oxidize large molecules into smaller intermediates, however full mineralization in the chemical treatment is often cost-intensive and thereby undesired. Therefore, combining chemical and biological processes for a complete mineralisation can be a favourable approach [165]. Even though chemical treatment processes can be highly effective for the removal of waterborne contaminants, the ratio between matrix and contaminant concentrations and the diversity in contaminant chemical structures complicates the design of cost-effective and sustainable treatment processes. Processes targeting specific contaminants do not provide the removal of a broad spectrum of contaminants, whereas more generic processes inevitably result in the reaction with matrix constituents reducing the removal efficiency for target contaminants. As a result, each individual situation therefore requires the assessment of the most adequate and cost-effective treatment system.

Recently, chemical treatment processes of a more generic character like ozonation and AC filtration have been well studied and locally implemented at WWTPs in post-treatment steps for pharmaceutical removal [73]. First results of their full scale implementation indicate that these processes can result in the effective removal of pharmaceuticals. Nevertheless, ozonation still raises concerns on the formation of potentially toxic transformation products, whereas AC filtration requires the regeneration or replacement of the AC questioning the sustainability and cost-effectiveness of the process. In addition, a one to one translation of these results to other places is hampered by differences in local raw wastewater qualities and the site-specific performance of primary and secondary treatment processes. For example, OM concentrations in secondary clarified effluents from individual Swiss, Japanese and U.S. WWTPs are two to ten times lower compared to those from individual

Australian, German, U.S. and Dutch WWTPs (Chapter 4) [109, 111, 117, 150, 193, 265, 298, 329]. At high OM concentrations or in the presence of specific molecular size OM fractions, pharmaceutical removal efficiencies in ozonation or AC filtration drastically decrease [151, 327]. An investigated solution to overcome this problem is the combination of pre-ozonation followed by AC filtration in which the incoming OM is partially converted by ozonation into smaller molecule sizes which only limitedly compete with pharmaceuticals for AC sorption sites [218, 328]. This is however a resource intensive and costly treatment and might not be the most sustainable combination of processes.

In other chemical processes employing oxidants such as hydrogen peroxide or Fenton's reagent for pharmaceutical removal OM matter is also known to reduce process efficiencies since it acts as an oxidant scavenger [22]. This suggests that pre-treatment processes targeting OM removal could be of great interest as they increase chemical treatment of pharmaceuticals. Some pre-treatment processes have been investigated like the use of anion exchange. This process effectively removed the OM humic acid fraction of a WWTP effluent and resulted in an 84% lower energy demand for the subsequent UV/H_2O_2 treatment process aiming at pharmaceutical removal [109]. Nevertheless, the costs associated to the combination of anion exchange and subsequent UV/H_2O_2 treatment are relatively high as they increase the wastewater treatment costs by approximately 75% [280]. Overall, there is a need for more cost-effective and sustainable treatment processes including combinations of chemical and biological processes, as currently there are only a few available for wastewater treatment [198].

6.4 Synergy between biological and chemical treatment processes for pharmaceutical removal

Multiple combinations of biological and chemical treatment processes have been demonstrated to be advantageous over single processes [120, 235]. In industries this concept is well introduced as combined process are used to treat textile, paper mill, winery, distillery and olive mill wastewaters [198]. The capacity of chemical treatment processes targeting non-biodegradable compounds to increase

their biodegradability by for instance a strong oxidant attack is the most known combination. Chemical treatment is effective for oxidation of large molecules into smaller intermediates, but not for full mineralization, whereas biological treatment can effectively degrade and mineralize intermediate and small size molecules [165]. The tipping point after which further chemical treatment is no more attractive and biological treatment performs better is depicted in Figure 6.1. Moreover, as biological treatment is generally cheaper and more sustainable than chemical treatment, the latter is typically used as a short pre-treatment increasing the biodegradability and reducing the overall treatment costs [52]. Although in industries the combination of biological and chemical treatment processes is well introduced [198], it is only limitedly applied in wastewater treatment. Chapters 3, 4 and 5 of this dissertation describe combinations of biological and chemical processes for pharmaceutical removal from wastewater with a focus on an unique combination in each chapter. The studied combinations are further discussed in this section.

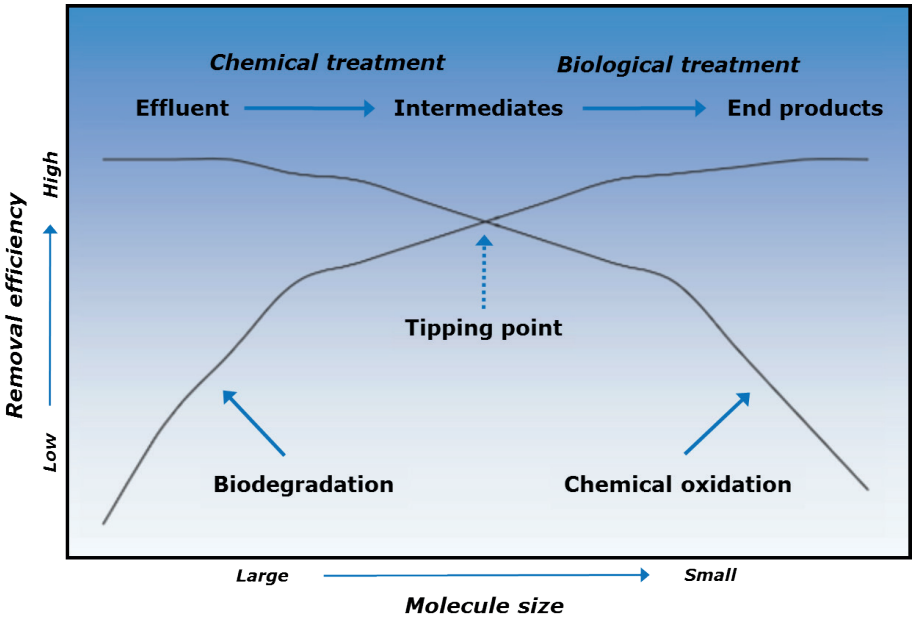

Figure 6.1 The concept of combined chemical and biological treatment (adapted from [165])

6.4.1 Chemical pre-treatment for enhanced biological pharmaceutical removal

Photocatalysis utilizing TiO_2 to catalyse the reaction of UV-light with organic contaminants is a well-known treatment process and widely employed in diverse water treatment systems [52, 103, 126]. Sunlight based TiO_2 photocatalytic processes have been successfully demonstrated, but are limitedly applied compared to systems using UV-lamps as the UV content of solar irradiation reaching the earth surface is only 3-5% [10, 22]. The use of UV-LED as alternative to traditional energy intensive UV-lamps has greatly increased the process resource efficiency [194]. Furthermore, the ongoing research on immobilized forms of TiO_2 is hypothesized to further improve the sustainability and cost-effectiveness of TiO_2 photocatalysis [16]. Successful removal of pharmaceuticals from wastewater by TiO_2 photocatalysis is reported, however the type and concentration of TiO_2 and the process reaction time highly influence the degree of removal, i.e. good removal is achieved at high TiO_2 levels and long irradiation times [272]. Moreover, TiO_2 photocatalytic pharmaceutical removal is associated to the formation of toxic transformation products. These undesirable aspects can hamper the implementation of this promising technology, but can possibly be overcome when combining photocatalysis with biodegradation.

