Polar Micropollutants in Wastewater Treatment Plants: Microbial Activity and Sampling Strategies as Causes for the Variation of Elimination Efficiencies

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"Every honest researcher I know admits he's just a professional amateur. He's doing whatever he's doing for the first time. That makes him an amateur. He has sense enough to know that he's going to have a lot of trouble, so that makes him a professional."

Charles Franklin Kettering (1876-1958)

Zusammenfassung

Eine Vielzahl hydrophiler Xenobiotika wird in kommunalen Kläranlagen nur unvollständig abgebaut und stellt ein potentielles Risiko für aquatische Ökosysteme dar. Die Eliminationsleistung von Kläranlagen hat sich deshalb während des letzten Jahrzehnts zu einem Schwerpunkt in der aquatischen Umweltchemie entwickelt. Die vorliegende Arbeit untersucht in diesem Zusammenhang die Variation biologischer Abbauleistungen kommunaler Kläranlagen für polare Xenobiotika. Es soll überprüft werden, ob die Abbauleistung von Xenobiotika mit derer klassischer Nährstoffe in Beziehung gesetzt werden kann. Als zusätzlicher Faktor zur Erklärung von unterschiedlichen Eliminationsleistungen wurden Probenahmestrategien hinsichtlich der Erstellung von Massenbilanzen analysiert.

Fünf Pharmaka sowie drei Aminopolycarbonsäuren wurden als Testsubstanzen ausgewählt. Diese umfassen ein Spektrum an mikrobiologischer Abbaubarkeit bedingt durch die Reaktivität ihrer unterschiedlichen molekularen Strukturen. Ein kombinierter Versuchsaufbau aus Respirometrie und Abbautests ermöglichte, neben der Bestimmung von Abbaukinetiken, auch die Charakterisierung von Belebtschlamm. Der Einfluss von Mischungsprozessen auf die Abbauleistungen von Kläranlagen wurde mittels hydraulischer Aufenthaltszeitverteilungen modelliert. Darauf basierend wurde eine Methode zur Beurteilung und Erstellung von Eliminationsleistungen aus Zulauf-Ablauf Massenbilanzen entwickelt.

Die Resultate zeigen deutliche Unterschiede in den Belebtschlammcharakteristika und dem Abbaupotential von Xenobiotika zwischen verschiedenen Kläranlagen. Die aktive heterotrophe Biomasse wurde als steuernder Faktor für die Biodegradation der untersuchten leicht und moderat abbaubaren Xenobiotika identifiziert. Dies kann als Hinweis auf kometabolische Abbauprozesse durch unspezifische Enzymaktivitäten gewertet werden. Die beobachtete Abnahme der Eliminationsleistung mit steigendem Schlammalter konnte anhand modifizierter Kinetiken pseudo-erster Ordnung beschrieben werden.

Die Modellierung von Mischungsprozessen mittels hydraulischer Aufenthaltszeitverteilungen macht deutlich, mit welcher Unsicherheit Massenbilanzen in Kläranlagen behaftet sind. Die Ergebnisse demonstrieren, dass dies vor allem auf inadäquate Probenahmestrategien zurückzuführen ist. Insbesondere Massenbilanzen auf der Basis von kurzen Probenahmezeiträumen und 24-h Mischproben resultieren in fehlerhafte Eliminationsraten. Der vorgestellte Ansatz benutzt die Verteilungen von Aufenthaltszeiten als Leitprinzip, um Zulauf- und Ablauffrachten zueinander in Beziehung zu setzen. Die Methode kann als Grundlage zur akkuraten Schätzung von Eliminationsleistungen herangezogen werden und bietet eine Erklärung für das Vorkommen negativer Eliminationsleistungen.

Abstract

A variety of hydrophilic xenobiotics pass biological wastewater treatment without being fully degraded thus exposing aquatic ecosystems to possible adverse effects. Because of this, xenobiotic removal efficiencies of wastewater treatment plants (WWTPs) have become a major concern in water research and environmental engineering during the last decades. In this context, the presented thesis deals with the variation of xenobiotic elimination efficiencies during activated sludge treatment focusing on governing microbial and hydraulic characteristics. It investigates the relationship between classical degradation processes of nutrients/organic carbon and xenobiotic breakdown. As an additional cause to explain the difference in removal performances, sampling strategies aiming at full-scale mass balances have been investigated.

Five pharmaceutically active compounds and three aminopolycarboxylic acids were chosen as test substances covering a range of biodegradability caused by the reactivity of their molecular structures. An experimental set-up combining respirometry and degradation test techniques allowed to characterize the activated sludge as well as to monitor xenobiotic breakdown. Additionally, the impact of hydraulics and contact time on full-scale WWTP elimination efficiencies was addressed by a residence time distribution (RTD) modeling approach. Based on these results, a novel approach has been developed to reliably set up and assess elimination efficiencies from full-scale mass balances.

Results show that microbial biokinetics and xenobiotic removal vary largely between WWTPs. The active heterotrophic biomass has been identified as a determining factor in the breakdown of readily and intermediate biodegradable micropollutants. This can be seen as strong indication for co-metabolic transformation processes via non-specific enzyme activity. Elimination efficiencies were observed to decrease with increasing sludge retention time and described by pseudo first-order kinetics modified to include the amount of active biomass.

The characterization of mixing regimes by an RTD approach showed that xenobiotic mass balances are associated with great uncertainties. The results of this thesis demonstrate that this is most notably due to inadequate sampling strategies. In particular mass balances on the basis of short-term sampling with 24-h composite samples result in erroneous elimination efficiencies. The method developed in this project applies hydraulic residence time distributions as a guiding principle to adapt individual sampling schemes which allows to match influent with effluent loads. The approach can be used to reliably estimate removal performances and to explain the occurrence of negative elimination efficiencies reported for municipal WWTPs.

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Glossary

APCA	Aminopolycarboxylic acids
ASM	Activated sludge model
BOD	Biological oxygen demand
BOD_{theo}	Theoretical biological oxygen demand
COD	Chemical oxygen demand
COD_{theo}	Theoretical chemical oxygen demand
CSTR	Continuous flow stirred tank reactor
DTPA	Diethylenetriaminepentaacetic acid
EDTA	Ethylendiaminetetraacetic acid
EPS	Extracellular polymeric substances
HPLC	High pressure liquid chromatography
HRT	Hydraulic retention time
K_d	Water-solid distribution coefficient
K_{ow}	Water-octanol distribution coefficient
LOQ	Limit of quantification
NTA	Nitrilotriacetic acid
OUR	Oxygen uptake rate
OUR_{ex}	Exogenous oxygen uptake rate
PFR	Plug flow reactor
PhAC	Pharmaceutically active compound
RTD	Residence time distribution
SRT	Sludge retention time
TSS	Total suspended solids
VSS	Volatile suspended solids

b_h	Heterotrophic decay rate
f_{at}	Fraction of active heterotrophic biomass
f_{cv}	COD/VSS ratio
f_i	VSS/TSS ratio
f_h	Endogenous respiration rate
f_{sup}	Non biodegradable particulate COD
f_{sus}	Non biodegradable soluble COD
k_{biol}	Biological degradation date constant
K_s	Readily biodegradable substrate half-saturation coefficient
μ_{max}	Maximum heterotrophic Growth rate
S_p	Sludge production
X_{bh}	Active heterotrophic biomass
Y_h	Heterotrophic yield

Chapter 1

1 Introduction

1.1 Elimination of Micropollutants during Wastewater Treatment

The quality of surface waters has improved in the last 30 years through systematic connection of sewer systems to biological wastewater treatment plants (WWTPs). While these measures aimed at reducing oxygen depletion and eutrophication in rivers, the focus in aquatic pollution recently shifted to the hazard caused by persistent polar organic pollutants such as pharmaceuticals, hormones, personal care products, pesticides or industrial chemicals (Kümmerer, 2004; Reemtsma et al., 2006; Bester et al., 2008; Stamatis et al., 2010; Ternes, 1998; Ternes & Joss, 2008). This trend has been encouraged to a large extent by the development of new analytical techniques that allow to determine micropollutants at very low trace quantities by liquid chromatography coupled with mass spectrometry (Gentili, 2007). Several ecotoxicological effects (Brian et al., 2007; Sumpter, 1995), endocrine disrupting properties as well as the appearance of polar micropollutants in drinking and surface water have caught the attention of water research and legislative authorities (Jones et al., 2005). A number of these hydrophilic compounds elude classical elimination pathways such as the binding to sewage sludge and often pass sewage treatment processes without being fully degraded (Egli, 2001; Joss et al., 2006; Lin et al., 2009).

While high concentrations could be expected for persistent compounds, a variety of monitoring campaigns showed that readily and intermediate biodegradable xenobiotics also occur up to the $\mu g\ L^{-1}$ level in WWTP effluents due to insufficient removal during wastewater treatment (Heberer, 2002; Metcalfe et al., 2003; Schmidt et al., 2004). WWTPs can therefore be seen as major point sources for xenobiotics to receiving waters, where dilution may be the main mechanism by which their concentration decreases.

The release of high amounts of micropollutants from WWTP effluents prompted researchers to investigate the governing factors responsible for micropollutant transformation. Depending on the reactivity of their molecular structure, xenobiotics can be degraded under aerobic but also under anoxic and anaerobic conditions. For the bulk of non-adsorbing micropollutants, aerobic treatment has been identified as the major degradation pathway (Carballa et al., 2004). This also applies for the compounds selected in this study. However, the governing microorganisms and processes responsible for micropollutant degradation have rarely been addressed. Since activated sludge microorganisms adapt to the composition of incoming raw wastewater and to the operating conditions, it can be expected that micropollutant removal capacities vary to a large extent between different treatment sites and can therefore usually not be transferred to other WWTPs.

Hence, it is not surprising that contradictory and highly variable elimination efficiencies have been reported. Zhang et al. (2008) have evaluated a variety of studies showing that re-

ported removal efficiencies of the persistent anti-inflammatory drug diclofenac ranged from 0 % to >70 %. Furthermore, the removal of the antiepileptic drug carbamazepine, which is without controversy considered as hardly biodegradable, has been found to range from -193 to 35 % (Andersen et al., 2003; Bendz et al., 2005), of which 8 out of 30 determinations were negative. Another example from Gabet-Giraud et al. (2010) who investigated the removal of beta-blockers in 14 WWTPs indicated total elimination efficiencies (first and third quartiles) between 10 and 80 % for metoprolol or 35 and 90 % for atenolol. The wide range of reported total elimination efficiencies for other pharmaceuticals has been compiled in a review by Onesios et al. (2009) who analyzed more than 80 full-scale studies. Surprisingly, a considerable high number of negative elimination efficiencies are reported for which poor or no explanation was provided.

The reasons for the variations in organic micropollutant removal have only been insufficiently investigated to date. There is a lack of understanding of the main governing factors that prevents water researchers from reliably anticipating xenobiotic removal under transient conditions and in untested WWTPs. The key to understand these variations and processes must thus be searched in i) activated sludge properties and ii) a reliable sampling method to assess this removal. It is highly probable that these governing factors help explaining commonly reported variation of total elimination efficiencies in WWTPs. This would allow to benchmark biodegradation potentials of different activated sludges. Large-scale assessment of WWTP emissions to the river network systems cannot exclusively rely on cost and work-intensive measurement campaigns. It thus displays the basis for extrapolating elimination potentials of aerobic biological treatment to individual WWTPs.

At present, great effort is being made to investigate the option of upgrading municipal WWTPs with advanced treatment steps such as membrane bioreactors, ozonation or activated carbon. These techniques have been recently employed to tackle the issue of incomplete micropollutant removal (Zimmermann et al., 2011; Miège et al., 2009). While these approaches can be appropriate for point sources (Buerge et al., 2003; Heberer & Feldmann, 2005) like hospital wastewaters, they currently are still too costly and energy-consuming to be installed at a larger scale on a standard basis (Castiglioni et al., 2006). Recent developments showed that biofiltration with biological active carbon might be a more cost-effective alternative (Reungoat et al., 2010).

The trend in developed countries is towards the installation of such additional treatment steps. Upgrading existing infrastructure can here only be based on acceptable benefit/cost ratios. Therefore, a detailed knowledge is required to assess the xenobiotic removal potential of conventional WWTPs and to identify major point sources. Treatment plants constitute here the link between sewer network and receiving waters, hence in-depth knowledge about the removal capacities is essential to improve water qualities at the river basin scale.

1.2 Scientific Context and Problem Statement

This section gives a brief introduction to the context of WWTP removal efficiency estimation and modeling. Two approaches have been followed to investigate the fate of xenobiotics during aerobic wastewater treatment: i) determining biodegradation kinetics at lab-scale under controlled conditions and ii) estimating full-scale elimination efficiencies. However, because both approaches are cost and work intensive, it has been attempted to relate process parameters to the attenuation of micropollutants without relying on expensive measurements. In this context, the sludge retention time (SRT) is certainly the predictor variable most discussed in the recent literature. This parameter as well as its interrelation with biokinetic properties of activated sludge is addressed in section 1.2.1.

Further, full-scale measurements are applied to benchmark xenobiotic removal capacities in WWTPs. This approach takes the transient wastewater composition and the prevailing operating parameters into account. The obtained data is required to validate or falsify the predictions of biodegradation models. The total percentage of a xenobiotic compound eliminated during wastewater treatment is generally obtained from influent-effluent mass balances. This becomes particularly important when representative sampling is required to reliably estimate removal efficiencies. The temporal variation of micropollutants in inlet and outlet as well as mixing regimes in the reactor tanks are inherently associated with this aspect. An overview of the assumptions and limitations of this approach are presented in section 1.2.2. A number of new advanced treatment techniques but also hypotheses concerning the conventional activated sludge process have been evaluated by the determination of total elimination efficiencies. Therefore, to ensure the correct assessment of these techniques, it is crucial to take their associated uncertainty into account. In view of that, the present thesis dedicates the chapter 4 to this aspect.

Besides biodegradation, adsorption onto biomass also is a relevant elimination process during the course of activated sludge treatment. This applies not only to hydrophobic substances but can also become significant for charged molecules that can adsorb onto the cell surface of microbial solids.

The partitioning of micropollutants between the water and the solid phase is mostly assessed by the water-solid distribution coefficient K_d. Recent studies reported that the here investigated pharmaceuticals exhibit low K_d values and that their sorption is usually not significant for the overall elimination (Alder et al., 1990; Ternes et al., 2004). Among the investigated compounds, sulfamethoxazole showed the highest K_d of 260 L kg_{TSS}^{-1} on secondary sludge (Göbel et al., 2005) which would lead to an overall elimination of <10 %. Detailed testing of adsorption kinetics onto biomass was not subject to this study and was therefore - based on previous studies - assumed to be of marginal importance.

1.2.1 Sludge Retention Time as Predictor Variable for Xenobiotic Biodegradation

The SRT is defined as the average residence time of a biomass floc in a recycling reactor system before being withdrawn from secondary clarification (Eq. 1.1). It is used as a design criterion in the planning of WWTPs and is inherently related to the microbial growth activity. Activated sludge microorganisms grow on the incoming substrate load which can be roughly estimated from population equivalents. The surplus of biomass (excess sludge) is withdrawn from the system in order to keep the biomass concentration (expressed as total suspended solids, TSS) at a constant level. It is usually disposed to sludge digesters for biogas production. The fraction withdrawn is commonly termed excess sludge and can be set equal to the sludge production (S_p). The SRT [d] is defined by (Gujer, 2007):

$$SRT = \frac{\text{mass of sludge in reactor}}{\text{mass of sludge wasted per day}} = \frac{V_t \cdot TSS_t}{S_p} \tag{1.1}$$

where V_t is the tank volume [m^3], TSS_t are the total suspended solids [kg m^{-3}] and S_p is the sludge production [kg d^{-1}].