Chapter 3 therefore describes a mild photocatalytic TiO_2 process followed by biological treatment for pharmaceutical removal as a resource efficient alternative to single TiO_2 photocatalysis. Similar to the literature, a selection of pharmaceuticals was removed by the employed photocatalysis whereas other persisted [15]. Unexpectedly, in the subsequent biological treatment the degradation of atorvastatin, caffeine, gemfibrozil and ibuprofen enhanced after photocatalytic pre-treatment, even though most of them were not targeted by photocatalysis. In addition, the biodegradation of diclofenac was observed after photocatalytic pre-treatment whereas it persisted in biological control experiments without pre-treatment. Based on the literature on photocatalytic products and biodegradation pathways it was postulated that intermediates formed during photocatalysis stimulated the biodegradation of pharmaceuticals. Hence, besides the benefits of combining chemical pre-treatment with biological treatment for cost-effective mineralization of large

molecules it can be stated that the biological treatment of pharmaceuticals also benefits from the presence of photocatalytic products for the enhanced biodegradation of parent compounds, i.e. pharmaceuticals. Next to the ongoing advancements on TiO_2 immobilization and UV-LED application, employing a mild photocatalysis with subsequent biological treatment further increases the sustainability. Overall, the enhanced biodegradation and improved sustainability are expected to stimulate the implementation of this combined treatment process. It should be noted that our findings were obtained using a clean matrix and powdered TiO_2, therefore further research on real WWTP effluents and using immobilized TiO_2 is strongly recommended. Moreover, the toxicity of the final effluent should be carefully assessed albeit potentially toxic photocatalytic products will most likely be removed during the biological treatment.

6.4.2 Biological pre-treatment for enhanced chemical pharmaceutical removal

The strong oxidative power of ozone towards a broad spectrum of micropollutants including pharmaceuticals is well studied and locally implemented as post-treatment in wastewater treatment [73]. Equal to the approach of combining chemical treatment followed by biological treatment used in industries, ozonation is followed by sand-filtration for the initial chemical oxidative of large molecules and the subsequent biodegradation for their further degradation or even mineralization [111, 165]. This is an effective strategy when concentrations of matrix constituents like OM, nitrite or other compounds competing for ozone with the target contaminants are present at minute levels. In case matrix composition and concentration are in competition with contaminants for ozone other strategies should be employed. A three-step design was therefore presented in Chapter 4 incorporating biological treatment, ozonation and biological treatment as the investigated secondary clarified effluent comprised relatively high OM levels, i.e. 17 mg TOC/L. These OM levels required an opposing paradigm to the industrial design scheme where chemical treatment supports the biological treatment. In the proposed BO_3B design biological pre-treatment facilitates the ozone treatment by removing matrix constituents that

scavenge the ozone reaction with biorecalcitrant pharmaceuticals prior to entering the ozone reactor. As 38% TOC removal was accomplished during biological pre-treatment, this design allowed a minimal ozone input. The BO_3B process effectively removed biodegradable and biorecalcitrant pharmaceuticals, as the removal mechanisms of biological treatment and ozonation targeted different pharmaceuticals. Finally, the ozonation transformation products are biodegraded in the last biological treatment step before being discharged into the environment. Although the BO_3B design incorporates three reactors, the estimated costs are low and would increase the costs for current treatment by less than 15%. The BO_3B process is therefore a versatile alternative combination of treatment processes compared to combinations such as ozonation and AC filtration which have been demonstrated to effectively remove pharmaceuticals [218]. However, the combination of biological treatment with ozonation instead of AC filtration with ozonation makes the process more sustainable as no regeneration or replacement of AC is needed and cost associated to biological treatment are generally lower than those for AC filtration.

6.4.3 Simultaneous biological and chemical treatment for pharmaceutical removal

Chemical treatment processes applied for the removal of organic compounds also exhibit disinfection properties. In drinking water production disinfection is one of the main reasons why chemical processes are employed [242, 294]. As disinfection has negative effects on microorganisms applied in biological treatment, there are only a few examples of versatile combinations of simultaneous biological and chemical treatment processes. A well-known combination are algal treatment systems, such as the photobioreactors investigated in Chapter 5. Treatment systems employing algae are a combination of chemical and biological processes as the metabolism of algae requires photons, i.e. light. Thus, photodegradation and biodegradation can occur simultaneously. We observed effective removal by photodegradation alone or in combination with biodegradation of pharmaceuticals such as diclofenac and metoprolol, respectively. Although the findings of the investigated combination are

promising, major drawbacks have been identified. For instance, only a selection of pharmaceuticals can be effectively removed by photolysis as not all compounds are susceptible to photolysis, or at an insignificant rate for application in wastewater treatment [12, 140, 143, 160]. General applicability of photolytic processes is also hampered by the differences in wastewaters as matrix constituents like OM, nitrate and bicarbonate influence the photolysis of pharmaceuticals in different ways requiring a site-specific evaluation of the photolytic efficiency [143]. This might explain why Matamoros, et al. [174] did not observe photodegradation of ibuprofen in their algal photobioreactor running on primary clarified wastewater whereas in Chapter 5 we found that in anaerobically treated black water ibuprofen was photodegraded. Irradiance intensities are also highly important as they influence the photodegradation efficiency of pharmaceuticals [12]. Hence, solar based systems will perform poorly at higher latitudes indicating the need of artificial irradiance.

Although photolytic and biological pharmaceutical removal are complementary processes for pharmaceutical removal, they do not target all pharmaceuticals. Biorecalcitrant and photorecalcitrant pharmaceuticals, such as carbamazepine, persist in algal treatment processes. Hence, algal treatment processes exhibit great benefits with respect to resource recovery, and for that purpose should be considered in wastewater treatment. While they can recover nutrients and remove pharmaceuticals simultaneously, additional treatment is required for a full pharmaceutical removal.

Another well-studied combination of simultaneous biological and chemical processes is the intimately coupled photocatalysis and biodegradation [307]. Carriers with an photocatalytic outer surface and attached microorganisms inside the carrier have demonstrated an effective removal of contaminants like the antibiotic tetracycline. The main advantage of this combination is the close distance between the reactive photocatalytic surface and the microorganisms which facilitates the instant biodegradation of formed photocatalytic products. To date, this combination is mainly tested on lab-scale, therefore it is unknown how effective this process will be on full-scale running on real wastewater.

6.5 Outlook, opportunities for lower pharmaceutical emissions

The ongoing contamination of worldwide freshwater bodies by countless micropollutants, including pharmaceuticals, jeopardizing the environment and human health is one of the main challenges humanity is facing [233]. Discharge of treated and untreated domestic wastewater is one of the major micropollutant sources as micropollutants are ineffectively removed during conventional wastewater treatment [234]. This dissertation and research by others has demonstrated the capacity of various treatment processes to remove pharmaceuticals from wastewater minimizing the further contamination of worldwide freshwater bodies. The use of complementary biological and chemical treatment processes has been found highly effective as major disadvantages of single processes like resource intensiveness or transformation product discharge can be tackled by combined treatment processes. A clear outcome of this dissertation is the inability of single processes for the cost-effectively removal of a broad array of structurally highly different molecules in a complex matrix like wastewater effluent. Both biological processes and chemical processes have their limitations in targeting specific pharmaceuticals and are therefore recommended to be combined to form an adequate barrier against micropollutants like pharmaceuticals.