The most important aspects in WWTP planning and SRT selection certainly is the quality of treated effluent concerning the removal of chemical oxygen demand (COD) and/or nitrification/nitrogen/phosphorus removal. Short SRTs of 1-5 days lead usually to sufficient COD removal. It is commonly accepted that WWTPs with short SRTs would not nitrify due to the absence of autotrophic bacteria. Heterotrophs are dominant which exclusively use organic carbon for energy and biomass synthesis with low oxygen demands.

The nitrification process whereby free and saline ammonia is oxidized to nitrite and nitrate occurs only at SRTs that are much longer than those required for COD removal only. Nitrification is mediated by autotrophic microorganisms that have significant lower biomass growth coefficients due to higher energy requirements. Autotrophs grow much slower than heterotrophs and are therefore only prevented from wash-out and present in WWTPs that operate at intermediate and long SRTs. The critical SRT required for nitrification usually ranges around 8-15 days (Gujer, 2007; Ekama & Wentzel, 2008), depending on the temperature. Once nitrifying bacteria are established, ammonia elimination is virtually complete given sufficient aeration is provided. Further details of the SRT in a wider context are presented elsewhere (Henze, 2008; Gujer, 2007).

Recent research proposed this concept of a critical SRT could also be valid for organic micropollutants such as pharmaceuticals and personal care products (Clara et al., 2005; Kreuzinger et al., 2004). This hypothesis has been frequently cited to explain the variability in removal efficiencies at different WWTP sites. It is believed that specialized bacteria

species using micropollutants as substrate also require a critical SRT to maintain themselves in the system in a relevant number. As a consequence, a higher diversified microbial community or comparably a broadened enzyme spectrum is expected at high SRTs. This aspect has however not been directly investigated in most of the studies. There are only few studies dealing with biodiversity using direct measurements such as 16S rRNA analyses. Kraigher et al. (2008) found a significant shift in the microbial structure of activated sludge only when were fed continuously at pharmaceutical concentrations >50 $\mu g\ L^{-1}$, which is unlikely to occur in municipal dominated wastewaters. Moreover, Saikaly et al. (2005) found a reduction of microbial diversity with increasing sludge ages (tested without xenobiotics).

Regarding this concept, a major constraint is that autotrophs grow per definition uniquely on inorganic carbon and ammonia which is usually present in the $mg\ L^{-1}$ range in raw wastewaters. Laboratory analyses demonstrated that bacteria species isolated from activated sludge communities are capable of degrading selected xenobiotics under controlled conditions (Gauthier et al., 2010; Nörtemann, 1992). Nonetheless, the evidence of existence and evolution of these species in mixed-culture communities under realistic conditions is still lacking. Due to the broad range of molecular structures of xenobiotics present in wastewaters, the identification or quantification of such microbial groups and even the proof of their existence is a challenge.

In this context, co-metabolic degradation processes were reported as a breakdown mechanism for micropollutants at trace levels. It can be assumed that organic xenobiotics may follow similar breakdown pathways as dominant substrates present in wastewater due to molecular similarities or functional groups (Stasinakis et al., 2010) and may therefore be subject to non-specific enzyme cleavage. Hence, no specialized species would be responsible but microorganisms that are common in activated sludge. It seems therefore reasonable to assume that (parts of) heterotrophic organisms are able to fulfill this requirement. This is consistent with findings by other authors: highest biotransformation rates of endocrine disruptors were found at a low SRT of 3 days (Stasinakis et al., 2010). Gaulke et al. (2009) reported no difference for 17α-ethinylestradiol at two different SRTs suggesting that heterotrophic bacteria being capable of degrading pharmaceutically active compounds (PhACs) are present both at low and high SRTs. She questions "*the hypothesis that there are critical SRTs for EE2 removal in WWTPs as a result of estrogen degradation by slow growing bacteria.*"

The fraction of active heterotrophs on the TSS can be described as a function of the SRT (Ekama & Wentzel, 2008; Koch et al., 2000). Given the dependency of microbial growth activity and SRT, highest fractions of active heterotrophs occur with low SRTs. This would plausibly explain findings which indicate faster micropollutant removal at low SRTs. Hence, no specialized bacteria would be necessary for the transformation of these

compounds. However, due to wide range of molecular properties of micropollutants found in domestic and industrial wastewater and different results reported, it is challenging to derive a general statement. The effect of the SRT in combination with primary substrate interaction, microbial diversity and enzymatic activities are largely unknown. The SRT alone does not reveal any direct information about the microbial or enzyme spectrum but can be used as an indicator for heterotrophic active fractions. Chapters 2 and 3 investigate the effect of variable amounts of active heterotrophic biomass as one possible governing factor for xenobiotic biotransformation of selected aminopolycarboxylic acids and PhACs.

1.2.2 Xenobiotic Elimination Efficiencies using Full-Scale Mass Balances

Apart from lab-scale testing, full-scale studies provide another possibility to estimate the removal of a xenobiotic chemical in a WWTP. Most investigations deploy mass balances by influent/effluent comparison. The difference between the influent and effluent load is assumed to be eliminated during the course of wastewater treatment. Mostly, sampling is done in the influent after the sand trap and in the effluent after the secondary clarifier.

A problem encountered is, in particular for short-term campaigns, to obtain representative samples for a reliable mass balance. Reactor tanks are mostly well-mixed systems. Pulses or concentrations patterns of inlet concentration can usually not be tracked in the effluent. It is therefore challenging to sample the same water volume in order to match effluent loads to influent loads. Although the approach of mass balancing the loads of short-term monitoring campaigns is commonly accepted, it essentially relies on two basic assumptions. The flow as well as xenobiotic concentrations are assumed to be perfectly constant in influent and effluent (sufficient condition). In this case, no time shift would be necessary between the start of influent and effluent sampling and any water package can be sampled to obtain the same elimination efficiency. This would also be valid for ideal periodic influent and effluent patterns, such as diurnal variation. Under these conditions it would be sufficient to sample one in- and effluent periodical pattern. In the likely case that variation occurs, it is assumed that this variation is propagated through the reactor tanks and can be sampled in the effluent with a certain temporal offset while no mixing is occurring (necessary condition).

However, such stable conditions, constant periodic loads and no mixing do not reflect reality in WWTPs and that is why it is necessary to consider the hydrodynamic behavior in the reactor tanks. Transient flow and concentrations have been shown to occur in influent and effluent (Nelson et al., 2011; Rieckermann et al., 2011) and consequently sampling strategies and calculations have to be adapted to ensure reliable removal estimations.

Some studies considered a temporal shift between influent and effluent sampling (Bernhard et al., 2006; Carballa et al., 2004), e.g. the mean hydraulic retention time (HRT),

which is calculated by the quotient of tank volume and flow through (single pass or with sludge recycling). The HRT is used to estimate how long a water package is retained on average in the reactor tanks (see chapter 4). It thus varies with the flow conditions. The idea that a water package passes the plant reactors by a time shift of the HRT does not apply to conventional completely-mixed WWTP reactors. Water volumes are mixed and consequently released as a distribution in the effluent.

The temporal variation of micropollutant emissions from WWTPs is largely determined by hydraulic mixing processes in reactor tanks and clarifiers. Not only biodegradation kinetics of micropollutants but also their release to receiving waters via wastewater effluents are a function of hydraulic residence times. A detailed understanding of these effluent variations is required in order to reliably assess micropollutant emissions and above all to adapt sampling methods. Generally, a high temporal sampling resolution is needed to obtain concentration values representative for the transient influent and effluent conditions.

A variety of approaches are available to describe mixing processes in reactor tanks. Two of the most common and established models for mixing regimes are *plug flow* reactors (PFRs) and *completely mixed* tanks (also referred to as continuous stirred tank reactors (CSTR, Levenspiel (1972)). Ideal PFRs are characterized by perfect mixing in the radial dimension but no axial dispersion and a constant volume. A distinct influent concentration signal (e.g. Dirac pulse) would be released in the effluent maintaining its shape. The time lag Δt between which the pulse enters and leaves the reactor tank can be precisely determined (Figure 1.1, bottom). This model applies mostly to pipe-shaped reactors, but can be also combined with CSTR, e.g. in order to describe channeling effects. A ski-lift or conveyor would be an example illustrating plug flow characteristics (Gujer, 2008).

On the contrary, a CSTR is characterized by a completely mixed volume without concentration gradients. An influent pulse is completely mixed and released as a distribution in the effluent of which each water volume has a certain specific residence time t_s. In wastewater engineering, often a cascade of CSTRs is applied (tanks-in-series approach; Figure 1.1, top). This approach is widely used to describe hydraulics in WWTP full-scale reactors (De Clercq et al., 1999; Coen et al., 1998). The according mathematical description can be found elsewhere (Coker, 2001). A number of modifications and other models exist describing non-ideal mixing characteristics as well as short-circuiting or mixing-bypasses which cannot be specified here in detail but are presented elsewhere in literature (Levenspiel, 1999).

In order to characterize a mixing regime in tank reactors, tracer tests are routinely performed (Olivet et al., 2005; Fall & Loaiza-Navía, 2007). Artificial tracers are highly soluble persistent substances like for instance lithium and bromide salts or fluorescent dyes that are injected either as a Dirac pulse or continuously in the influent to calibrate models to the effluent signal. A tracer test is however often time- and work-intensive. In this context,

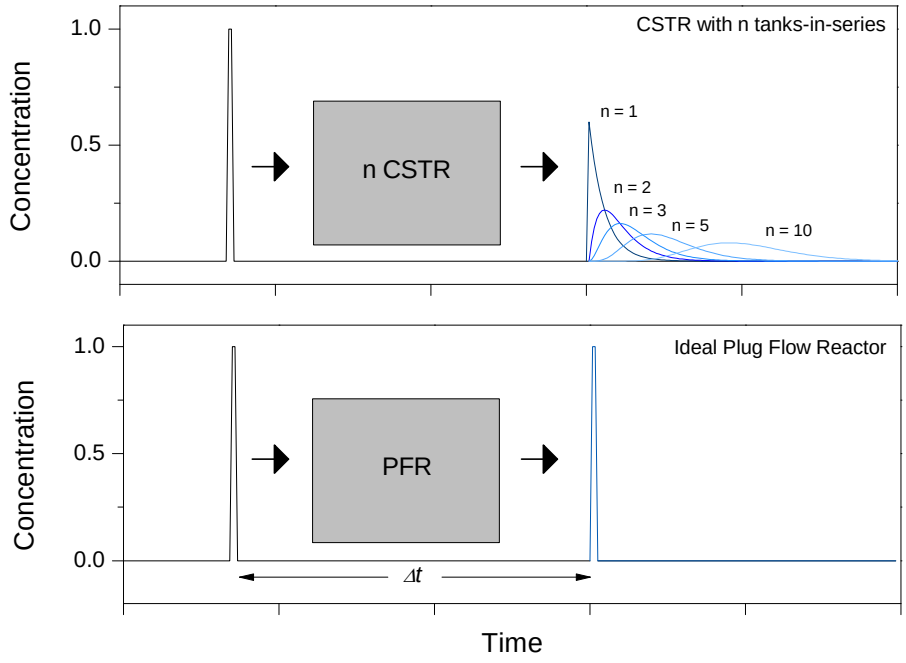

Figure 1.1: Schematic comparison of ideal CSTR and plug flow reactor responses to a Dirac pulse during constant flow conditions

Ahnert et al. (2010) showed that mixing models can also be successfully calibrated using intense signals of routine parameters such as wastewater temperature or conductivity. The degrees of freedom for modeling a CSTR system are the number of tanks, the tank volumes (usually fixed), their internal connection (De Clercq et al., 1999) and the recycled flow of activated sludge.

Prediction modeling of xenobiotic removal efficiencies is often carried out by solving (pseudo) first-order kinetics for the HRT (Maurer et al., 2007; Wick et al., 2009). As mentioned before, a single retention time applies only for a perfect PFR but not to well-mixed conventional WWTP bioreactors. Considering (variable) residence time distributions (RTDs) is therefore much more adequate for estimating total removal calculations via degradation kinetics. To couple the biodegradation of xenobiotics to variable RTDs, (partial) differential equations are used to compute the biodegradation for each specific residence time t_s of a RTD. This allows a dynamic estimation of total removal efficiencies under variable conditions such as e.g. rain events assuming that lab-scale biodegradation rate constants k_{biol} are representative for full-scale plants.

Taking all this information into account makes the variability of published removal rates more understandable. Although the RTD concept is well understood and widely used in hydraulic engineering, the link between environmental chemistry and xenobiotic fate assessment regarding full-scale sampling strategies has not been established to date. A detailed case study about the implication of micropollutant transport in WWTP reactors on full-scale mass balance sampling strategies is presented in chapter 4.

1.3 Research Objective

The number of publications dealing with the fate of xenobiotics in WWTPs and their ranking among the recent top-cited articles reveals that the topic is still a very actual issue. As shown before, micropollutant biodegradation has been tested in a broad variety of studies. However, experimental approaches focus almost exclusively on the investigated micropollutant. Other crucial aspects such as activated sludge properties and the presence of additional substrates are rarely addressed. These approaches do not yield information on i) governing factors that actually determine biodegradation rates and ii) extrapolation of the latter to individual WWTPs. This becomes especially important since it is well known that wastewater, including the loads of xenobiotics (Fuerhacker et al., 2003), the quality of activated sludge and operational conditions vary in time (e.g. rainfall events, season) as well as between treatment sites. A better understanding of the true performances of WWTPs in pollutant removal is necessary to assess the immission situation in surface waters as also requested by the European Water Framework Directive. The micropollutant removal efficiency cannot rely exclusively on expensive chemical analysis. Model simulation based on simpler plant performance characteristics is needed to predict the (dynamic) emission of polar pollutants. These as well as accidental concentration peaks in WWTP effluents are also of concern for acute ecotoxicological effects in receiving waters dominated by wastewater.

A conspicuous fact reported in literature is the significant variability of elimination efficiencies for different WWTPs. Most work carried out focused on full-scale elimination efficiencies by inlet-outlet mass balances. A major drawback of this approach is that these findings can hardly be extrapolated to changed influent conditions, for instance in terms of concentration and flow, and thus represents only a snap-shot. Moreover, governing parameters that may explain removal variability during aerobic treatment have not been identified so far.

To tackle this issue, the present thesis investigates activated sludge properties parallel to biodegradation experiments. To date only few attempts have been made to relate xenobiotic biodegradation to activated sludge characteristics or responsible microbial groups. As shown before, recent studies suggested the SRT as a predictor variable for the elimination

efficiency of xenobiotics. Its applicability as a proxy variable is however controversially discussed in literature (Clara et al., 2005; Gaulke et al., 2009; Kreuzinger et al., 2004; Saikaly & Oerther, 2004; Saikaly et al., 2005; Stasinakis et al., 2010) and was consequently also one objective of this study.

Other factors such as raw water composition, active biomass, enzyme diversity, operating conditions and inadequate sampling were hypothesized as possible causes for this variability. This study raises the question whether a good removal performance of organic matter and nutrients can also give information concerning xenobiotic degradation. Based on this, the relationship between process parameters, microbial activity, COD removal as well as micropollutant removal was investigated.

In order to characterize activated sludge, respirometry was chosen which is well established in wastewater engineering. It allows to characterize activated sludge by the oxygen consumed during substrate oxidation. The removal performance of the sludges can be described in a far more precise manner than by just calculating sludge age.

As mentioned above, degradation rates for the use in modeling have often been estimated from established biodegradability test methods. The biodegradation rate constant is a key parameter and degradation models therefore require an accurate input for this parameter (Temmink & Klapwijk, 2003). For this purpose, an array of biodegradation rates has been determined in activated sludges of six Luxembourg WWTPs that differed largely in their size, layout, sludge age and capacity utilization. One of the plants, WWTP Mamer, was taken as an example to study the effect of hydraulic transport processes as well as sampling strategies to reliably establish mass balances of xenobiotics in full-scale WWTPs.

In summary, this thesis aims at bridging the gap between environmental chemistry and chemical engineering by pursuing the following objectives:

- Identify activated sludge characteristics that are significant to xenobiotic breakdown by use of respirometry.
- Refine and explain variation of biodegradation rates and elimination efficiencies between different WWTP sites.
- Scrutinize the link of the SRT, HRT and activated sludge properties to the xenobiotic elimination potential regarding plant optimization.
- Describe the hydraulic transport and mixing of polar micropollutants in reactor tanks of full-scale WWTPs regarding dynamic emission modeling and mass balance sampling strategies.

The following three chapters present the major results of the project which have to be seen in the context briefly outlined above. The first two research articles deal with varying

biodegradation kinetics of three aminopolycarboxylic acids and five PhACs in aerobic activated sludge systems and their relationship to possible predictor variables (chapter 2 & 3). The third research article (chapter 4) proposes a novel method to reliably assess full-scale elimination efficiencies. Moreover, the effect of RTDs under transient flow and concentration conditions on the biodegradation of polar micropollutants was shown by a modeling approach. The main conclusions and an outlook containing questions that were raised during the project as well as aspects that should be further investigated are summarized in chapter 5.

Chapter 2

2 Influence of Microbial Activity on Polar Xenobiotic Degradation in Activated Sludge Systems

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Influence of Microbial Activity on Polar Xenobiotic Degradation in Activated Sludge Systems

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Abstract The influence of activated sludge quality on the co-metabolic biodegradation of three aminopolycarboxylic acids was investigated for a variety of Luxembourg sewage treatment plants. A combination of biodegradation experiments and respirometric techniques are presented as a reliable approach for the estimation of biokinetics and biological xenobiotic degradation rates that allow for identification of governing parameters such as microbial activity and active biomass. Results showed that biokinetics and degradation rates vary greatly between different plants. The fraction of active biomass on the total suspended solids ranged between 16.9 and 53.7 %. Xenobiotic biodegradation rates correlated with microbial activity suggesting a relationship with WWTP performance for carbon and nutrient removal. The biokinetic information can be used to increase the prediction accuracy of xenobiotics removal by individual WWTPs.

Keywords Activated Sludge; Active Biomass; Co-metabolism; Respirometry; Xenobiotics

Introduction

The fate of polar xenobiotics in the course of wastewater treatment has turned into a major issue of water research during the last decade. These micropollutants often pass wastewater treatment processes without being completely degraded. Their main degradation pathway was reported to be biodegradation during biological treatment (Thomas & Foster, 2004).

In order to assess the fate of micropollutants, biodegradation rates are usually obtained in lab-scale studies. However, their applicability remains limited to the investigated sludge and test conditions. The transfer of those biodegradation rates to other WWTPs is not accurate. Moreover, biodegradation rates observed in standardized tests such as e.g. OECD procedures do not adequately account for prevailing conditions at full scale treatment like

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co-substrates, hydraulic and solids retention times. Additionally no biokinetic information about activated sludge such as growth rates, active biomass or respiration is considered.

The influent composition, hydraulic load and operational parameters as well as the design of WWTPs select for microorganisms best adapted to reproduce (Comeau et al., 2008). As a consequence, activated sludge characteristics are expected to vary to large extents between different sites. This complicates to transfer biodegradation rates between WWTPs and the need to refine xenobiotic degradation experiments by considering biokinetics is apparent.

Activated sludge has recently been characterized with respirometric tests in a cost-effective and adequate manner (Young & Cowan, 2004; Plattes et al., 2007). It enables the monitoring of the OUR which is a measure for the microbial activity in oxidative substrate removal. Supporting this hypothesis, the maximum OUR was found to correlate with catalase activity in municipal activated sludges (Guwy et al., 1998). Modeling simulations of the OUR generated by defined substrate additions allow for the estimation of the most relevant biokinetic parameters (Koch et al., 2000).

Simultaneously, the biodegradation of three well known polar xenobiotics from the class of aminopolycarboxylic acids (APCAs) was tracked. They were chosen in this study because of their differences in biodegradability, broadly available data on biodegradation and widely spread occurrence in wastewater (Knepper, 2003; Schmidt et al., 2004). They are extensively used in industrial applications and detergents. Nitrilotriacetic acid (NTA) is easily biodegradable under aerobic conditions. Diethylenetriaminepentaacetic acid (DTPA) is less biodegradable while ethylenediaminetetraacetic acid (EDTA) is known for its persistence and is barely eliminated during biological treatment (Bucheli-Witschel & Egli, 2001). NTA, EDTA and DTPA strongly bind metal ions and are subsequently present as complexes. Their fate can significantly depend on the type of metal (Sillanpää et al., 2001). APCAs are non-volatile, very hydrophilic and not expected to adsorb on sludge solids (Alder et al., 1990). The present study investigates the link between classical substrate degradation processes and the removal of APCAs using a combination of respirometry and biodegradation experiments. Biokinetic parameters of six Luxembourg WWTPs were investigated yielding reliable xenobiotic predictor qualities.

Methods

Sludge Sampling

Activated sludge grab samples were taken from aerated biological basins of six WWTPs and decanted into the respirometer vessel the same day. The sludge was brought to endogenous respiration over night but not longer than 14 h. Triplicate measurements of the total suspended solids (TSS) were measured with each sampling.

Respirometer

Respirometric experiments were conducted in a custom made 3 L closed bioreactor with a surrounding chamber for temperature control purchased from Ochs GmbH. The beaker was filled with 2.4 L of activated sludge sample. Agitation was provided by a magnetic stirrer and the liquid was assumed to be completely mixed. Temperature was maintained at 20 ± 1°C by means of a RM6 Lauda thermostat. A Metrohm GPD 751 Titrino served as pH control at 7.5 ± 0.2 during the experiment by addition of hydrochloric acid or sodium hydroxide. The sample was aerated using a Schego M2K3 air pump and two aeration stones. Dissolved oxygen (DO) limits were set to 3 and 6 $mgO_2\ L^{-1}$, respectively. The DO concentration was measured with an oxygen sensor from Hach Lange LDO. The OUR was calculated from the depleting DO concentration in the non-aeration phase by moving window 10-point linear regressions. Automatic aeration and data acquisition were accomplished by a program written in LabVIEW (National Instruments). To inhibit nitrification, N-allylthiourea was added to a concentration of 10 mg L^{-1} (Dircks et al., 1999). The input of atmospheric oxygen via the liquid surface was taken into account. ($K_La = 5.01 \cdot 10^{-4}\ min^{-1}$).

Biokinetic Parameter Estimation

Four parameters were chosen to be derived from experimental OURs: the maximum heterotrophic specific growth rate μ_{max}, the readily biodegradable substrate half-saturation coefficient K_s, the heterotrophic yield Y_h and the active heterotrophic biomass X_{bh}. The decay rate was set to default value and assumed to be constant in the course of the tests. Defined volumes (2–4.5 ml) of a 26 g L^{-1} $NaCH_3COO \cdot 3H_2O$ solution corresponding to 50–130 mgBOD L^{-1} were injected before and after every biodegradation experiment into the respirometer to account for possible biokinetic changes during the experiment. The obtained OUR responses were simulated with Activated Sludge Model (ASM) No.1 (Vanrolleghem et al., 1999) that is implemented in the wastewater treatment modeling software GPS-X from Hydromantis. The target parameters were estimated by minimizing the absolute difference between measured and simulated data (Plattes et al., 2007). Heterotrophic yields could be manually calculated from the ratio of theoretical and experimental biological oxygen demand BOD by the following equation:

$$Y_h = \frac{BOD_{theo} - \int OUR_{ex}}{BOD_{theo}} \tag{2.1}$$

where Y_h is the heterotrophic yield [gCOD $gCOD^{-1}$], BOD_{theo} is the theoretical biological oxygen demand [mg L^{-1}] and OUR_{ex} is the exogenous oxygen uptake rate [$mgO_2\ L^{-1}\ h^{-1}$].

Biodegradation experiments

A mixture of EDTA, NTA and DTPA was added to the respirometer resulting in a concentration of 1 mg L^{-1} for each substance. Sodium acetate, ammonium chloride and sodium dihydrogen phosphate monohydrate served as co-substrate with a C:N:P ratio of 100:5:1 as it is typical for municipal WWTPs (Janke et al., 2008). The ratio of xenobiotic to co-substrate was 1:550. The same (co-)substrates were used for all experiments. This provides the basis for a reliable comparability between the different sludges. The OUR was kept at its maximum during the period of the experiment by supplementary additions of co-substrate. Every 30 min a sample of 5 ml was drawn (n = 11), filtered and directly analyzed.

APCA Analysis

NTA, EDTA and DTPA were analyzed using a Dionex HPLC System, consisting of an autosampler AS 40, an IP 20 isocratic pump and a Shimadzu SPD-10AV UV/Vis detector. A C_{18} column (Hypersil 150 x 4; 5 µm HyPurity Elite C_{18}) was used. Acidic water (HNO_3) containing 7.4 mmol L^{-1} of tetrabutylammoniumhydrogensulfate and 2.6 mmol L^{-1} of tetrabutylammoniumhydroxide as ion pair reagents served as eluent with a flow of 0.9 ml min^{-1}. A chromatographic separation of the three compounds could be achieved with an isocratic eluent program. A precolumn derivatization was done by adding 40 ml of a 37 mmol L^{-1} Fe(III)/130 mmol L^{-1} TBAOH solution to 0.5 ml of sample volume to convert all APCAs into their Fe(III)-complexes. Samples were heated for 20 min at 60°C in a water bath and afterwards detected at 260 nm.

Results and Discussion

Variation of Biokinetics in Activated Sludge

The metabolic activities of the investigated sludges showed clear differences. Fast oxidative substrate removal resulted in a high maximum OUR, whereas less active microorganisms had a slower oxygen consumption (Figure 2.1). In all experiments the OUR could be reliably modeled using the ASM 1. Simulations were only accepted if their percentage of explained variation exceeded 85 %. The constantly decreasing phase at the end of the exogenous phase (Figure 2.1, right) is probably due to the production and transformation of storage polymers (Dircks et al., 1999) which is not considered in ASM 1.

Results showed that the fraction of active heterotrophic biomass X_{bh} of TSS varied largely between the six investigated sludges (Table 2.1). It ranged from 16.9 to 53.7 % where higher fractions of X_{bh} promote low solids retention times (SRT). The very low fractions in WWTP Martelange and WWTP Boevange may be caused by their low BOD sludge loads

(e.g. Boevange is operating at 20 % capacity). No significant differences in half saturation coefficients and yields were visible. Heterotrophic yields corresponded to default values given in ASM 1 ($Y_h = 0.67$) and in the literature ($Y_h = 0.71$, Dircks et al. (1999)). The maximum growth rates $\mu_{max,h}$ were found to range from 0.95 to 1.78 d^{-1} and were located below defaults (3–6 d^{-1}). Nevertheless, they increased, even though only slightly, with increasing X_{bh} and OUR.

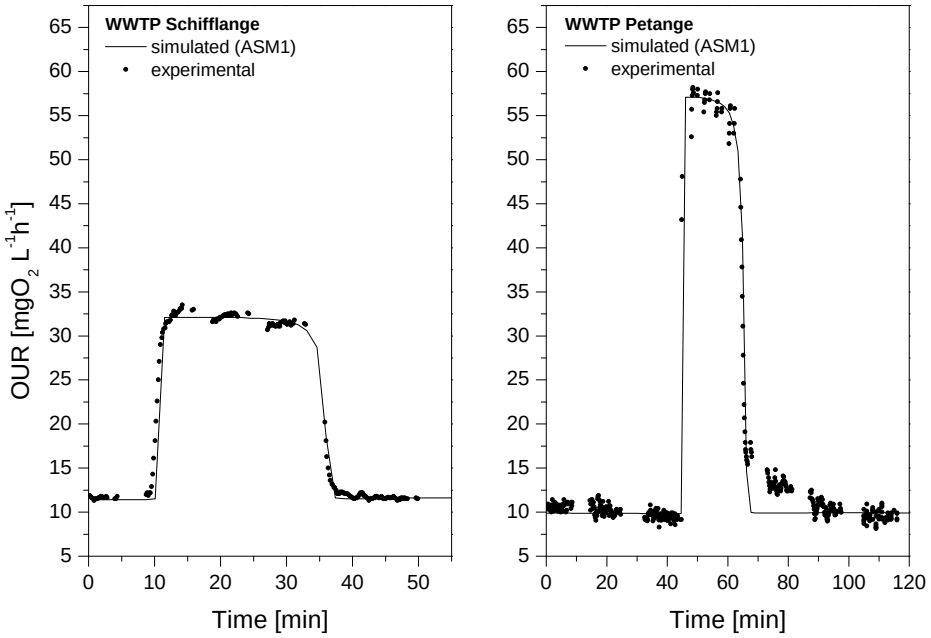

Figure 2.1: OUR response of two respirometry experiments with activated sludge from WWTP Schifflange (left) and Mamer (right); generated by addition of sodium acetate.

In this context, the maximum OUR as an expression of microbial activity can be only regarded as a relative measure since it is substrate-specific. The absence of, for example, ammonium or phosphorus in preliminary tests resulted in a significantly lower maximum OUR than with a C:N:P mixed substrate. The OUR_{max} can be used as indicator for the rate of substrate uptake whereby the overall BOD removal remains constant.

In addition, activated sludge with high concentrations of active biomass seemed to promote also increased levels of activity per g X_{bh}. The enhanced metabolic activity may be caused by enzyme production, specialization or transport processes and access to energy sources. Since microorganisms are present as clustered flocs in WWTPs, the location of the active cells may also be decisive, especially in the presence of large amounts of inert solids.

WWTPs	SRT [d]	TSS [g L^{-1}]	OUR_{max} [mgO_2 $L^{-1}h^{-1}$]	Y_h [gCOD $gCOD^{-1}$]	μ_{max} [d^{-1}]	K_s [mgCOD L^{-1}]	X_{bh} [g L^{-1}]	X_{bh}/TSS [%]
Schifflange	27	2.91	61.2	0.68	1.15	0.93	0.858	29.5
Reckange	15	3.68	58.1	0.65	1.03	0.11	0.959	26.1
Petange	19	3.08	97.5	0.66	1.34	0.26	1.223	39.8
Martelange	45	6.58	40.2	0.68	1.22	0.36	1.114	16.9
Boevange	54	2.49	22.2	0.63	0.95	0.31	0.501	20.1
Mamer	3-6	3.03	163.1	0.71	1.78	0.30	1.628	53.7

Table 2.1: Biokinetics in activated sludge of six WWTPs; measured on the day of sampling; D = Denitrification; P = Primary treatment. Designs and population connected (PE): Schifflange (D, 69200); Reckange (3500); Petange (P, D, 42000); Martelange (D, 4300); Boevange (D, 3200); Mamer (P, D, 20300).

Xenobiotic Biodegradation Kinetics

Two model approaches, first-order (Eq. 2.2) and pseudo first-order (Eq. 2.3) reactions were applied to fit data from biodegradation experiments (Table 2.2). APCAs are assumed to be degraded as secondary substrate and do not result in any significant microbial growth. First-order kinetics are independent of the start concentration and biokinetics. They are used in comparison of activated sludge parameters and degradation rate constants in order to avoid auto-correlation effects with X_{bh} (cf. Figure 2.3). In pseudo first-order kinetics, the transformation potential for xenobiotics can be attributed to the microorganisms present that are usually expressed in parameters of volatile or total suspended solids (VSS, TSS). The amount of active biomass can be used for a more adequate determination of the degradation rate constant k_{biol} by substituting TSS or VSS in (Eq. 2.3). However, this approach is limited to the assumptions that X_{bh} as the main governing parameter remains constant in the course of the batch test. Variable levels of activity cannot be considered.

$$\frac{\Delta C}{\Delta t} = -k_{biol} \cdot C_s \tag{2.2}$$

$$\frac{\Delta C}{\Delta t} = -k_{biol} \cdot X_{bh} \cdot C_s \tag{2.3}$$

where C is the total compound concentration [mg L^{-1}], t is the time [h], k_{biol} is the degradation rate constant [h^{-1} and L gX_{bh}^{-1} h^{-1}], X_{bh} is the amount of active heterotrophic biomass [g L^{-1}] and C_s is the soluble compound concentration [mg L^{-1}].