Table 6.2 Synergy between biological and chemical processes for pharmaceutical removal

Process combination		Synergy	Chapter
Mild UV-LED TiO_2 photocatalysis	Biological treatment	Enhanced biodegradation of biodegradable and otherwise recalcitrant pharmaceuticals	3
Biological treatment	Ozonation	Reduced ozone input by biological TOC removal	4
Ozonation	Biological treatment	Biological removal of ozonation products	4
Algal photobioreactor: Simultaneous photolysis and biological treatment		Single reactor system with multiple pharmaceutical removal mechanisms	5

Different combinations of biological and chemical treatment processes have been investigated in this dissertation. Synergy was found for all the studied combinations, though the way this synergy was expressed differed per combination (Table 6.2). Mild photocatalytic pre-treatment was found to enhance the subsequent biological treatment, even for pharmaceuticals that were not targeted by photocatalysis. Biological pre-treatment removing ozone scavenging OM increased the cost-effectiveness of ozonation for pharmaceutical removal where after potentially toxic ozonation products were biodegraded during subsequent biological treatment. Simultaneously photodegradation and biodegradation in algal photobioreactors demonstrated that in this specific case biological and chemical treatment can also be integrated into a single reactor. Complementariness was found in all the studied combinations increasing the spectrum of targeted pharmaceuticals and the cost-effectiveness of the combination compared to single processes. Based on these results and the literature on combined treatment processes for various contaminants it is postulated that other combinations than the three studied in this dissertation can be cost-effective for the removal of pharmaceuticals. Moreover, the differences among the synergies found in the studies presented in this dissertation suggest that there may be even a wider range of synergy when other combinations of biological and chemical processes are included. Therefore it is recommended to further study combined treatment processes to identify optimal solutions for the wide suite of wastewater effluents to be treated.

The worldwide variety among raw wastewater compositions and treatment plant configurations which both affect the effluent quality, complicates the design and development of a robust universal treatment process for pharmaceutical removal. Rather, there should be a focus on site-specific solutions as there is a wide arsenal of biological and chemical treatment processes available. A better understanding of underlying process mechanisms is required to further improve and extend the selection of process combinations enabling the design of tailor made treatment processes for locations where the currently known combinations are not cost-effective. Therefore, further research is recommended on understanding and

improving biological and chemical treatment processes and the synergy when combining processes, as mentioned earlier in this chapter.

For locations where the wastewater effluent composition is well known, e.g. the WWTP of Bennekom studied in this dissertation, investigated combinations can be scaled up towards pilot installations. Upscaling of the BO_3B treatment process and the algal treatment process is viable as lab-scale systems were successfully tested on real wastewater. Moreover, the BO_3B process was successfully run in a continuous set-up for almost one year. In addition, the BO_3B process performs better than other combinations for estimated costs and sustainability aspects [109, 125]. Hence, the promising results motivate the upscaling of this combined treatment process, especially with respect to the energy savings for ozonation by TOC removal in a biological pre-treatment. Main points of attention concern the design and operation of the biological reactors for the removal of TOC, pharmaceuticals and transformation products. Full-scale sand-filters are regularly back-flushed to prevent clogging, however, the lab-scale reactors used in this dissertation were not regularly back flushed to prevent the wash-out of specific TOC and pharmaceutical degrading microorganisms. Thus, on pilot-scale the need for regular back flushing should be well evaluated or performed in a manner that valuable biomass will not be flushed out. Moreover, the lab-scale reactors were operated with a counter current air flow to achieve fully aerobic conditions favouring the pharmaceutical degradation and to prevent clogging of the reactors. For larger scale reactors it might be more challenging to obtain fully aerobic conditions in a cost-efficient way and this aspect requires attention during the reactor design. Ozonation on full-scale is already applied for pharmaceutical removal, and experiences from these projects can be used for a BO_3B pilot. Furthermore, a broader array of contaminants should be studied in a pilot plant, including non-pharmaceutical micropollutants, to assess the effectiveness of the BO_3B process for numerous compounds at real WWTP effluent concentrations. Lastly, bioassays with *D. magna, P. subcapitata and V. fischeri* showed no acute toxic effects when exposed to the BO_3B process effluent, however these assays cannot be regarded as a full toxicological assessment. Chronic toxicity assays and specific toxicity assays,

e.g. genotoxicity or carcinogenicity assessment, should therefore be conducted to obtain a better understanding of the toxicity potential of the BO_3B process effluent.

The algal treatment process is currently being up scaled at the NIOO building (Wageningen, the Netherlands). By means of vacuum toilets a concentrated black water stream is collected separately from the other wastewater streams and anaerobically digested after which it is fed into the algal treatment system. In tubular algal reactors located in a greenhouse the resource recovery, i.e. algae growth and harvesting, is further studied on pilot-scale. When the resource recovery on pilot scale will be successful, further investigations into the removal of pharmaceuticals is highly recommended as these will be present in high concentrations due to the separate collection of the black water.

Pharmaceutical removal by the combination of photocatalysis and biodegradation was only investigated in a clean matrix and therefore requires further lab-scale testing with real wastewater before upscaling. Next to the effects of matrix constituents on the pharmaceutical removal the immobilization or recovery of TiO_2 should be investigated as the current process with powdered TiO_2 is not sustainable. Various methods to immobilize TiO_2 have been developed, each with its own characteristics like isoelectric point which do influence the photocatalysis of for instance pharmaceuticals [15]. Understanding the correlation between different TiO_2 immobilization methods and the removal of pharmaceuticals in a subsequent biological treatment process is therefore highly relevant.

The BO_3B process seems the most versatile treatment process for implementation on a short horizon taking into account the observed synergies between the investigated biological and chemical processes and the upscaling potential. Especially for wastewater effluents with relatively high OM concentrations the complementariness between biological treatment and ozonation seems most useful for application, like for instance at WWTP Bennekom.

The detection and identification of numerous pharmaceuticals in wastewater only revealed the tip of the iceberg as it is already know that in the human body, in the sewer and during wastewater treatment countless metabolites and transformation products can be formed. Indications that some of these compounds might be more

harmful than the parent compounds, i.e. the consumed pharmaceuticals, implies that further research on this topic is needed. Understanding the behaviour, fate, effects and treatability of pharmaceuticals took an enormous effort of the scientific community over the last decades and is still ongoing. Hence, fully understanding the role and impact of metabolites and transformation products is even more challenging and is expected to provide numerous research needs to be addressed over the coming decades. In addition to pharmaceuticals high numbers of other micropollutants are also present in wastewater and should be taken into account in future research on the design of removal processes [234]. Moreover, non-chemical contamination like antibiotic resistant genes and bacteria can pose a serious threat to public health and should therefore also be studied [41, 48]. Ideally, the disinfection properties of chemical treatment processes can be applied to target micropollutant and antibiotic resistant genes and bacteria, for example, as ozonation employed for micropollutant removal also demonstrated disinfection of secondary clarified effluent [20].

Pharmaceuticals are only one group of all the different waterborne contaminants discharged with the effluent. Future research should therefore mainly focus on effect based removal strategies as the final goal of wastewater treatment processes is to provide a safe effluent posing none or minimal effects to the subsequent compartments of the water cycle. Advanced analytical methods at leading institutes on micropollutant research can detect and quantify over 300 different micropollutants present in aqueous matrices in a single run [177]. Even though this is a great achievement and the amount of detectable compounds will most likely even further increase, these methods do not provide an accurate assessment of the risk of the tested sample, i.e. the posed toxicity of the sample constituents mixture towards the ecosystem or humans [208]. Development of comprehensive and practically applicable assays to screen for toxicological effects, especially for chronic effects, is therefore highly needed. Going beyond the performance assessment of treatment processes based on chemical parameters, effect based removal strategies incorporating *in vivo* and *in vitro* bioassays can provide a better insight in the obtained removal efficiency.