Naturally, the TSS is bigger than the amount of active biomass which, as a consequence, underestimates the actual degradation rate constants.

WWTP	NTA				DTPA			
	pseudo first-order		first-order		pseudo first-order		first-order	
	k_{biol} [L g X_{bh} h^{-1}]	± error	k_{biol} [h^{-1}]	± error	k_{biol} [L g X_{bh} h^{-1}]	± error	k_{biol} [h^{-1}]	± error
Schifflange	0.197	0.032	0.185	0.030	0.073	0.013	0.047	0.009
Reckange	0.049	0.018	0.047	0.029	0.041	0.002	0.069	0.013
Petange	0.849	0.095	1.036	0.117	0.128	0.019	0.122	0.003
Martelange	0.084	0.017	0.106	0.014	0.056	0.008	0.029	0.005
Boevange	0.211	0.027	0.115	0.014	0.055	0.007	0.028	0.004
Mamer	1.238	0.179	2.030	0.293	0.429	0.072	0.703	0.118

Table 2.2: Pseudo first-order and first-order biodegradation rates of NTA and DTPA; all r^2 >0.8.

Variation of APCAs Biodegradation

NTA and DTPA show substantial variation within their k_{biol}. The pseudo first-order k_{biol} of NTA in WWTP Mamer was more than one order of magnitude higher than in WWTP Reckange. The rate constants of DTPA were accordingly lower, but within the same dimension of variability. EDTA was of minor interest due to its persistence. Its k_{biol} remained steadily less than 0.009 L gX_{bh}^{-1} h^{-1} which is, however, more likely to be traced back to photolysis of Fe(III)EDTA. Pseudo first-order k_{biol} obtained in respirometric batch tests were chosen to compare the absolute xenobiotics removal capacity of the six WWTPs (Figure 2.2). Half-lives ranged from 0.5 to 9 h for NTA and from 1.5 to 16 h for DTPA.

The complexing agents were added as sodium salts. It can be expected that Na^+ is substituted by Fe^{3+} due to its high complexation constant and speciation kinetics. Fe(III)-complexes are likely to dominate since all investigated WWTPs use $FeCl_3$ for phosphorus precipitation upstream of biological treatment. As a matter of fact, it is improbable that significant variation of biodegradation is a result of differences in ligand speciation.

Linking Xenobiotic Elimination to Activated Sludge Activity

The principal hypothesis was that the biological removal capacity for APCAs is influenced or even governed by microbial activity. Starting from a critical specific OUR_{max} >70 mgO2 L^{-1} h^{-1}, differences measured in microbial activity correlated with variable k_{biol} of NTA and DTPA resulting in a positive relationship (Figure 2.3). Increased microbial activity

seemed to promote faster xenobiotics removal. The level of enzyme production, which can be expected to increase with increasing activity, may be responsible for enhanced APCAs degradation. However, below this OUR no significant changes in k_{biol} were observed, although NTA alone can be used as sole carbon source (Egli et al., 1990). EDTA (not shown) is not influenced at all.

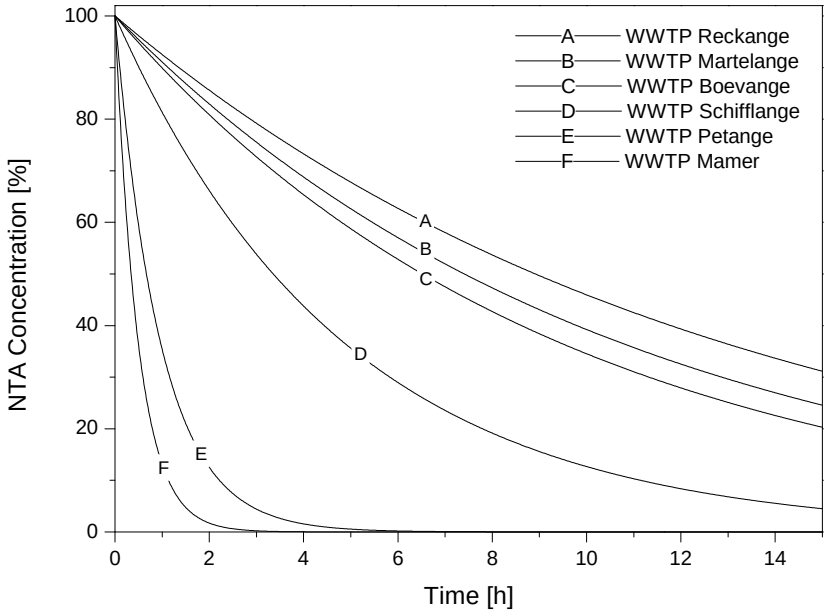

Figure 2.2: Pseudo first-order fits of NTA (using X_{bh}) obtained in respirometric batch tests; $C_0 = 1$ mg L^{-1}; test period $t = 7.5$ h; co-substrate: C:N:P mixture with a ratio of 100:5:1; number of data points per fit: n = 11 (not shown); $r^2 > 0.8$ for all fits.

Moreover, the influence of the activity appears to be substance specific. NTA is more strongly affected by changes in OUR_{max} than DTPA. Also the ratios of k_{biol} NTA/DTPA (calculated from Table 2.2) were observed to vary from 2.8 to 8.5. The value of WWTP Reckange (0.68) seems unrealistic, since a ratio <1 reveals that NTA is faster degraded than DTPA. Nonetheless, the k_{biol} of both substances seem to increase after reaching the same critical OUR.

Results can be seen as a strong indicator for biodegradation of APCAs via co-metabolism as a governing process. The latter provides no significant advantage for microorganisms in terms of energy or catabolism (Rittmann, 1992) but can be related to the presence of non-specific enzymes. Key enzymes involved in activation reactions of NTA breakdown, e.g. oxygenases, are very likely to occur in a fairly high number of WWTPs (Bucheli-

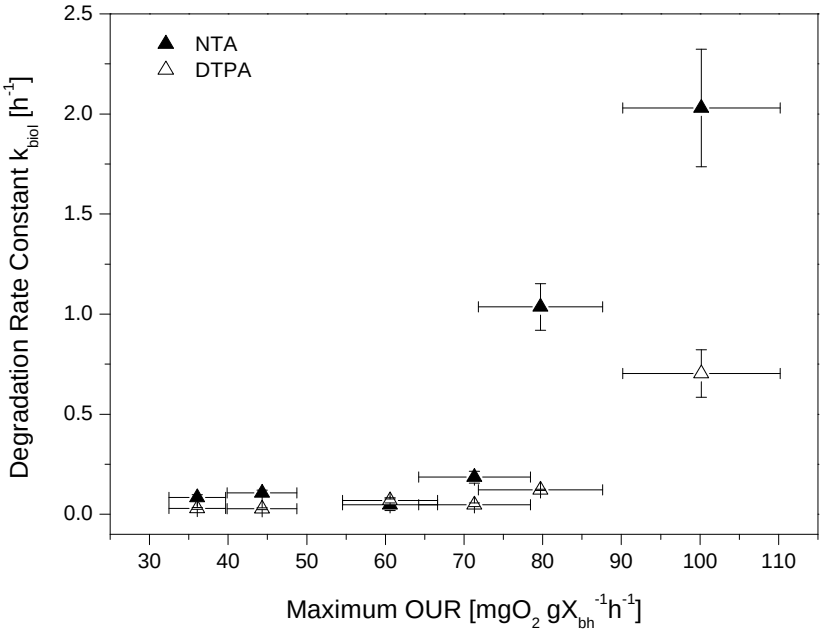

Figure 2.3: Influence of microbial activity expressed as OUR_{max} on the biodegradation of NTA and DTPA; first-order kinetics were used to avoid auto-correlation effects with X_{bh} comprised in OUR; the y-error bars represent uncertainty of moving window linear regressions and measuring devices.

Witschel & Egli, 2001; Yuan & VanBriesen, 2008). However, investigating these requires their quantification and identification which cannot be achieved by means of this approach. Hence, it is assumed that no occurrence of specialists in the microbial consortium is required for co-metabolic biodegradation. NTA and DTPA were clearly more effectively removed at low SRTs that are usually related to high microbial activities. In contrast, other authors observed higher biodegradation of xenobiotics, such as pharmaceuticals, at high SRTs (Clara et al., 2005). In this context, estimations about xenobiotic biodegradation and microbial behavior and consortium are difficult to derive from the SRT since it is only a hydraulic parameter.

Eventually, APCA degradation rates must be seen in the context of activated sludge quality. Here, the OUR_{max} as a possible predictor variable is suitable to xenobiotics that follow co-metabolic degradation pathways. For the latter, the microbial activity is a central parameter and can be used to benchmark their degradation rates for individual WWTPs. It is suggested that xenobiotic elimination capacity has to be assessed individually for each plant according to its performance. At this point, results provided an approach for a model that is transferable between WWTPs with known biokinetics. Nevertheless, a

broader statistical basis is required as well as the need to clarify under which conditions co-metabolic processes start to act. Moreover, the transfer of the biodegradation rates from batch tests to full scale WWTPs is still limited as long as no further investigations are made on co-metabolic processes especially in regard of inhibiting substances and available biodegradable co-substrates in real wastewaters.

Conclusions

Xenobiotic biodegradation experiments combined with respirometry was proposed as a reliable methodology for the estimation of biokinetics and APCAs degradation rates. Both were found to vary to a large extent between the six investigated different WWTPs. The variability of degradation rates could be explained by differences in microbial activity for the most part where the link to elimination of carbon and nutrients and thus co-metabolism is suggested. Increased activity resulted in faster acetate and also in enhanced xenobiotics removal. Activated sludge biokinetics allow for extrapolating the variable fate of APCAs to individual WWTPs of different design and performance.

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Chapter 3

3 Active Heterotrophic Biomass and Sludge Retention Time (SRT) as Determining Factors for Biodegradation Kinetics of Pharmaceuticals in Activated Sludge

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Active Heterotrophic Biomass and Sludge Retention Time (SRT) as Determining Factors for Biodegradation Kinetics of Pharmaceuticals in Activated Sludge

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Abstract The present study investigates the biodegradation of pharmaceutically active compounds (PhACs) by active biomass in activated sludge. Active heterotrophs (X_{bh}) which are known to govern COD removal are also suggested as a determining factor for biological PhAC removal. Biodegradation kinetics of five polar PhACs were determined in activated sludge of two wastewater treatment plants which differed in size, layout and sludge retention time (SRT). Results showed that active fractions of the total suspended solids (TSS) differed significantly between the two sludges, indicating that TSS does not reveal information about heterotrophic activity. Furthermore, PhAC removal was significantly faster in the presence of high numbers of heterotrophs and a low SRT. Pseudo first-order kinetics were modified to include X_{bh} and used to describe decreasing PhAC elimination with increasing SRT.

Keywords Pharmaceuticals; Biodegradation; Activated Sludge; Active Heterotrophic Biomass; Sludge Retention Time

Introduction

The removal of pharmaceutically active compounds (PhAC) during wastewater treatment has become a major concern in water research during the last decade. Biodegradation during activated sludge treatment has been identified as a major elimination pathway in particular for hydrophilic non-persistent PhACs in a variety of studies. To assess PhAC breakdown in individual activated sludges, biodegradation rates are mostly determined in lab-scale tests where microbial biomass is a key parameter. Biomass is usually approximated by the

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amount of total (or volatile) suspended solids (TSS) which can be easily determined by routine measurements. Recent studies proposed pseudo first-order reaction kinetics to describe PhAC removal (Maurer et al., 2007; Wick et al., 2009). This reaction is governed by the amount of biomass and the biodegradation rate constant k_{biol}. However, a major drawback of utilizing TSS is that only a fraction of the suspended solids can be considered as viable biomass while an inert fraction is also present (Cronje et al., 2002). Only the viable fractions are responsible for biological removal processes and biodegradation rates should therefore be expressed in terms of active biomass. While this has been successfully achieved e.g. for COD and NH_4^+ transformations by classifying activated sludge bacteria into heterotrophic and autotrophic fractions, the issue of identifying bacteria (groups) that are responsible for xenobiotic biodegradation processes still remains. In this context, slow growing specialized bacteria and diversified enzymes were suggested to enhance PhAC removal. These bacteria are believed to be retained in sludge in significant numbers in wastewater treatment plants (WWTPs) that operate above a critical sludge retention time (SRT) of 10 days (referred to 10 °C) (Clara et al., 2005). The concept of critical SRTs was developed for implementing the nitrification process in biological wastewater treatment systems and has been adopted for PhAC removal (Kreuzinger et al., 2004). In contrast, Stasinakis et al. (2010) found the highest biotransformation rates of endocrine disruptors at a low SRT of 3 days and Gaulke et al. (2009) found no difference for 17α-ethinylestradiol at two different SRTs suggesting that heterotrophic bacteria capable of degrading PhAC are present at both low and high SRTs. Furthermore, biodegradation rates of aminopolycarboxylic acids, which have been suggested to be promoted by heterotrophic microbial activity, were significantly higher at low SRTs (Majewsky et al., 2010). The SRT is a design criterion for WWTPs and strongly related to microbial growth. Nonetheless, the relation between SRT, microbial community structure and xenobiotic degradation performance is not fully understood and controversial findings have been reported (Clara et al., 2005; Gaulke et al., 2009; Kraigher et al., 2008; Saikaly et al., 2005; Schaar et al., 2010; Stasinakis et al., 2010).

The presented study focuses on the active heterotrophic biomass which governs COD removal, suggesting a determining factor for biological PhAC removal as well. It aims at contributing to the refinement of biodegradation rate estimations and at explaining variability of the latter between WWTPs. The interrelationship of PhAC removal capacity with operational process parameters such as SRT and hydraulic retention time (HRT) was investigated. A spectrum of five different hydrophilic pharmaceuticals was chosen (Table 3.1) that contains a variety of molecular structures with heterocyclic and aromatic rings and different functional groups. The selected substances carbamazepine (CBZ), diclofenac (DCF), sulfamethoxazole (SMX) and paracetamol (PCT) as well as caffeine (CAF) have been widely detected in concentrations up to the $\mu g\ L^{-1}$ level in wastewater influents (Heberer, 2002;

Zhang et al., 2008). These compounds range from persistent to easily biodegradable chemicals. Partitioning of the investigated compounds onto biomass particles by adsorption is usually not significant in the overall elimination (Ternes et al., 2004) and was therefore not subject to this study. Only sulfamethoxazole has a tendency to adsorb on secondary sludge particles with a solid–water distribution coefficient (K_d) of 260 L $kgTSS^{-1}$ (Göbel et al., 2005). This would account for a maximum 10 % of the total elimination efficiency, dependending on the sludge production (Ternes et al., 2004).

PhAC biodegradation kinetics were determined in activated sludge from two Luxembourg WWTPs that differed in size, layout, HRT, SRT and organic loading rates, suggesting significant differences in the level of heterotrophic microbial activity. A simultaneous estimation of viable heterotrophic biomass fractions and degradation kinetics was achieved by combining batch experiments with respirometry. Recent research successfully demonstrated the applicability of such a set-up in order to account for both, micropollutant biodegradation and process kinetics of activated sludge (Olmez-Hanci et al., 2011). The present study raised the question, whether pharmaceutical attenuation can be attributed to heterotrophic activity and therefore linked to treatment process parameters.

Materials and Methods

Sampling & Bioreactor

Activated sludge (2 x 20 L) was taken from the aerated tanks of the two Luxembourg WWTPs studied, Mamer and Boevange, in May 2009. Aliquots (2.4 L) were used for respirometer experiments. The respirometer used to study the removal of the PhACs consisted of a 3 L jacketed bioreactor (Ochs GmbH, Germany) maintained at a temperature of 20 ± 1 °C. A Metrohm GPD 751 Titrino controlled the pH at 7.5 ± 0.2 during the experiment by addition of hydrochloric acid or sodium hydroxide. The dissolved oxygen concentration, monitored using a LDO probe from Hach-Lange, was automatically maintained between 3 and 6 mgO_2 L^{-1} by an aeration pump controlled by a program written in LabView®. The oxygen uptake rate was calculated from the depleting dissolved oxygen concentration during non-aeration phases by moving window linear regressions (n = 10). The input of atmospheric oxygen via the liquid surface was taken into account in the model used for simulation ($K_La = 4.5 \cdot 10^{-3}$ min^{-1}).

Biodegradation Experiments

The pharmaceuticals carbamazepine, diclofenac, sulfamethoxazole, paracetamol and caffeine (purchased from Dr. Ehrenstorfer GmbH, Germany) were added as a mixed stock solution

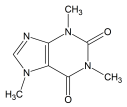
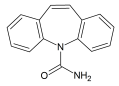
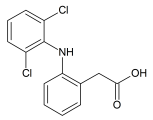
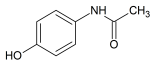
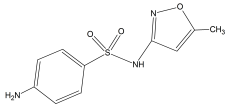
	CAS	Log K_{ow}[a]	Molecular weight[a] [g mol^{-1}]	Water solubility[a] [mg L^{-1}]	K_d secondary sludge [L kgTSS^{-1}]	Structure	Application
Caffeine	58-08-2	-0.07	194.19	$2.16 \cdot 10^4$	-		Psychostimulant
Carbamazepine	298-46-4	2.45	236.28	112	1.2[c]		Anti-epileptic drug
Diclofenac	15307-79-6	0.7[b]	296.16	2.37	16[c]		Non-steroidal anti-inflammatory drug
Paracetamol	103-90-2	0.46	151.17	$1.4 \cdot 10^4$	< 1[c]		Non-steroidal anti-inflammatory drug
Sulfamethoxazole	723-46-6	0.89	253.28	610	260[d]		Antibiotic

Table 3.1: Molecular structures, physico-chemical properties and application of the selected compounds; [a] SRC (2010); [b] Jones et al. (2002); [c] Ternes et al. (2004); [d] Göbel et al. (2005).

(c = 1.2 mg L^{-1} in H_2O, V_{added} = 2 ml) to the bioreactor resulting in an initial concentration of 1 µg L^{-1} (V_{sludge} = 2.4 L). In order to make biodegradation rates directly comparable, the same synthetic substrate was used together with PhAC spikes in each experiment. The synthetic substrate consisted of a mixture of sodium acetate, ammonium chloride and sodium dihydrogen phosphate monohydrate with a ratio of C:N:P of 100:50:1, corresponding to typical carbon to nutrient ratios occurring in domestic wastewater. The substrate was added (V_{added} = 21.2 ml) at a concentration of $COD_{theoretical}$ = 736.8 mgO_2 L^{-1}, thereby avoiding nitrogen or phosphorus from becoming limiting factors. The amount added ensured that the synthetic primary substrate was permanently present in excess during the period of the biodegradation test (5-6 h) and controlled by real-time OUR measurements. Samples of 10 ml were taken from the reactor every 30 min over a period of 5 hours (n = 11). Experiments were repeated three times for each activated sludge and mean values were used for the estimation of the apparent biodegradation rate constants.

Analytical Methods

Aqueous samples collected during the biodegradation experiments (10 ml) were filtered twice (0.2 mm; 0.45 µm) and adjusted to pH 3 using dilute hydrochloride acid. Mecoprop D-3 and di-hydrocarbamazepine were added as internal standards (c = 100 ng L^{-1}) to correct for losses during solid phase extractions (Weigel et al., 2004; Radjenović et al., 2007). Samples were then enriched using Oasis HLB 60 mg cartridges from Waters. The target compounds were eluted using 6 ml ethylacetate. The eluates were evaporated to dryness under a gentle nitrogen flow and then reconstituted in 1 ml of methanol. Pharmaceutical concentrations were measured using a LC-MS/MS system consisting of a Finnigan TSQ Quantum Discovery MAX from Thermo equipped with a Surveyor MS Pump Plus (flow rate of 200 µl min^{-1}), a polar endcapped C_{18} column Gold aQ (100 x 2.1 mm, particle size 3 µm) and an autosampler HTC PAL from CTC Analytics. The injection volume was 50 µl and the eluent gradient was from 70:30 H_2O/MeOH to 0:100 within 22 min. Limits of quantification were determined experimentally and lay by 50 ng L^{-1} for all investigated substances.

Results and Discussion

WWTP Characterization

Layout & Operation

Activated sludges from two WWTPs were chosen for biodegradation experiments that differed in size, design and operation (Table 3.2). WWTP Mamer operates at full capacity with 20,300 population equivalents, an organic loading rate of 0.095 kg BOD $kgTSS^{-1}$ d^{-1}

and a low SRT of 6 days. In contrast, the organic loading rate in WWTP Boevange is 6 times lower. In this plant the SRT was 54 days. Both plants operate with denitrification but only WWTP Mamer is additionally equipped with a primary clarifier. The incoming wastewater of both plants has a largely domestic origin.

WWTP	PE	SRT	Average Flow[a]	HRT[b]	Capacity Utilization	Sludge Load[c]
		[d]	[m^3 h^{-1}]	[h]	[%]	[kg BOD kg TSS^{-1} d^{-1}]
Mamer	20,300	6	136 ± 54	16.7 ± 3.7	100	0.095 ± 0.022
Boevange	2,700	54	65 ± 6	58.4 ± 6.6	20	0.016 ± 0.005

Table 3.2: General information on the investigated WWTPs; [a] during dry weather conditions; [b] calculated from flow through and tank volume; single pass; [c] calculated from daily BOD and TSS values (n=36); [d] calculated from daily averages; PE = Population equivalents.

Heterotrophic Biomass X_{bh}

The two investigated sludges showed significant differences concerning the amounts of active heterotrophic biomass (Table 3.3). It was found at 1.5 ± 0.1 g L^{-1} in WWTP Mamer (n = 13) compared to 0.6 ± 0.1 g L^{-1} in WWTP Boevange (n = 9). Both values varied only marginally during the three week measurement period. The formation of different fractions of active biomass is most likely due to available biodegradable substrates present in incoming wastewater, here referred to as the organic loading rate. Their activity adapts to the available substrates (Lemmer et al., 1994; Ni et al., 2008) and therefore can be expected to vary significantly between WWTPs.

In contrast, very similar values were found for the TSS with 2.4 ± 0.3 g L^{-1} and 2.5 ± 0.1 g L^{-1}, respectively. This leads to significantly different fractions (f_{at}) of X_{bh} / TSS: 62.9 ± 5.8 % of the TSS are active heterotrophs in WWTP Mamer but only 25.2 ± 6.3 % in WWTP Boevange. The large difference might be also favored by the absence of a primary clarification at WWTP Boevange. It can be expected that more inert particles enter the reactor tanks and contribute to a lower f_{at}. These inactive fractions can consist of i) endogenous residues, ii) inert organic and inorganic material, iii) the (in this case) inhibited autotrophs and iv) extracellular polymeric substances (EPS) that build flocs by holding various microorganisms together (Cronje et al., 2002; Wilén et al., 2008). These results indicate that TSS does not contain any information about the level of microbial activity and can therefore lead to biased estimates when used in rate calculations, as often

WWTP	TSS [g L^{-1}]	X_{bh} [g L^{-1}]	Fraction f_{at} [%]	Y_H [mgCOD $mgCOD^{-1}$]
Mamer	2.4 ± 0.3	1.5 ± 0.1	62.9 ± 5.8	0.69 ± 0.02
Boevange	2.5 ± 0.1	0.6 ± 0.1	25.2 ± 6.3	0.61 ± 0.04

Table 3.3: Estimation of active heterotrophic fractions f_{at} and yields Y_H in activated sludge from WWTP Mamer (n = 13) and WWTP Boevange (n = 9) using respirometry; ± one standard deviation; sampling period: April / May 2009.

done in modeling approaches. Furthermore, heterotrophic yields differed only slightly with Y_H = 0.69 ± 0.02 mgCOD $mgCOD^{-1}$ in WWTP Mamer and Y_H = 0.61 ± 0.04 mgCOD $mgCOD^{-1}$ in WWTP Boevange.

PhAC Biodegradation

Pseudo First-Order Kinetic Parameter Estimation

Pseudo-first order reaction kinetics (Eq. 3.1) was applied to describe pharmaceutical removal in batch tests. Thereby, degradation kinetics was assumed to depend on the degradation rate constant k_{biol} and the amount of active heterotrophic biomass that is expected to be constant over the duration of the experiment. The biodegradation rate constant k_{biol} is derived from fitting the analytical solution of Eq. 3.1 to the measured data (n = 11) by minimizing chi square χ^2 with an optimization routine provided in the evaluation software Origin® (Additive) while holding the heterotrophic biomass and the initial concentration constant:

$$\frac{\Delta C_t}{\Delta t} = -k_{biol} \cdot X_{bh} \cdot C_0 \tag{3.1}$$

where $\Delta\ C_t\ /\ \Delta\ t$ is the reaction rate [ng L^{-1} h^{-1}], C_t is the dissolved pharmaceutical concentration [ng L^{-1}], t is the time [h], k_{biol} is the degradation rate constant [L gX_{bh}^{-1} h^{-1}], X_{bh} is the amount of active heterotrophic biomass [g L^{-1}] and C_0 is the initial dissolved pharmaceutical concentration [ng L^{-1}].

PhAC Biodegradation Results

Results show that pseudo first-order kinetics was well suited to describe biological degradation of the selected compounds. The coefficients of determination r^2 ranged from 0.78 to 0.98 (Table 3.4) and average values of three experiments per plant (n = 3) showed standard deviations of <15 %. It can be observed that the degradation of paracetamol, caffeine, sulfamethoxazole and diclofenac was significantly enhanced in batch tests with ac-

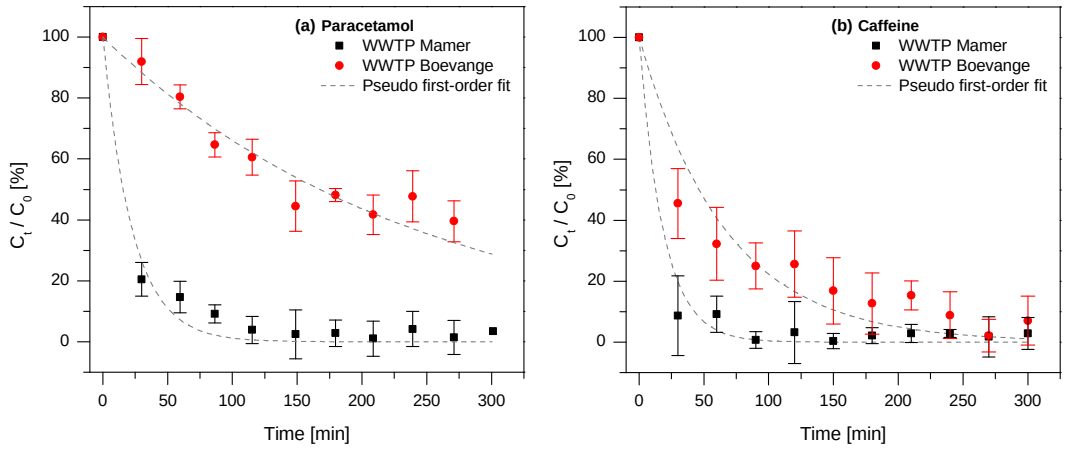


Figure 3.1: Biodegradation and pseudo first-order fits of paracetamol (a) and caffeine (b); data points n = 11; number of replicates n = 3; error bars indicate standard deviation

tivated sludge from WWTP Mamer compared to sludge from WWTP Boevange (Figure 3.1 & 3.2) for identical experimental conditions. The removal of carbamazepine was not significant in both sludges considering the standard deviation of three replicates (data not shown). As expected, a clear order of biodegradability could be observed with caffeine as easily biodegradable substance, sulfamethoxazole as semi-persistent, and diclofenac as well as carbamazepine as persistent compounds. Paracetamol was expected to be readily biodegradable as observed in the activated sludge of WWTP Mamer. However, in sludge from WWTP Boevange, its k_{biol} is close to that of sulfamethoxazole.

	Degradation rate constant [L gX_{bh}^{-1} h^{-1}]				Ratio [-]
	WWTP Mamer	r^2	WWTP Boevange	r^2	
Carbamazepine	0.007 ± 0.001[a]	0.81	0.010 ± 0.001[a]	0.78	0.7 ± 0.2
Diclofenac	0.029 ± 0.002	0.87	0.025 ± 0.002	0.82	1.2 ± 0.2
Sulfamethoxazole	0.307 ± 0.022	0.94	0.245 ± 0.014	0.89	1.3 ± 0.1
Paracetamol	1.654 ± 0.181	0.97	0.415 ± 0.034	0.89	4.0 ± 0.8
Caffeine	2.030 ± 0.185	0.98	1.500 ± 0.147	0.92	1.4 ± 0.2

Table 3.4: Pseudo first-order biodegradation rates constants of carbamazepine, diclofenac, sulfamethoxazole, paracetamol and caffeine; calculated using the amount of active heterotrophic biomass; data points per fit: n = 11; [a] not significant.

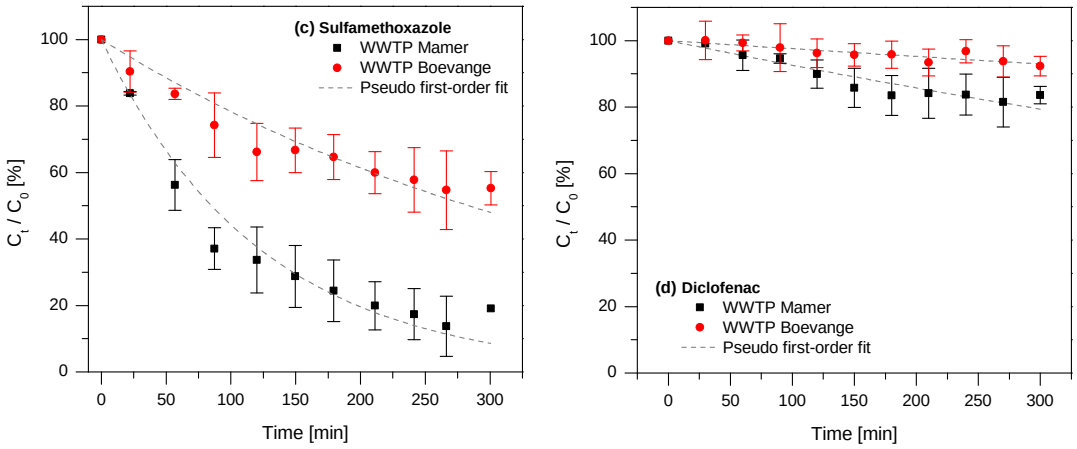


Figure 3.2: Biodegradation and pseudo first-order fits of sulfamethoxazole (a) and diclofenac (b); data points n = 11; number of replicates n = 3; error bars indicate standard deviation.

Suggesting heterotrophs to be governing, the k_{biol} of pseudo first-order kinetics would result in identical values in both sludges and hence in a ratio of 1 since k_{biol} is directly proportional to X_{bh} (Eq. 3.1). As it can be seen from Table 3.4, the differences in xenobiotic degradation efficiency can be largely explained by X_{bh} for 4 of the 5 substances considered. In fact, the ratios of the kinetic rate constants $k_{biol,Mamer}$ / $k_{biol,Boevange}$ range around 1 except for paracetamol (ratio = 4.0 ± 0.8). As mentioned above, its k_{biol} is unexpectedly low in WWTP Boevange.