It is difficult to value the environmental and public health benefits for diminished pharmaceutical emissions via wastewater effluents, especially as the chronic effects of exposure to mixtures of pharmaceuticals at low concentrations are so far limitedly understood. Conducting a thorough cost-benefit analysis is therefore very difficult, or even impossible. Nevertheless, indications that pharmaceuticals can severely affect the aquatic ecosystem and the worldwide need for wastewater reuse motivates investments to mitigate the problem of effluent pharmaceutical emissions. Estimated costs for the BO_3B process implementation would result in increased wastewater treatment costs of 15% for the Netherlands. Expressed per population equivalent this is around €5 per year, which is small compared to the yearly tariff for wastewater treatment of €55 per population equivalent. Moreover, this increase is only 0.014% of the Dutch average yearly spendable income [43]. Similarly, estimated tariff increases of additional pharmaceutical removal processes in the literature are €5-20 per person per year and only 0.1% per person for primary energy consumption [125]. Deciding to wait for a detailed cost-benefit analysis further jeopardizes the environment and the public health. The unknown risks and the limited costs associated to additional wastewater treatment processes for pharmaceutical removal costs therefore justify their implementation.

Interventions at other stages in the chain from pharmaceutical manufacturing to drinking water treatment can be undertaken rather than the end-of-pipe solutions which are the focus of this dissertation and most literature. On the one hand, WWTPs are a main hub in the water cycle which motivates effective end-of-pipe solutions as they can safeguard the next compartments in the water cycle such as surface, ground and drinking water. On the other hand, measures taken at earlier stages in the pharmaceutical chain can positively influence subsequent stages reducing the overall impact. Initiated by the Dutch national government and the wastewater and drinking water authorities in the Netherlands, a chain-approach is formulated aiming at increased awareness of all chain-actors and when possible facilitating interventions to reduce the negative impacts on subsequent [221, 290]. For example, in the province of Flevoland, the Netherlands, pharmacists and family doctors were informed by the local water authorities on the treatability and environmental impact of

pharmaceuticals [181]. They agreed that in case there are biodegradable alternatives available to prescribe these over non-biodegradable pharmaceuticals, thereby reducing the pharmaceutical loads into the environment. A successful experiment was performed In the hospital of Deventer by distributing urine collection bags to patients who ingested contrast media for X-ray scanning [67]. Contrast media are known to persist during wastewater treatment which favours their separate collection and treatment. Patients were asked to collect their urine during 24 hours after ingestion and dispose the bags with the municipal solid waste. After collection this waste is incinerated whereby the contrast media are effectively eliminated. Another aspect of the Dutch chain approach is the support of Green Pharmacy, i.e. the development of degradable pharmaceuticals. Moreover, the chain approach aims at implementing the environmental effect based assessment during the regulatory approval of pharmaceuticals. Raising awareness among the general public on the proper disposal of pharmaceuticals and the environmental and human health related effects of pharmaceuticals is also included in the chain approach. Even though the removal of pharmaceuticals at WWTPs will most likely remain the main stage in the entire pharmaceutical chain, improvements at earlier stages like the examples mentioned above can significantly impact the quantity and quality of the loads entering WWTPs and contribute to reduced emissions to the environment and are therefore strongly recommended.

Switzerland is to date the only example where the discharge of micropollutants including pharmaceuticals is restricted by legislation which forces the monitoring and 80% removal of 5 out of 12 micropollutants [232]. Also for other countries, like the Netherlands, stricter legislation on WWTP effluent quality is required to manage the discharge of pharmaceuticals and other contaminants into the environment. The current knowledge on the negative effects and possible risks of mixtures of discharged contaminants on the environment and the public health motivates the implementation of the precautionary principle. By this policy makers can enforce more restrictive discharge regulations as without further legislation implementation of additional treatment processes is expected to be minimal.

In a wider perspective, alternative sanitation systems should be seriously considered as the current wastewater collection and treatment systems are not optimal for resource recovery, water usage minimization and pharmaceutical removal. Alternative sanitation systems such as the decentralised sanitation and reuse concept are based on separation at source principles which promote the separation of individual wastewater streams, their specific treatment and the use of cost-effective wastewater collection systems [136, 200]. On neighbourhood and office building scale this concept has been locally implemented and demonstrated to be a sustainable alternative allowing resource recovery and the demand minimization of precious drinking water for toilet flushing [251, 285, 318]. This concept offers opportunities for a more cost-effective removal of pharmaceuticals as by the use of vacuum toilets a highly concentrated black water stream is obtained with much higher pharmaceutical concentrations compared to conventional centralised systems [62]. Although the design of most alternative sanitation systems aims at resource recovery and water demand minimization, a better pharmaceutical removal has been observed in such an alternative sanitation full scale system than in conventional WWTPs [36]. Additional treatment steps aiming specifically at pharmaceuticals is postulated to be more cost-effective than similar steps at conventional WWTPs as the high pharmaceutical concentration and the low flow rate of the black water stream ease the specific targeting of pharmaceuticals. The large-scale implementation of alternative sanitation systems requires a willingness to innovate of the main actors regarding legislative and construction aspects of sanitation systems, i.e. municipalities, water boards and construction companies. The rigorously different way of wastewater collection in alternative sanitation systems, i.e. two streams of which one collected by vacuum, requires the costly replacement of the current sewer infrastructure. The limited willingness to innovate and the high replacement costs of conventional sewers hamper the rapid implementation of alternative sanitation systems. However, for new to build neighbourhoods and offices this concept offers serious advantages over the conventional gravitational sewer and wastewater treatment system, including benefits for pharmaceutical removal, and is therefore strongly recommended.

Knowing that pharmaceuticals are only one of the contaminants jeopardising freshwater bodies, a more general approach towards all chemical and non-chemical contaminants including other micropollutants like hormones, pesticides and personal care products and antibiotic resistant bacteria and genes should be applied. Hence, well-designed treatment systems targeting multiple contaminants can prevent the costly implementation of additional treatment processes for each individual type of contaminant. Only with a broader scope going beyond pharmaceuticals the further contamination of the environment by effluent emissions can be prevented. The current indications that numerous contaminants pose adverse effects to the environment and human health demands an immediate action. The lack of legislation should not be used as an excuse to wait, but calls upon the responsibility of water authorities and other stakeholders to act now. As long as chronic effects on the environment and public health are insufficiently understood the precautionary principle should be applied to strive for the safe reuse of wastewater effluent and the harmless discharge into the environment. For the implementation of the precautionary principle, combined biological and chemical treatment processes are foreseen to play a crucial role in the cost-effective removal of contaminants from wastewater effluents.