Heterotrophic PhAC Biodegradation and Sludge Retention Time

The SRT is a process parameter that is inherently related to microbial growth activity. The latter increases with increasing biodegradable COD available in incoming wastewater. By definition, the SRT decreases with increasing sludge production and therefore high active fractions of X_{bh} are usually found at low SRTs. The relation between the active heterotrophic fraction f_{at} and SRT has been described as follows (Ekama & Wentzel, 2008):

$$f_{at} = f_i \cdot \left(\frac{1}{1 + f_h \cdot b_h \cdot SRT + \frac{f_{sup} \cdot (1 + b_h \cdot SRT)}{f_{cv} \cdot Y_{Hv} \cdot (1 - f_{sup} - f_{sus})}} \right) \tag{3.2}$$

where f_{at} = fraction of active heterotrophs on TSS, $f_i = VSS/\text{TSS}$ ratio of activated sludge; f_h = endogenous residue fraction, b_h = heterotroph decay rate [d^{-1}], SRT = sludge retention time [d], $f_{cv} = COD/VSS$ ratio [mgCOD $mgVSS^{-1}$], Y_{Hv} = yield coefficient

[mg VSS mgCOD^{-1}], f_{sup} = fraction of non biodegradable particulate COD, f_{sus} = fraction of non biodegradable soluble COD. The amount of X_{bh} [g L^{-1}] can be estimated from:

$$X_{bh} = f_{at} \cdot TSS \tag{3.3}$$

With regard to the tested sludges, faster PhAC removal was observed for the activated sludge with the higher fraction of X_{bh} and lower SRT (Figure 3.1, 3.2). By substituting Eq. 3.2 and 3.3 for X_{bh} in pseudo first order kinetics (Eq. 3.1), the PhAC elimination can be described as a function of the SRT given that X_{bh} is the determining factor:

$$\frac{\Delta C_t}{\Delta t} = -k_{biol} \cdot f_i \cdot \left(\frac{1}{1 + f_h \cdot b_h \cdot SRT + \frac{f_{sup} \cdot (1 + b_h \cdot SRT)}{f_{cv} \cdot Y_{Hv} \cdot (1 - f_{sup} - f_{sus})}} \right) \cdot TSS \cdot C_s \tag{3.4}$$

To apply this approach to the two selected WWTPs, typical wastewater characteristics for raw and settled wastewater were taken from Ekama & Wentzel (2008) (Appendix A). For elimination calculations, Eq. 3.4 was solved for the mean hydraulic retention time (single pass) estimated for both plants by daily average dry weather flow and volumes of the aerated tanks. It should be noted here, that the HRT is only a simplified average parameter that does not address mixing in the tank reactors. A refined estimation of removal efficiencies could be achieved for instance by using the hydraulic residence time distributions as input. Biodegradation rate constants k_{biol} and TSS were taken from Table 3.3 and 3.4. A rate constant of k_{biol} = 0.001 L gX_{bh}^{-1} h^{-1} was used for carbamazepine since its removal in the batch tests was not significantly different from 0. Biodegradation rate constants were assumed to be constant and also representative of full-scale plants.

Predicted elimination efficiencies and f_{at} as a function of the SRT can be seen in Figure 3.3 and 3.4. The inert fraction is complementary to f_{at} and therefore increases with increasing SRT. The model matches well the measured f_{at} of WWTPs Mamer and Boevange. Investigating the modeled PhAC elimination, no effect can be expected for the readily biodegradable paracetamol and caffeine. Their removal remains constantly at 100 % for the given HRT. Also, for carbamazepine no significant effect of the SRT is expected due to the persistence of the compound. Its removal remained constantly <5 %. In contrast, a significant decrease in the removal efficiency can be anticipated for diclofenac in both plants and for sulfamethoxazole in WWTP Mamer.

Although WWTP Boevange has a clearly lower f_{at}, significantly higher total removal efficiencies are obtained. This is a result of the different HRTs in the aerated tanks. In Boevange it is 3 times the HRT of the Mamer plant with 29.2 ± 2.7 h and 7.3 ± 3.5 h, respectively. Hence, the long retention time in the Boevange plant compensates the low X_{bh}

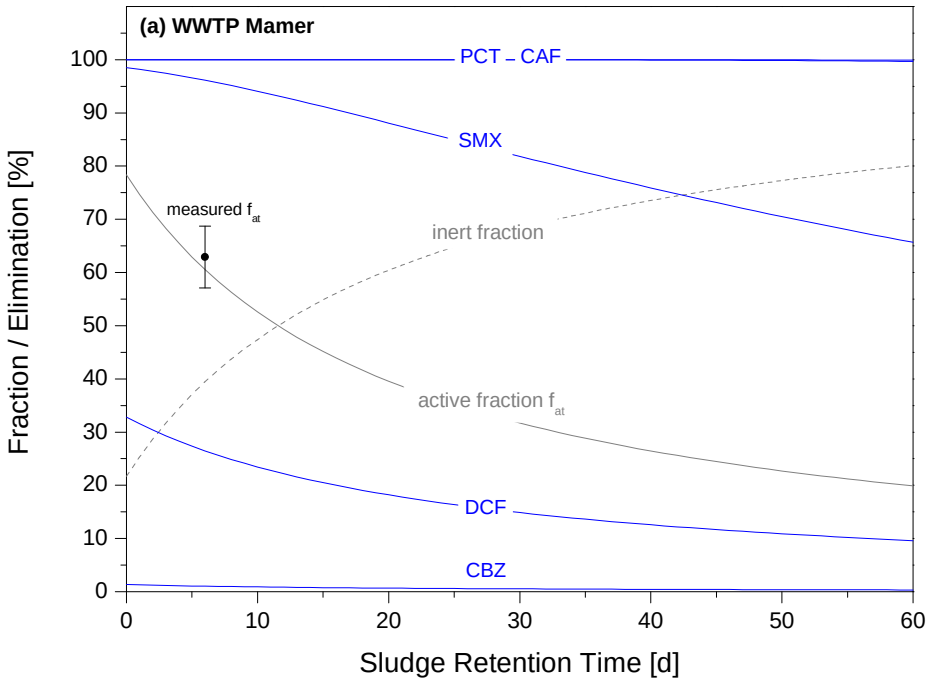


Figure 3.3: Active heterotrophic and inert fractions and elimination efficiencies of caffeine (CAF), paracetamol (PCT), sulfamethoxazole (SMX), diclofenac (DCF) and carbamazepine (CBZ) in dependency of the sludge retention time in WWTP Mamer, HRT = 7.3 ± 3.5 h.

resulting in higher total removal efficiencies. Both SRT and HRT are governed by plant design and hydraulic/organic loading with very limited room of maneuver for tuning once the plant has been build. Calculations show that significant differences in removal efficiencies can only be expected for substances with intermediate degradability. As capacity utilization changes over the life-time of a treatment plant, process proxies give the opportunity to estimate xenobiotic emission data.

Results suggested heterotrophic activity as governing factor for the removal of the selected PhACs since autotrophs were inhibited during the experiments. Increased degradation rates of the selected PhACs were observed in the sludge with the lower SRT and higher fractions of X_{bh} except for carbamazepine. This is consistent with the fact that increasing heterotrophic biomass fractions are linked to decreasing SRTs and therefore, highest active fractions of X_{bh} occur at low SRTs. Microbial communities evolve according to the prevailing environmental conditions and thus largely depend on the incoming wastewater composition, also referred to as organic loading rate. However, it is questionable if they adapt to pharmaceu-

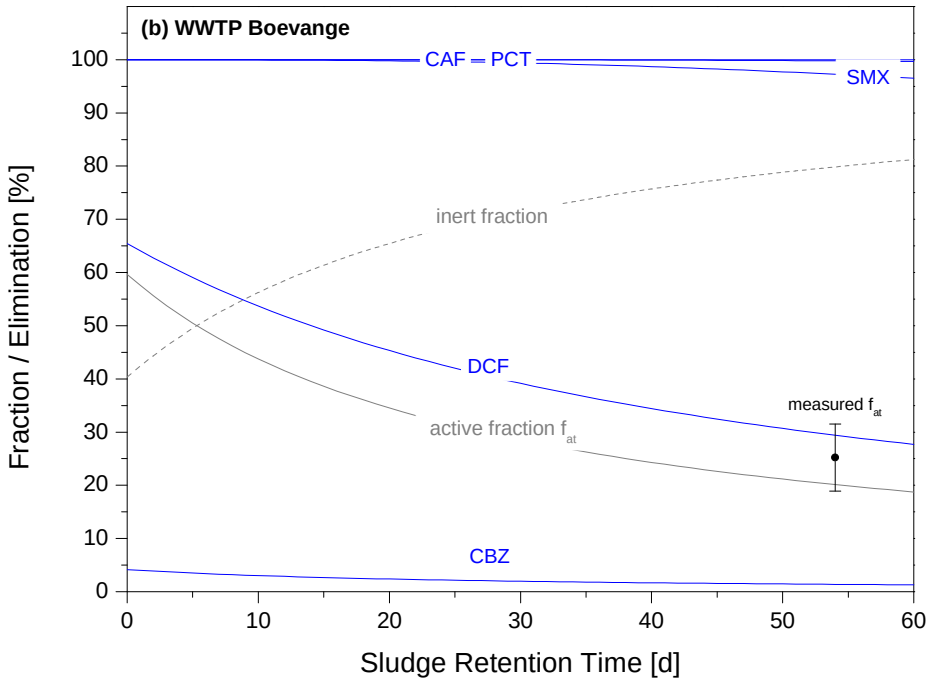


Figure 3.4: Active heterotrophic and inert fractions and elimination efficiencies of caffeine (CAF), paracetamol (PCT), sulfamethoxazole (SMX), diclofenac (DCF) and carbamazepine (CBZ) in dependency of the sludge retention time in WWTP Boevange, HRT = 29.2 ± 2.7 h.

tical compounds present in wastewater only in very small quantities. Kraigher et al. (2008) showed that a significant structural shift in the bacterial community caused by permanent PhAC presence occurred only at concentrations >50 µg L^{-1}, which are unlikely to occur in municipal WWTPs receiving domestic wastewaters.

It can be rather expected that the selected compounds may follow similar breakdown pathways as dominant substrates present in wastewater (Stasinakis et al., 2010) and may therefore be subjected to non-specific enzyme cleavage. In this case, enzyme production and microbial activity are determining factors. The results of this study can be seen in this context, where the active heterotrophs are proposed to reduce the variability of k_{biol} for sulfamethoxazole, caffeine, diclofenac and carbamazepine via pseudo first-order normalization. The increased biodegradation rate constant for paracetamol could however not be explained by the data of this study. It may be caused by specific differences in enzymatic profiles and/or phylogenetic composition of activated sludge. The SRT does not give any direct information about the microbial or enzyme spectrum but can be used as a proxy for

heterotrophic active fractions. Taking the latter as a driver for PhAC elimination, however, questions the hypothesis of enhanced xenobiotic elimination at high SRTs. It should be noted that the presented results focused on a selection of substances with limited representativeness. However, it appears that readily biodegradable substances, such as caffeine and paracetamol (in this study) or ibuprofen and natural hormones (Kreuzinger et al., 2004) are almost constantly eliminated up to 100 %. The persistence of carbamazepine seems also not to be linked to the sludge age. These findings indicate that the SRT is rather suited to reveal information about the removal capacity for intermediate biodegradable substances.

Apart from that, the SRT was reported to influence also microbial floc-structures, EPS production (Liao et al., 2006) as well as active biomass fractions in aerobic granules (Ni et al., 2008). These aspects have not been scrutinized in view of xenobiotic breakdown. Furthermore, investigations including enzyme analyses on a larger sample of sludges and substances are needed to better understand the role of heterotrophs for metabolic cleavage of xenobiotics.

Conclusions

This study presented arguments for active heterotrophs to be largely responsible for PhAC degradation. The modeling of two WWTPs showed that PhAC attenuation of intermediate biodegradable substances is expected to be decreased at higher SRTs due to a lower active biomass presence. In consideration of the HRT, the total removal efficiency of carbamazepine as well as caffeine and paracetamol was not affected by varying the SRT. A long HRT was found to compensate for low biodegradation rate constants. Nevertheless, optimization for maximum PhAC removal based on these process parameters can be hardly implemented in existing plants. Model simulations and proxy process parameters can be used to identify WWTPs with low PhAC removal capacity and to evaluate their impact as point sources for receiving waters.

Acknowledgements

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Appendix A

Parameter	Symbol	Unit	WWTP Boevange (raw wastewater)	WWTP Mamer (settled wastewater)
Pseudo first-order reaction constant	k_{biol}	L gX_{bh}^{-1} h^{-1}	See Table 3.4	See Table 3.4
VSS/TSS ratio of activated sludge[a]	f_i	mgVSS mgTSS^{-1}	0.75	0.83
Endogenous residue fraction[a]	f_h	—	0.2	0.2
Endogenous respiration rate[a]	b_h	h^{-1}	0.1 (0.24)[b]	0.2 (0.24)[b]
COD/VSS ratio[a]	f_{cv}	mgCOD mgVSS^{-1}	1.48	1.48
Yield coefficient[a]	Y_{Hv}	mgVSS mgCOD^{-1}	0.45	0.45
Non biodegradable particulate COD[a]	f_{sup}	—	0.15	0.04
Non biodegradable soluble COD[a]	f_{sus}	—	0.07	0.12
Total suspended solids	TSS	g L^{-1}	2.5	2.4
Hydraulic retention time[c]	t	h	29.2 ± 2.7	7.3 ± 3.5

Table A.3.1: Wastewater parameters and mean hydraulic retention time (single pass) used for WWTP Boevange (raw wastewater) and WWTP Mamer (settled wastewater); [a] Ekama & Wentzel (2008); [b] Corrected for temperature, standard value at 20°C in brackets; [c] Calculated from flow through and tank volume of aerated treatment of daily average values of 3 weeks.

Chapter 4

4 Xenobiotic Removal Efficiencies in Wastewater Treatment Plants: Residence Time Distributions as a Guiding Principle for Sampling Strategies

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Xenobiotic Removal Efficiencies in Wastewater Treatment Plants: Residence Time Distributions as a Guiding Principle for Sampling Strategies

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Abstract The effect of mixing regime and residence time distribution (RTD) on solute transport in wastewater treatment plants (WWTPs) is well understood in environmental engineering. Nevertheless, it is frequently neglected in sampling design and data analysis for the investigation of polar xenobiotic removal efficiencies in WWTPs. Most studies on the latter use 24-h composite samples in influent and effluent. The effluent sampling period is often shifted by the mean hydraulic retention time assuming that this allows a total coverage of the influent load. However, this assumption disregards mixing regime characteristics as well as flow and concentration variability in evaluating xenobiotic removal performances and may consequently lead to biased estimates or even negative elimination efficiencies.

The present study aims at developing a modeling approach to estimate xenobiotic removal efficiencies from monitoring data taking the hydraulic RTD in WWTPs into consideration. For this purpose, completely mixed tanks-in-series were used to address hydraulic mixing regimes in a Luxembourg WWTP. Hydraulic calibration for this WWTP was performed using wastewater conductivity as a tracer. The RTD mixing approach was coupled with first-order biodegradation kinetics for xenobiotics covering three classes of biodegradability during aerobic treatment. Model simulations showed that a daily influent load is distributed over more than one day in the effluent. A 24-h sampling period with an optimal time offset between influent and effluent covers only half of the influent load in a dry weather scenario. According to RTD calculations, an optimized sampling strategy covering four consecutive measuring days in the influent would be necessary to estimate the full-scale elimination efficiencies with sufficient

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accuracy. Daily variations of influent flow and concentrations can substantially affect the reliability of sampling results. Commonly reported negative removal efficiencies for xenobiotics might therefore be a consequence of biased sampling schemes. In this regard, the present study aims at contributing to bridge the gap between environmental chemistry and engineering practices.