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Nomenclature

AnBW	Anaerobically treated Black Water
AOP	Advanced Oxidation Process
BOD	Biological Oxygen Demand
BOM	Biodegradable Organic Matter
BO_3B	Biological–Ozone–Biological
CAF	Caffeine
CAS	Conventional Activated Sludge
CBZ	Carbamazepine
COD	Chemical Oxygen Demand
CW	Constructed Wetland
DAD	Diode Array Detector
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DM	Dry Matter
HRT	Hydraulic Retention Time
IBP	Ibuprofen
LC	Liquid Chromatograph
MET	Metoprolol
MS	Mass Spectrometer
NAP	Naproxen
OM	Organic Matter
PRO	Propranolol
SPE	Solid Phase Extraction
TiO_2	Titanium dioxide
TOC	Total Organic Carbon
UV	Ultraviolet
WWTP	Wastewater Treatment Plant

Samenvatting

Wereldwijd worden in toenemende mate geneesmiddelen geconsumeerd, en de verwachting is dat dit op de korte termijn niet afneemt. Enkele uren tot dagen na inname van geneesmiddelen scheidt het menselijk lichaam deze uit via urine of ontlasting. Door geavanceerde afvoermechanismen in het menselijk lichaam worden geneesmiddelen (gedeeltelijk) uitgescheden als zogenoemde metabolieten, d.w.z. in een andere moleculaire vorm dan het originele geneesmiddel. Geneesmiddelen en metabolieten komen via het toilet en het riool uiteindelijk in de rioolwaterzuiveringsinstallatie terecht.

Huidige rioolwaterzuiveringsinstallaties, gebruikmakend van actief slib, zijn ontworpen om bulkverontreinigingen, zoals organisch materiaal, stikstof en fosfaat, te verwijderen. Deze installaties zijn echter niet ontworpen op het verwijderen van het brede spectrum aan geneesmiddelen dat in het afvalwater aanwezig is. Deze groep stoffen met zeer uiteenlopende en complexe moleculaire structuren, aanwezig in nano- tot microgrammen per liter, wordt daardoor doorgaans slechts minimaal verwijderd. De geneesmiddelen waarvan wel biologische afbraak wordt waargenomen worden in de regel niet gemineraliseerd tot CO_2 en H_2O, maar enkel omgezet tot transformatieproduct met een licht veranderde structuur ten opzichte van het originele geneesmiddel. Dit resulteert in de emissie van ontelbare geneesmiddelen, metabolieten en transformatieproducten naar het milieu.

De aanwezigheid van deze potentieel gevaarlijke stoffen in het milieu is een van de grote uitdagingen van deze tijd omdat ze een serieuze bedreiging vormen voor de kwaliteit van het aquatisch milieu en de volksgezondheid. Negatieve gevolgen zoals de vervrouwelijking van vissen zijn goed beschreven in literatuur en vormen een bedreiging voor het gehele ecosysteem. Aanzienlijke concentraties aan geneesmiddelen in drinkwaterbronnen afkomstig van rioolwaterzuiveringsinstallaties vormen een gevaar voor de volksgezondheid indien drinkwater niet voldoende gezuiverd wordt. Daarnaast belemmert de aanwezigheid van geneesmiddelen het hergebruik van afvalwater, terwijl wereldwijde waterschaarste juist gedeeltelijk opgelost zou kunnen worden door hergebruik van afvalwater. De opsomming van de

hierboven beschreven negatieve aspecten motiveert de verwijdering van geneesmiddelen uit afvalwater voordat deze het aquatisch milieu bereiken.

Als een van de hoofdschakels in de waterketen zijn rioolwaterzuiveringsinstallaties een optimale locatie voor een barrière tegen geneesmiddelenemissies naar het milieu. Meerdere biologische en chemische processen zijn bestudeerd voor de verwijdering van geneesmiddelen, waarvan sommige reeds lokaal geïmplementeerd zijn. Desalniettemin tonen veel van deze processen een onvolledige verwijdering van geneesmiddelen of worden ze gekenmerkt door hoge kosten en lage duurzaamheid. Oftewel, er is een tekort aan kost-effectieve processen voor geneesmiddelenverwijdering. Deze dissertatie gaat daarom in op verschillende processen voor de kost-effectieve verwijdering van geneesmiddelen uit afvalwater met een focus op de synergie tussen biologische en chemische zuiveringsprocessen voor een verbeterde geneesmiddelen verwijdering (Hoofdstuk 1).

De doorgaans lage kosten voor biologische zuiveringsprocessen begunstigen de toekomstige implementatie ervan en stimuleren het onderzoek ernaar. Van alle parameters die invloed hebben op de werking van biologische processen worden redox-condities gezien als een van de belangrijkste. Uit batch- en kolomexperimenten met sediment van helofytenfilters is gebleken dat geneesmiddelen het best worden verwijderd onder aërobe, d.w.z. zuurstofrijke, sulfaat reducerende en methanogene condities (Hoofdstuk 2). Microaërofiele en nitraat reducerende condities zijn minder effectief. Biologische afbraak en sorptie aan sediment zijn geïdentificeerd als onderliggende verwijderingsmechanismen van geneesmiddelen en worden beide beïnvloed door de heersende redox-condities. Van de onderzochte geneesmiddelen vertoonde propranolol de hoogste sorptiecoëfficiënt. Voor propranolol vond onder de meest optimale redox-conditie verzadiging van de sediment sorptieplekken plaats na 300 porie volumewisselingen. Dit toont aan dat in biologische filtratieprocessen geneesmiddelenverwijdering middels sorptie aan sediment slechts een minimale rol speelt ten opzichte van biodegradatie. De persistentie van biorecalitrante geneesmiddelen zoals carbamazepine in biologische zuiveringsprocessen benadrukt de onvolkomenheid van deze processen en onderschrijft de noodzaak voor additionele niet-biologische zuiveringsstappen.

Milde UV-LED TiO_2 fotokatalyse gecombineerd met een nageschakeld biologisch zuiveringsproces toonde een verbeterde geneesmiddelenverwijdering ten opzichte van beide afzonderlijke processen (Hoofdstuk 3). Drie van de negen onderzochte geneesmiddelen werden verwijderd door milde fotokatalyse. Vier geneesmiddelen vertoonden een verbeterde geneesmiddelenverwijdering tijdens biologische zuivering na milde fotokatalytische voorbehandeling. Verrassend genoeg, was slechts één van deze vier geneesmiddelen verwijderd gedurende milde fotokatalyse. Bovendien werd diclofenac biologisch afgebroken na voorbehandeling terwijl er zonder voorbehandeling geen afbraak plaats vond. Afgaande op literatuur is gepostuleerd dat afbraakproducten van de drie door milde fotokatalyse verwijderde geneesmiddelen hebben geleid tot de activering van enzymatische systemen die betrokken zijn bij de initiële reacties voor de biodegradatie van organische moleculen.