Keywords Hydraulic Residence Time; Sampling; Xenobiotics; Conductivity; Removal Efficiency; Optimal Experimental Design

Introduction

The elimination of micropollutants in wastewater treatment plants (WWTPs) became a major concern during the last decade. A variety of polar micropollutants such as pharmaceutical compounds pass biological wastewater treatment without being fully degraded (Bernhard et al., 2006; Reemtsma et al., 2006). In order to estimate micropollutant emissions to receiving waters, removal performance of WWTPs is usually assessed by either full-scale balancing or by the determination of biodegradation rates at lab-scale (Vieno et al., 2007; Wick et al., 2009). Both estimation approaches rely essentially on the time that the water remains in the plant, normally referred to as the hydraulic retention time (HRT). The latter is an easily accessible parameter since it can be calculated from flow through and tank volumes. Most work carried out uses 24-h composite samples assuming quantitative coverage of influent loads in the effluent with a temporal shift proportional to the HRT or very stable influent concentrations over relevant periods. However, taking into consideration variable influent conditions and that residence time distributions (RTD) of perfect plug flow tanks do not apply to conventional WWTPs reactors, mass balancing based on influent-effluent comparison may lead to biased or even negative removal efficiencies. Hence, an adequate description of the hydraulic characteristics is critical for designing sampling campaigns and predicting dynamic xenobiotic emission.

The characterization of mixing regimes in wastewater treatment plants with RTDs and pulse-response techniques is well explored and common practice (De Clercq et al., 1999; Gujer, 2008; Levenspiel, 1999). It was successfully applied to describe mixing-regimes in a variety of tracer test studies (Fall & Loaiza-Navía, 2007; Capela et al., 2009). The RTD is hereby fitted by the number and size of tanks-in-series, the type of the mixing regime (completely mixed, plug-flow etc.) as well as the flow conditions. Artificial tracer tests with appropriate substances like e.g. lithium and bromide salts or fluorescent dyes are commonly used for hydraulic characterization of mixing regimes (Olivet et al., 2005). However, recent studies showed that the latter can also be realized with data from routine measurements of WWTPs such as temperature or conductivity (Ahnert et al., 2010).

The fact that the effluent concentration dynamics of hydrophilic micropollutants are largely governed by hydraulic mixing is often poorly considered and can lead to increased uncertainty and misinterpretation of the sampling results. Generally, WWTP performances are routinely evaluated by comparison of long-term influent-effluent data, e.g. for chemical oxygen demand (COD) or NH_3-N, and is therefore believed to be applicable to xenobiotics as well. However, the measurements and analyses of xenobiotics are cost- and work-intensive which is why often only a short sampling period (mostly 24 h in influent and effluent) is used as a tradeoff between cost and data density. In such a case, the effect of influent variations on sampling results is naturally potentially much larger.

In this context, the present study aims to bridge the gap between hydrodynamic behavior and biodegradation in municipal WWTP to assess xenobiotic removal efficiencies and derive adequate sampling strategies. So far, the RTD concept has not been applied in combination with short-term xenobiotic mass balance calculations.

For this purpose, hydraulic mixing regimes were characterized by use of an RTD approach and coupled to first-order biodegradation kinetics. Biodegradation was modeled for three different levels of xenobiotic biodegradability which are representative for persistent as well as moderately and easily biodegradable compounds. Simulations were applied to derive optimal sampling strategies and to minimize sampling errors. Moreover, an RTD-based method is proposed for an adequate estimation of overall removal efficiencies as well as a guidance tool for designing measurement campaigns at full-scale WWTPs.

Material and Methods

Sampling & WWTP Data

Wastewater conductivity was measured in the influent (after sand trap) and the effluent (after secondary clarification) of the Luxembourg WWTP Mamer with YSI 600 OMS probes over a period of three weeks (sampling interval $\delta t = 10$ min). Hourly inflow data and tank volumes were obtained from the plant operators (Water Syndicate SIDERO) (Figure 4.1).

Modeling

Plant Layout & Calibration

The plant layout of WWTP Mamer was reproduced in the wastewater modeling software GPS-X from Hydromantis (Hamilton, Canada) (Figure 4.1). It is equipped with standard activated sludge models (Gujer et al., 1999; Henze et al., 1987) allowing dynamic simulation of WWTPs. Completely mixed tanks-in-series with rectangular primary clarifiers and circular secondary clarifiers were selected. For the latter, a 1-D model of settler mass balance

equations is used for ten horizontal layers of equal depth. Volumes, sequence and tank operation were adjusted according to the data supplied by the plant managers (Table 4.1).

Measured wastewater conductivity was used as a tracer for model calibration at the given flow conditions of a three week period. Inlet conductivity was fed to the model as input and the predicted effluent conductivity was iteratively fit to the measured effluent data to determine the number of completely mixed tanks-in-series and to estimate the sludge recirculation flow used in the model. The difference between measured and predicted values was minimized by the chi square χ^2 (Eq. 4.1) within GPS-X.

$$\chi^2 = \sum_{i=1}^{N} \frac{1}{\sigma^2} \cdot (y_i - \hat{y}_i)^2 \tag{4.1}$$

where χ^2 = chi square, N = set of observations, σ = standard deviation of the measurements, y_i = measured values, $\hat{y}_i$ = predicted values.

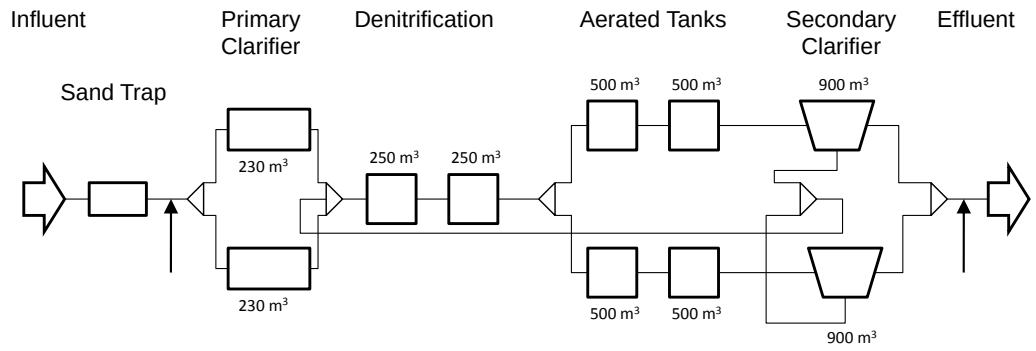

Figure 4.1: Layout, tank volumes and tanks-in-series used in the model to describe the mixing regime of WWTP Mamer; arrows indicate point of sampling for conductivity.

Residence Time Distribution (RTD)

The distribution of residence times of a xenobiotic within the plant was determined by model simulations. To this purpose, concentrations pulses were created in the influent (duration: 24 h, following typical sampling periods of composite samples). The fraction of soluble inert COD S_i served as model substance for xenobiotics in GPS-X. The COD S_i fraction is neither degraded nor produced in the model and can therefore be used to determine RTDs. Residence time distributions were obtained for various flow conditions including dry weather conditions and a storm event. They were exported to MatLab (MathWorks), fitted

with a linear interpolant function ($r^2 = 1$) and integrated using the curve fitting toolbox. Stepwise integrals were determined for equal time steps which allow the calculation of the released fraction of S_i on the total S_i per step. Mass balances of S_i were checked to assure that 100 % of the S_i influent signal has been released.

Biodegradation

Organic micropollutant biodegradation in activated sludge is typically described with pseudo first-order or first-order reaction kinetics (Joss et al., 2006; Schwarzenbach et al., 2003). Here, first-order biodegradation rate constants k_{biol} of 0.05, 0.5 and 5 h^{-1} were chosen as representative values for three classes of biodegradability (persistent, moderately and easily biodegradable polar xenobiotics) to account for biological removal during the course of aerobic wastewater treatment. It was assumed that no significant degradation occurs during denitrification and in the clarifiers. To simulate xenobiotic biodegradation during aerobic conditions, first-order reaction kinetics was implemented into GPS-X:

$$r_{si} = -k_{biol} \cdot S_i \tag{4.2}$$

where r_{si} = reaction rate [ng L^{-1} h^{-1}], k_{biol} = biodegradation rate constant [h^{-1}], S_i = soluble xenobiotic concentration [ng L^{-1}].

To calculate the xenobiotic effluent loads as a function of the RTD, Eq. 4.2 is solved analytically for each residence time step t_s of the RTD (temporal resolution = 1 min) and multiplied by the flow. The degraded effluent load for a given degradation rate constant is then the sum of all partial loads over the selected time span e.g. 24 hours:

$$L_{eff} = \sum S_i \cdot e^{-k_{biol} \cdot t_s} \cdot Q \tag{4.3}$$

where L_{eff} = total xenobiotic effluent load [g d^{-1}], t_s = residence time step of the RTD and Q = flow [L h^{-1}].

The total elimination efficiency is then calculated by mass balancing of the xenobiotic influent and effluent load:

$$E = (\frac{L_{inf} - L_{eff}}{L_{inf}}) \cdot 100 \tag{4.4}$$

where E = elimination efficiency in [%], L_{inf} = xenobiotic influent load [g d^{-1}].

Sampling Scenarios

Two model scenarios were set up to derive optimized sampling strategies taking WWTP Mamer as an example. In scenario 1, a perfect steady-state xenobiotic influent loading was assumed on the basis of 8-h composite samples (total load: 2.96 ± 0.8 g d^{-1}; corresponding to measured loads of the pharmaceutical diclofenac; Table A.4.1; constant flow = 200 m^3 h^{-1}). In scenario 2, an example data-set for realistic influent variability was created from measured influent concentrations of two days on the basis of 2-h composite samples (see Appendix A). Since inlet concentration data was only available for two days, but a time series of four days was required, the scenario was completed by using generated concentration data for two additional days. Concentrations (n = 12 per day) were randomly generated from a normal distribution with mean and standard deviation of the measured concentrations (703 ± 35 ng L^{-1}, n = 24). Corresponding measured hourly flow values of one week were used. The resulting average loads for these two days (day zero and three) were 2.1 ± 0.6 g d^{-1} and 2.2 ± 0.7 g d^{-1}, respectively.

Uncertainty Analysis

Monte Carlo simulations were performed to assess the uncertainty introduced by discrete sampling on the load estimation. Following Ort & Gujer (2006), the error of a 2-h composite sample was assumed to be ± 20 % (minimum error for sampling intervals >5 min). The flow error was estimated to be ± 10 %. The corresponding 2-h composite sample measurement values of day one and two were averaged for both flow and concentration in order to approximate a representative diurnal variation pattern (n = 12). Each concentration and flow value was varied by an error composed of the standard deviation of the 2-h composite sample measurement value as given before and a random error taken from a normal distribution assuming non-systematic error variability:

$$m^* = m_i + \sigma_i \cdot \epsilon \tag{4.5}$$

where m^*= varied value for flow and concentration, respectively, i = number of the 2-h composite sample (1-12), m_i = measured value of 2-h composite sample i, s = absolute standard deviation of 2-h composite sample i and ϵ = error taken from a Gaussian normal distribution (mean = 0, standard deviation = 1). An array of 10000 simulation runs assured to asymptotically approximate normal distributions. The resulting error associated with the determination of a load was evaluated by using the relative standard deviation and the 5 and 95 % percentile of the output distribution. Error propagation was calculated according to standard equations (Refsgaard et al., 2007).

Results and Discussion

The investigated WWTP runs at full capacity with 20,300 population equivalents (PE). It operates with primary clarifiers, denitrification and two lanes with aerobic treatment followed by secondary clarifiers. The mean HRT, calculated by the quotient of average flow (hourly values over three weeks) and tank volumes was found to be 16.7 ± 3.7 h over the whole plant and 7.3 ± 3.5 h (single pass; ± one standard deviation) in the aerated tanks during dry weather conditions (Table 4.1). It decreased during a storm event (flow = 503 ± 44 m^3 h^{-1}) to 4.6 ± 1.4 h and 1.9 ± 0.17 h, respectively.

WWTP Mamer	
Population equivalents	20,300
Capacity utilization [%]	100
Average flow during dry weather [m^3 h^{-1}][a]	136 ± 54
Average flow during rainfall event [m^3 h^{-1}][a]	503 ± 44
Hydraulic retention time[b] [h]	
Mean HRT during dry weather	16.7 ± 3.7
Mean HRT on measured storm event	4.6 ± 1.4
Mean HRT in aerated tanks only; during dry weather	7.3 ± 3.5
Mean HRT in aerated tanks only; during measured rainfall event	1.9 ± 0.17
Recycled fraction of activated sludge [%][c] (flow proportional)	0.8

Table 4.1: Operational data of the investigated WWTP; [a] flow conditions during the measurement campaign (3 weeks), daily mean during rainfall event; [b] calculated by the quotient of tank volume and flow through; [c] estimated from calibration.

Model Calibration

Calibration results show that modeled values matched measured effluent conductivity within i) the range of the effluent concentration and ii) the variation patterns of the effluent (Figure 4.2). Artifacts in the effluent conductivity caused by measurement interferences were deleted resulting in gaps in the consecutive time series. Figure 4.2 reveals that influent variations become dampened in the effluent but could be adequately reproduced by the model. The correlation coefficient was found to be R = 0.76 suggesting good tracking of

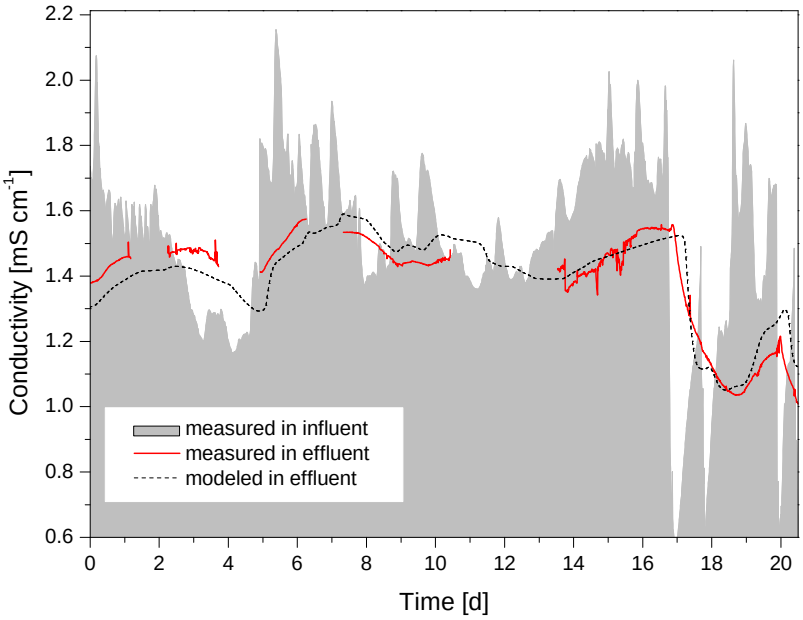

Figure 4.2: Hydrodynamic calibration of WWTP Mamer using natural conductivity. Input data: influent conductivity (gray) and flow (hourly values, not shown).

the conductivity variation. Nonetheless, small differences between modeled and measured values are observable that may be caused by not considering short circuits, stagnant zones and non-ideal mixing in the model. Their influence might change with variable hydraulic loading. The number of completely mixed tanks-in-series was determined to be n = 4 (2 denitrification / 2 aerobic treatment tanks as well as 2 clarifiers per lane, see Figure 4.1) by minimizing chi square between modeled and measured effluent conductivity values.

WWTP Mamer operates with $FeCl_3$ addition for phosphate precipitation before activated sludge treatment which may influence the conductivity. Moreover, the latter can be affected by ionic compounds being produced or removed during biological treatment or changes in the pH. However, this is apparently of minor importance to the effluent conductivity since measured outlet patterns correlated well with modeled values using the measured influent conductivity as input. Also, the pH was found to be stable during the measurement period with 7.9 ± 0.2 (n = 36).