Naast geneesmiddelen zijn er talrijke andere (onschadelijke) organische stoffen aanwezigheid in rioolwaterzuivering effluent. In het geval ozonisatie wordt toegepast als additioneel zuiveringsproces voor geneesmiddelenverwijdering, vangen deze stoffen een grote fractie van het ozon af waarmee de ozonisatie van geneesmiddelen sterk in effectiviteit reduceert. Hoge totale organisch koolstofconcentraties (TOC), zoals 17,3 mg TOC/L in het voor deze studie onderzochte afvalwater, resulteren derhalve in een hoog ozonverbruik waardoor de kost-effectiviteit van ozonisatie afneemt. Om die reden is een bio-ozon-bio-proces (BO_3B) bestaande uit twee identieke druppelfilters met zand als dragermateriaal en een ozonreactor ontworpen (Hoofdstuk 4). Met als doel het ontwikkelen van een kost-effectieve zuivering zijn verschillende hydraulische verblijftijden en ozondoseringen onderzocht. Over de eerste biologische zuiveringsstap is bij een relatief korte verblijftijd van 1,5 uur een TOC-verwijdering van 38% behaald, waardoor het ozon verbruik in de daaropvolgende ozonisatiestap evenredig verlaagd kon worden. Vergeleken met het actief-slibproces, zoals toegepast in huidige rioolwaterzuiveringsinstallaties, presteerde de eerste biologische zuiveringsstap van het BO_3B proces beter op geneesmiddelenverwijdering, ondanks de lage verblijftijd en lage biomassaconcentratie. Zelfs enkele moeilijk afbreekbare geneesmiddelen zoals sulfamethoxazol werden biologisch verwijderd. Met een lage ozondosis van 0.02 g O_3/g TOC werden in de ozonisatiestap biorecalcitrante geneesmiddelen zoals

carbamazepine effectief verwijderd. De 17% TOC-verwijdering over de laatste biologische zuiveringsstap is vermoedelijk toe te schrijven aan de biologische verwijdering van ozonisatie producten.

In algen-fotobioreactoren is het gelukt om uit anaëroob behandeld zwart water en uit pure urine geneesmiddelenverwijdering en nutriënten terugwinning gelijktijdig te bewerkstelligen (Hoofdstuk 5). Uit literatuur is bekend dat algen in staat zijn stikstof en fosfor op te nemen uit brongescheiden afvalwaterstromen. Uit onze experimenten blijkt dat ze daarnaast ook bijdragen aan geneesmiddelenverwijdering uit afvalwater. Controle-experimenten toonden aan dat zowel algen, bacteriën als ook de belichting van de reactor bijdragen aan de geneesmiddelenverwijdering in de algen-fotobioreactoren. Geneesmiddelen die vatbaar zijn voor licht zoals diclofenac werden verwijderd middels fotolyse, terwijl andere, zoals paracetamol en metoprolol, door een combinatie van fotolyse en biodegradatie verwijderd werden. Sorptie van geneesmiddelen aan algen bleek minimaal waardoor het gebruik van deze nutriëntrijke biomassastroom als meststof voordelen heeft over bemesting met urine.

De bevindingen van deze dissertatie geven inzicht in de tekortkomingen van individuele biologische en chemische processen voor geneesmiddelenverwijdering uit afvalwater, en benadrukken de waarde van combinaties van complementaire processen waarbij de nadelen van individuele processen wordt overstegen (Hoofdstuk 6). Omdat er geen universele kost-effectieve combinatie van processen bestaat, bepalen locatiespecifieke omstandigheden zoals de afvalwatercompositie welke combinatie lokaal het meest effectief is. De implementatie van aanvullende zuiveringsstappen dient daarom uit te gaan van op maat gemaakte combinaties. Hierbij kan worden geput uit het reeds bestaande arsenaal aan biologische en chemische processen voor afvalwaterzuivering en eventuele nog te ontwikkelen processen. Om de kost-effectiviteit van combinatieprocessen verder te verbeteren zal toekomstig onderzoek moeten focussen op de onderliggende verwijderingsmechanismen, waaronder de enzymatische routes waarlangs geneesmiddelen verwijderd worden en de formatie en afbraak van transformatieproducten.

Voor de in deze dissertatie bestudeerde verwijderingsprocessen is een vooruitzicht geschetst betreffende vervolgonderzoek en opschaling. Het BO_3B-proces

en het zuiveringsproces met algen zijn rijp om op pilot-schaal getest te worden. Voornamelijk de potentie van het BO_3B-proces is hoog omdat dit reeds een jaar lang succesvol op werkelijk afvalwater is getest. Gedurende deze periode is op kost-effectieve wijze een hoog verwijderingsrendement bereikt. Opschaling zou zich voornamelijk moeten richten op het bedrijven van de biologische zuiveringsstappen, omdat deze de hoge kosten van het ozonisatieproces reduceren.

Het zuiveringsproces met algen wordt momenteel bestudeerd op pilot-schaal. Hiervoor wordt aanbevolen, net als voor andere vervolgonderzoeken, naast geneesmiddelen ook andere microverontreinigingen te bestuderen. De combinatie van fotokatalyse en biologische zuivering vereist verder laboratoriumonderzoek, omdat deze combinatie enkel op een schone watermatrix getest is. Daarnaast dient er voor de verduurzaming van het proces naar TiO_2-immobilisatie gekeken te worden.

Vergaarde kennis van de afgelopen decennia onderstreept het belang van effect-gebaseerde verwijderingsstrategieën en dient te worden meegenomen in toekomstig onderzoek en implementatie. De meeste studies echter, inclusief deze dissertatie, zijn voornamelijk gestoeld op chemische parameters zoals geneesmiddelen concentraties en gaan daardoor voorbij aan de effecten van de onderzochte stoffen op het aquatische milieu.

Interventies in de geneesmiddelenketen van farmaceutische industrie tot drinkwaterproductie waarmee end-of-pipe-oplossingen minder nodig zijn, evenals de implementatie van striktere wet- en regelgeving omtrent het voorkomen van geneesmiddelenemissies, worden beide sterk aanbevolen om de geneesmiddelenemissies naar het milieu te verminderen. Daarnaast wordt ook het realiseren van brongescheiden sanitatiesystemen aanbevolen, waarmee in vergelijking tot gemengde afvalwaterstromen een kost-effectievere geneesmiddelenverwijdering kan worden bewerkstelligd.

Afsluitend kan gesteld worden dat de bevindingen beschreven in deze dissertatie aanvullend inzicht geven in het combineren van biologische en chemische processen ten behoeve van verregaande geneesmiddelenverwijdering uit afvalwater en daarbij onderdeel uitmaken van een groter geheel aan handelingen die noodzakelijk zijn ter voorkoming van verdere bedreiging die geneesmiddelenemissies vormen voor mens en milieu.

Резюме

Употребление лекарственных препаратов растет повсеместно и тенденции к снижению их использования в ближайшем будущем не предвидится. Спустя несколько часов, а то и дней, после приема медикаментов человеческий организм выводит их с мочой или фекалиями. Но благодаря совершенным «механизмам дренажа» человека, фармацевтические препараты частично выделяются, в виде метаболитов (то есть в другой молекулярной форме, чем исходный препарат). Через туалет и канализацию медикаменты и метаболиты попадают на пункты очистки сточных вод.

Нынешние очистные сооружения, работающие на базе активного ила, предназначены для удаления стандартных, объемных загрязнений, таких как органика, азот и фосфат. Концентрация этих веществ в сточных водах исчисляется миллиграммами на литр. Однако современные установки не рассчитаны на удаление широкого спектра фармацевтических продуктов, присутствующих в сточных водах. Таким образом, эта группа соединений, выраженная разнообразными и сложными молекулярными структурами и присутствующая в нано-микрограммах в литре жидкости, удаляется минимально. Кроме того, те лекарственные средства, у которых наблюдается биодеградация (биологический распад), как правило, не минерализуются в CO_2 и H_2O, а лишь преобразуются в продукт трансформации со слегка отличающейся от исходного лекарственного препарата химической структурой. Таким образом, в окружающую среду попадает бесчисленное количество медицинских препаратов, метаболитов и продуктов трансформации.

Присутствие этих потенциально опасных веществ в окружающей среде является одной из главных проблем нашего времени, поскольку они реально угрожают качеству состояния водной среды и, как следствие, здоровью человечества. Отрицательные последствия этих процессов, такие как, например, феминизация рыб (развитие у особи мужского пола вторичных половых признаков, характерных для женской особи), также представляют угрозу для всей экосистемы. Оказалось, что источники питьевой воды, уже содержат

высокие концентрации фармацевтических веществ, которые выделяются в экосистему очистными сооружениями. Вода, производимая подобными источниками и очищенная ненадлежащим образом, создает реальную опасность для здоровья населения. В связи с этим, присутствие фармацевтических препаратов в сточных водах препятствует их повторному использованию, в то время как глобальная нехватка воды на планете могла бы быть частично решена именно путем повторного использования сточных вод. Перечисленные выше негативные аспекты мотивируют на удаление медикаментов из сточных вод, прежде чем они достигнут водной среды.

Будучи одним из главных звеньев водной цепи, очистные сооружения являются оптимальным местом для перехвата фармацевтических препаратов до их попадания в окружающую среду. Уже изучен ряд биологических и химических процессов удаления медицинских средств, часть из них уже применяется. Тем не менее, многие из этих методов либо не способны удалять медицинские препараты полностью, либо являются дорогостоящими и недолговечными. Иными словами, сегодня существует реальная нехватка доступных, с экономической точки зрения, и эффективных процессов удаления фармацевтических препаратов. Поэтому данная диссертация посвящена исследованию различных процессов рентабельного удаления медицинских средств из сточных вод. В работе был сделан акцент на синергизм биологических и химических процессов очистки для улучшенного удаления лекарственных препаратов (глава 1).

В целом же низкая стоимость биологических процессов очистки сточных вод способствует их внедрению в будущем и стимулирует их исследования. Среди различных параметров, влияющих на биологические процессы, окислительно-восстановительные условия считаются одними из наиболее важных. Из batch и колоночных экспериментов, с использованием искусственных болот, стало понятно, что удаление фармацевтических препаратов проходило наиболее эффективно в аэробных, сульфатно-восстановительных и метаногенных условиях (глава 2). В то время как, микроаэрофильные и нитрат-восстановительные условия оказались менее эффективными. Биодеградация и сорбция были идентифицированы как основные механизмы удаления лекарственных препаратов. Также было установлено, что эффективность этих

двух процессов (биодеградации и сорбции) зависит от окислительно-восстановительных условий (redox). Из всех исследуемых лекарственных средств, пропранолол показал самый высокий коэффициент сорбции. Было также обнаружено, что насыщение сорбционных участков пропранолом происходит после 300-кратного изменения объема пор при наиболее благоприятных окислительно-восстановительных условиях. Это говорит о том, что в процессах биологической фильтрации сорбция фармацевтических препаратов имеет второстепенное значение по сравнению с биодеградацией. Устойчивость био-рекальцитрантов, таких как карбамазепин, к процессам биологической очистки подчеркивает их несовершенство и указывает на необходимость в дополнительных небиологических этапах очистки.

Так мягкий фотокатализ в присутствии УФ-светодиода и TiO_2 в сочетании с последующей биологической обработкой продемонстрировал улучшенное удаление лекарственных средств по сравнению с этими же процессами, проведенными по отдельности (глава 3). Три из девяти исследуемых фармацевтических препаратов были частично удалены путем мягкой фотокаталитической обработки. Биодеградация четырех других медицинских препаратов также улучшилась после мягкой предварительной обработки фотокатализом. И лишь один из этих четырех препаратов был частично удален во время легкого фотокатализа. Кроме того, мягкая предварительная фотокаталитическая обработка способствовала биодеградированию диклофенака, который продемонстрировал устойчивость к однократной биологической обработке. Также выдвинуто предположение, что продукты распада трех медицинских препаратов, удаляемые мягким фотокатализом, приводят к активации биологических систем, участвующих в первоначальных реакциях биодеградации органических молекул.

Наряду с фармацевтическими препаратами в сточных водах также присутствует множество безвредных органических веществ. В этом случае, в качестве дополнительного процесса очистки сточных вод, используется их озонирование. Однако эти органические вещества захватывают большую часть самого озона, что значительно снижает эффективность этого процесса. Высокие концентрации общего органического углерода (ООУ) (17,3 мг ООУ/литр) в

сточных водах, используемых для данного исследования, требуют ввода больших доз озона, что снижает экономическую эффективность озонирования. Поэтому в рамках исследования был разработан трехступенчатый био-озоно-биопроцесс (BO_3B), включающий озоновый реактор и два одинаковых фильтра с насадкой, использующих песок в качестве носителей биомассы (глава 4). В целях разработки рентабельной системы очистки были исследованы различные времена гидравлического удержания (ВГУ) и дозы озона. Первый биологический реактор удалял 38% ООУ при ВГУ в 1,5 часа, что пропорционально уменьшало потребность в озоне в последующем озоновом реакторе. Улучшенное удаление фармацевтических препаратов по сравнению с обычной очисткой сточных вод наблюдалось в первом биологическом реакторе, несмотря на короткое ВГУ и низкое количество биомассы в системе BO_3B. Эффективная биодеградация была продемонстрирована даже для таких трудноразлагаемых лекарственных средств, как сульфаметоксазол. Био-рекальцитранты, такие как карбамазепин, эффективно удалялись при низких дозах озона (до 0,2 г O_3/г ООУ). Удаление 17% ООУ в последнем биологическом реакторе свидетельствовало об удалении продуктов трансформации, образовавшихся во время озонирования.

Одновременное извлечение биогенов и удаление фармацевтических препаратов было обнаружено в фотобиореакторах с культивацией водорослей, работающих на анаэробно обработанной черной воде или моче (глава 5). Было обнаружено, что водоросли, которые, как известно, способны поглощать азот и фосфор из этих высококонцентрированных источников сточных вод, также способствовали удалению из них фармацевтических препаратов. Контрольные эксперименты еще раз подтвердили факт содействия водорослей, бактерий и света в удалении фармацевтических препаратов. Нужно также отметить поведение медицинских препаратов, оказавшихся чувствительными к фотолизу. Так, например, диклофенак, после применения этой химической реакции, фотодеградировал, тогда как парацетамол и метопролол удалились лишь при комбинирование био- и фото- деградаций. Сорбция фармацевтических препаратов для водорослей оказалась минимальной, что позволяет рекомендовать использование этой богатой питательными веществами биомассы

в качестве удобрения, поскольку она имеет большие преимущества перед применением для таких же целей мочи.

Таким образом, результаты данной работы дают представление о недостатках отдельно проведенных биологических и химических процессов удаления фармацевтических препаратов из сточных вод и подчеркивают ценность их комбинирования (глава 6). Специфические для каждой конкретной ситуации условия, такие как, например состав сточных вод, определяют, какая комбинация процессов будет наиболее выгодна в данном случае, поскольку универсальной экономически-эффективной комбинации процессов не существует. Поэтому внедрение дополнительных этапов очистки должно быть сосредоточено на поиске индивидуальных комбинаций для каждого конкретного случая. Для дальнейшего повышения рентабельности комбинированных процессов будущие исследователи должны сосредоточить свое внимание на основных механизмах удаления, включая ферментативные пути деградации фармацевтических препаратов, а также образование и удаление продуктов трансформации.

Изученные и описанные в этой диссертации процессы очистки представляют собой прекрасную перспективу для дальнейших исследований и укрепления opschaling (увеличения масштабов процессов). Так, например, процесс BO_3B и система очистки при помощи водорослей проведены в рамках пилотного проекта и готовы к последующим изучениям и экспериментам. Нужно отметить, что система BO_3B была успешно протестирована в лабораторных условиях на реальных сточных водах в течение года и продемонстрировала высокую эффективность удаления фармацевтических препаратов и оказалась экономически доступным способом.

Будущим исследователям также необходимо сфокусироваться на работе биологических реакторов, поскольку именно они способствуют рентабельности процесса BO_3B, и на удалении широкого спектра микрозагрязнений. Комбинация фотокатализа и биодеградации также требует дальнейших лабораторных исследований, поскольку для улучшения устойчивости системы должен быть решен вопрос иммобилизации TiO_2; к тому же, данная система была проверена только на чистой матрице.

Знания, полученные в течение последних десятилетий, подчеркивают важность усовершенствования стратегий очистки и нацелены на конечный эффект. Исследования же, проведенные в рамках этой диссертации, обращены на оптимизацию химических показателей, таких как концентрации отдельных фармацевтических препаратов, и не учитывают конечный эффект этих препаратов, например, на водную среду. Системы санитарии (source separation), работающие на разделении сточных вод на выходе «из дома», позволяют перехватить и экономически-эффективно удалить фармацевтические препараты еще на ранних этапах очистки сточных вод. Кроме того, source separation уменьшают потребность в очистке в конце технологического цикла, а также являются настоятельно рекомендуемыми для повсеместного внедрения. Более того, необходимо ужесточить законодательство, регулирующее концентрацию выбросов фармацевтических препаратов в сточные воды.

В заключение хотелось бы отметить, что результаты, полученные в данной диссертации, способствуют лучшему пониманию сочетания процессов биологической и химической очистки сточных вод от фармацевтического загрязнения. А также являются набором шагов, которые необходимо предпринять для предотвращения дальнейших выбросов фармацевтических препаратов и других загрязнителей, реально угрожающих окружающей среде и здоровью населения.

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Like in nature, the local environment plays a crucial role in the success of communities and individuals. Being a very happy individual, I would like to express my gratitude for having worked in a very nice environment at the sub-department of Environmental Technology. Populated with a diverse community of specialists, team-players, critical thinkers and beer-drinkers the department houses an excellent atmosphere to flourish in both the scientific and personal direction. Credits therefore to the chairmen Cees and Huub who manage to maintain this environment, but equal credits to all those colleagues who contribute(d) to this pleasant working place. As I know that I will fail to name everybody of the department and considering that real interaction is much more important than being mentioned on paper like in this dissertation, I would like to thank ALL colleagues A LOT for the great time at the department.

Success is not only dictated by the local environment, also the interaction between individuals highly contributes to the success of individuals and thereby of the community. I would like to thank two individuals in particular for their symbiotic interaction that highly contributed to the success of our local pharma-community.

Alette, starting together at the department at exactly the same date, you were a symbiont since day one. I was very happy that after our fruitful collaboration during my MSc thesis you had the trust in me to become your PhD candidate in the undiscovered field of pharmaceutical removal. As in a true symbiosis I think we have learned and achieved a lot in our (scientific)work and I am very pleased that most likely this symbiosis will not end soon as I have the feeling that there is way more we can achieve. Thank you for providing me oceans of freedom while also being such a good mentor to me, including your critical reflection on our work and on me as a person. Though I am not that fond of gardening, sewing and reading e-mail at 7 AM, I admire your enormous drive and dedication for the aspects in life you find important. This was very well expressed during your time off, I highly appreciated the contact we had in those months and truly believe that your dedication to work helped you to recover. Next to your dedication, the interest you show in a person beyond the work

related interest makes you a pleasant person to work with and is a quality that definitely improves the collaboration with others.

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As an unidentified, but difficult to culture, multilingual sub-species, the civilians occupying room 1.087 during the period 2013-2017 are kindly acknowledged for the distraction of my PhD work they offered me, their great sense of humour, the in-depth discussions on environmental-political-craziness and the meaning of life, and the talks about cows and calves. Without you girls and guys my PhD work would have been much more boring, and I know yours would be as well ;-) Apologies for the delay in your work caused by my distractivity, but I am pretty sure that all of you will make it (or already made it) as room 1.087 is simply the best!

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Curriculum vitae

Henrik Arnoud de Wilt was born on September 10th, 1987 in Utrecht, the Netherlands. In 2006 he finished his secondary education with a VWO beta-profile diploma at the Stichtse Vrije School in Zeist, after which he started his Bachelor's study Milieukunde with a specialisation in Milieutechnologie at Wageningen University. Arnoud completed his Bachelor's Degree at the sub-department of Environmental Technology in 2009, with a thesis on the feasibility of plant power technology on green roofs. After graduation, he was employed by DeSaH and worked on the construction and operation of a novel sanitation system including decentralised wastewater treatment at an Olympic boarding school in Crimea, Ukraine. In 2011 he

returned to the Netherlands to start his Master's study Environmental Sciences at Wageningen University, with a specialisation in Environmental Technology. During his studies Arnoud continued to remotely work part-time for DeSaH on the project in Ukraine. He graduated in 2013 with a thesis at the sub-department of Environmental Technology on the biological removal of pharmaceuticals from domestic wastewater and an internship at the Netherlands Institute of Ecology on removal of pharmaceuticals from source separated wastewater by algae. Under supervision of Dr Alette Langenhoff and Prof. Dr Huub Rijnaarts Arnoud started a PhD in 2013 on the removal of pharmaceuticals from wastewater at the sub-department of Environmental Technology of the Wageningen University. In 2016, parallel to his PhD, he started to work part-time for LeAF as a consultant on new sanitation and micropollutant removal. He will continue his career in the field of micropollutant removal from wastewater at Royal HaskoningDHV, while continuing his work for LeAF.

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- Advanced Course on Environmental Biotechnology (2015)
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Other PhD and Advanced MSc Courses

- Communication with the Media and the General Public, Wageningen University (2014)
- Competence Assessment, Wageningen University (2014)
- Voice Matters-Voice and Presentation Skills Training, Wageningen University (2015)
- Project and Time Management, Wageningen University (2015)
- Teaching and supervising Thesis students, Wageningen University (2015)

External training at a foreign research institute

- International Collaborative Research, University of Waterloo, Canada (2015)
- PhD study trip to Tsinghua University, Chinese Academy of Sciences, Tongji University and etc. China (2016)

Management and Didactic Skills Training

- Supervising six MSc students (2013-2017)
- Supervising two BSc students (2016-2017)
- Assisting practicals of the MSc courses 'water treatment' (2013-2016), 'Introduction environmental technology' (2013-2016) and Environmental project studies' (2014)

Oral Presentations

- *Pharmaceutical removal by algae grown on source separated wastewater*. 9th IWA Micropol & Ecohazard Conference, 22-25 November 2015, Singapore
- *Nabehandeling van geneesmiddelen; 3-staps Bio-Ozon-Bio systeem*. Kennisdag Geneesmiddelen, 1 December 2016, Wageningen, The Netherlands
- *Pharmaceutical removal in a Bio-Ozone-Bio process*. 10th Micropol & Ecohazard Conference, 17-20 September 2017, Vienna, Austria

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Dr. Monique Gulickx

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