Residence Time Distribution

After having calibrated the model, the RTD of an inert soluble xenobiotic was determined by use of a S_i injection pulse in the influent (duration: 24 h). Here, it should be kept in mind that the RTD is flow dependent. On that account, Figure 4.3 shows modeled distributions at various given flow conditions in order to illustrate the retention of different fractions of Si in the tanks. From this, percentiles can be determined giving the percentage of the released fraction of the influent water volume at a certain time t (Figure 4.4). The RTD becomes more left-skewed with increased flow resulting in smaller percentiles. For instance, during dry weather conditions around 20 % of the influent water volume have been released within 24 hours while during a rainfall event already 60 % have been emitted during the same time. When comparing those percentiles to the HRT from Table 4.1, the mismatch becomes apparent: at a mean HRT (16.7 h) only around 10 % of the water volume that entered the WWTP 16.7 hours ago has been released. A 24-h effluent (composite) sample shifted by a temporal offset of the HRT would contain only 30-40 % of the influent pulse. Consequently, a large proportion of sampled wastewater would originate from periods preceding the influent pulse during dry weather conditions. This aspect is addressed in detail in section "Sampling Scenarios".

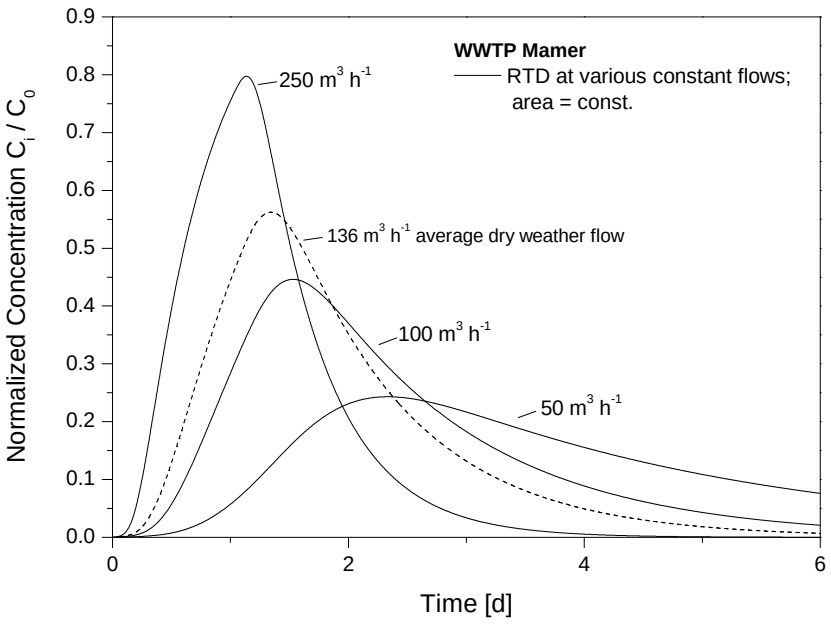

Figure 4.3: Residence time distribution of xenobiotics at flows varying from 50-250 m^3 h^{-1} as a result of an influent pulse in WWTP Mamer (duration: 24 h); areas of all curves are constant.

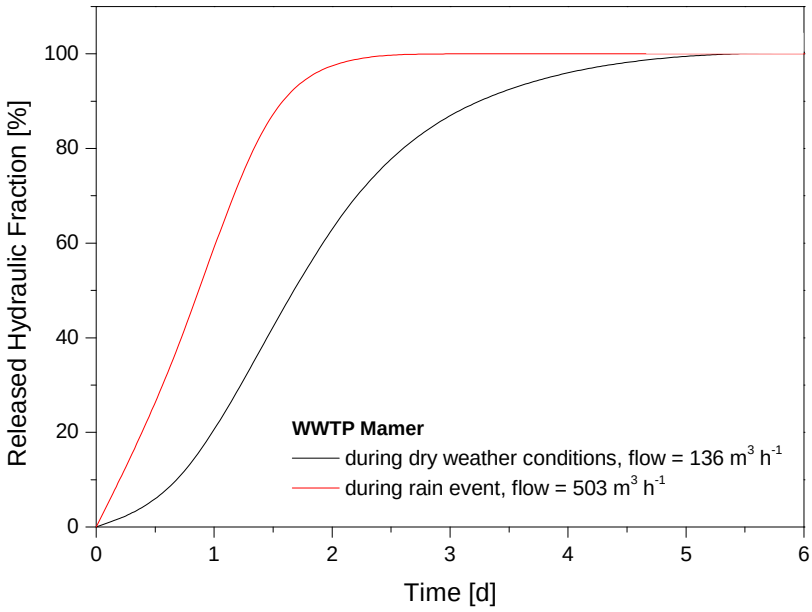

Figure 4.4: Exit-age distributions as a result of S_i influent pulse injections (duration: 24 h) showing the cumulative released water volume fractions in WWTP Mamer; flow was selected according to Table 4.1.

RTD Effects on Biodegradation

For prediction of the overall removal efficiency, first-order kinetics is usually solved for the HRT. As can be seen from Figure 4.3, a single mean HRT is not suited to describe the residence of wastewater volumes in reactor tanks, in particular for strongly skewed RTDs. The use of RTDs for removal calculations is therefore much more adequate. It allows depicting decreased removal efficiencies during rainfall events (Table 4.2). Under high flow conditions the RTD shifts toward a left-skewed distribution, i.e. that the RTD fractions of short retention in the plant increase. Hence, high flow leads to decreased retention times in the tanks and to a decreased removal assuming that degradation rates remain constant. A visualization of flow influence on elimination efficiency is shown in Figure 4.5, where the decrease of the elimination of a moderately biodegradable xenobiotic ($k_{biol} = 0.5$ h^{-1}) with increasing flow through has been plotted.

Using the model, elimination efficiencies can be described as a function of the flow. The dashed line indicates removal efficiencies calculated by use of the HRT. Compared to the solid line, which show the efficiencies based on the RTD, a clear mismatch of up to 45 % is evident. There, the mixing regime has a significant impact causing lower degradation. As a

consequence, the use of HRT and laboratory determined k_{biol} may lead to an overestimation of the actual removal performance (Table 4.2).

	Degradation rate constant k_{biol} [h^{-1}]	Total elimination efficiency Based on RTD [%]		Based on HRT [%]	
Xenobiotic		dry weather	rain event	dry weather	rain event
persistent	0.05	10	4	31	10
moderately biodegradable	0.5	55	21	98	63
easily biodegradable	5	98	78	100	100

Table 4.2: Elimination efficiencies calculated on the basis of the RTD and the HRT in the aerated tanks for three different first-order degradation rate constants; dry weather: constant flow = 136 m^3 h^{-1}; storm event: constant flow = 503 m^3 h^{-1}.

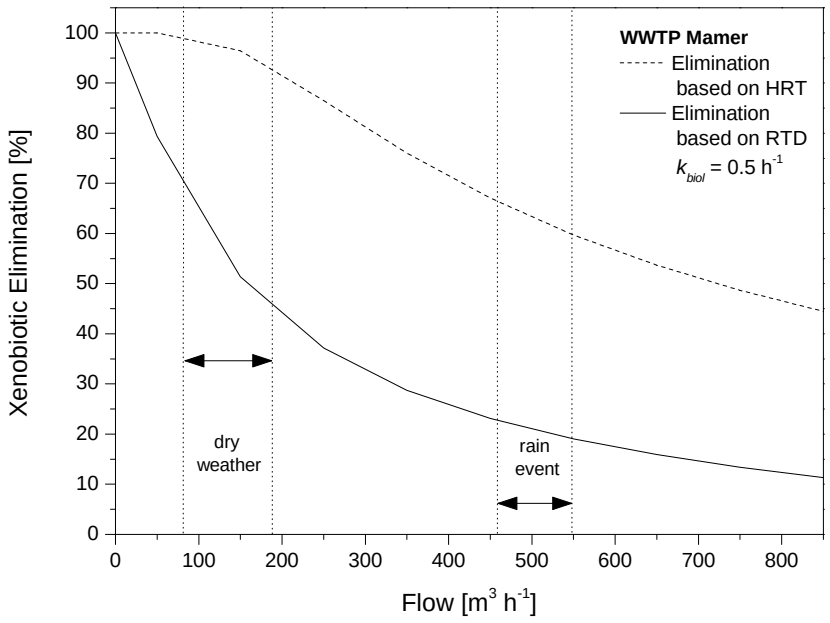

Figure 4.5: Comparison of modeled elimination efficiencies for a moderately biodegradable xenobiotic (k_{biol} = 0.5 h^{-1}) on the basis of HRT and RTD as residence times in aerated tanks at various constant flow conditions.

The fact that daily influent loads are discharged in the effluent over a period longer than one day is a major concern for the determination of full-scale elimination efficiencies

by influent-effluent mass balancing. To account for this hydraulic behavior and to derive more adequate sampling strategies, influent-effluent correspondence was investigated in the following scenarios.

Sampling Scenarios

The adequate setting of sampling intervals to address mixing regimes is crucial for the determination of elimination rates at full-scale. For example, it is remarkable that negative removal efficiencies for several (biodegradable) pharmaceuticals have been reported in a variety of studies (Onesios et al., 2009). Although knowing that certain parent compounds can be formed by the cleavage of conjugates (Ternes, 1998) also inadequate sampling strategies can yield erroneous mass balances when the water volumes sampled in influent and effluent do not correspond. The importance of adapting sampling mode and frequency to influent variability and catchment structure as well as the errors being associated with discrete sampling have been shown before (Minkkinen, 2007; Minkkinen & Esbensen, 2009; Ort et al., 2010a). Modeling simulations showed that a daily water volume is distributed over more than one day when discharged in the effluent. Consequently, a daily influent load cannot be completely covered by (composite) samples taken over a period of only 24 h at the outlet. However, an optimum temporal offset can be identified, by which a 24-h effluent sampling period is shifted from the beginning of the influent period to cover the maximum percentage of the released load. Given these findings, the following model scenarios were set up to derive an optimized sampling strategy for reliable xenobiotic mass balances at full-scale:

• **Scenario 1:** In this scenario, the influent concentrations are assumed to be sampled on three consecutive days on a basis of 8-h composite samples, while the effluent is sampled on one day only. The effluent sampling period (24 h) was shifted by the optimum offset (here: 18 h) from the beginning of the second measurement day and is indicated as vertical dashed lines in Figure 4.6. A perfect steady-state variation pattern was assumed with a constant flow (200 m^3 h^{-1}) and biodegradation was set to zero (k_{biol} = 0 h^{-1}).

The water volume sampled on day two corresponds to only 55.6 % of the sampled effluent water volume. Consequently, the remaining 44.4 % originate from preceding days and one following day, as can be seen from Figure 4.6. Based on the RTD approach, the fractions of each (daily) influent water volume released at the time of the effluent sampling can be calculated (Table 4.3).

Subsequently, to obtain a reliable mass balance, it is preferable to explain the origin of 80-90 % of the sampled effluent water volume. The number of influent sampling days needed to achieve this, can be derived from the cumulative proportions of each measurement day on the effluent load. In this case, influent sampling days 1-3 would allow explaining 91.0 %

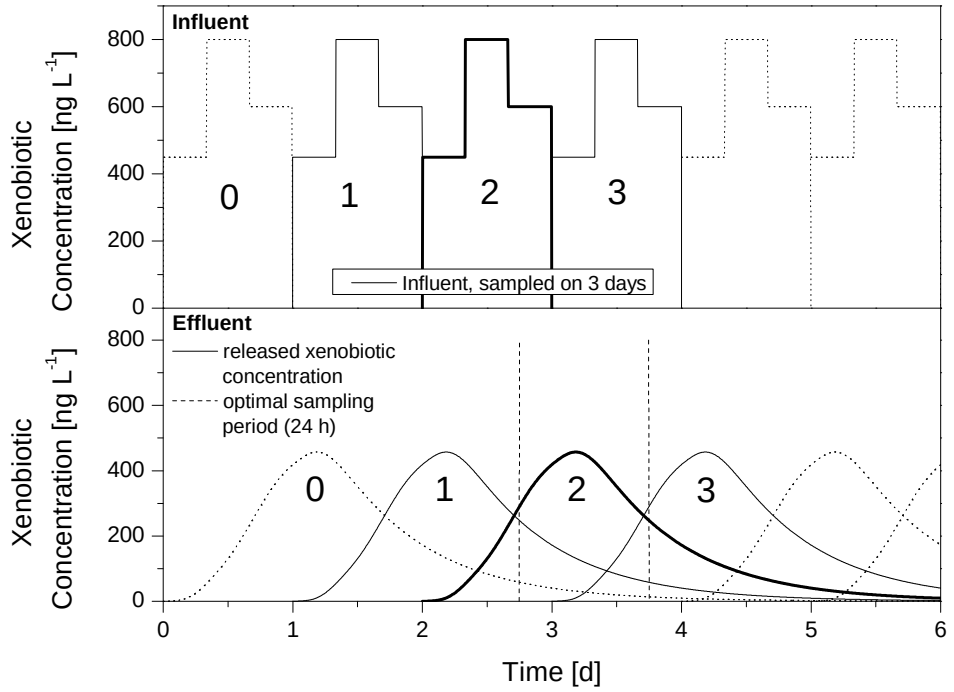

Figure 4.6: Sampling scenario 1: xenobiotic concentrations assumed to be sampled on three consecutive days in the influent (8-h composite samples) and one sampling day in the effluent shifted by the optimum offset (18 h from the beginning of influent day 2); flow = 200 m^3 h^{-1} (not shown); load fractions captured during the sampling period: 8.2 % of day 0, 24.2 % of day 1, 55.6 % of day 2 and 11.2 % of day 3.

of the effluent sampled during a 24-h period. An additional fourth day would result in 99.2 %. When sampling influent day 1-3, 9 % of the effluent sample originates from unknown water volumes (day zero, non-covered period). The proportions of the influent load on the effluent sample match exactly the captured fractions, since a perfect steady-state was assumed. A steady-state diurnal concentration pattern at constant flow was assumed in the influent, consequently resulting in periodic effluent concentrations (one effluent period may extent over more than 24 h). Under these simplifying conditions it would be sufficient to sample one in- and effluent periodical pattern with any time shift. Mass balance calculations would result in the same elimination efficiency. However, such stable conditions and constant periodic loads do not reflect reality. The concentrations of xenobiotics can vary largely during a diurnal cycle depending on their usage. Nelson et al. (2011) reported intense pulses that exceeded relative standard deviations of 100 % of their daily means for selected pharmaceuticals in WWTP effluents. Further, strong diurnal variation was shown

Influent measurement day	Influent load fraction captured by effluent sampling[b] [%]	Proportion explained of the effluent load[c] [%]
day 0[a]	8.2	8.2
day 1	24.2	24.2
day 2 (optimum offset)	55.6	55.6
day 3	11.2	11.2
non-covered period	0.8	0.8

Table 4.3: Load fractions of the consecutive influent measurement days captured during the effluent sampling period (24 h, from Figure 4.6) and their proportion of the effluent load; the optimum outlet sampling period was calculated to start by an 18-h time shift from the beginning of day two. [a] day 0 = day before the measuring period; [b] referred to the influent load of each measurement day; [c] referred to the calculated effluent load (2.96 g d^{-1}); in this case, the proportions of the effluent load are identical to the captured influent fractions since perfect steady-state conditions were assumed.

e.g. for benzotriazoles in influents (Ort et al., 2005). Hence, it can be expected that high variations in influent and effluent concentrations are very likely to occur. As a consequence, the variability of empirical data was used in Scenario 2 to adapt the sampling scheme to realistic conditions.

• **Scenario 2:** Here, realistic influent concentration patterns (2-h composite samples) during dry weather conditions were introduced as well as the corresponding measured flow (hourly values) during that period (Figure 4.7). A diurnal variation can be observed in the flow as well as one rainfall event on the fifth day. Again, biodegradation was set to zero (k_{biol} = 0 h^{-1}). The concentration variability of the influent load is propagated through the plant and can visibly be tracked in the released effluent concentrations. Applying the same sampling scheme as in scenario 1 (day 1-3), the origin of only 71.1 % of the effluent load could be explained with three consecutive inlet measurement days and added up to only 84.9 % when including day 0 (Table 4.4). Hence, 15.1 % stem from loads of days preceding the influent sampling period and are therefore unknown. Compared to scenario 1, scenario 2 shows a lower coverage during the effluent sampling time span. This is mainly due to the lower flow conditions that decelerate the xenobiotic release.

The actual elimination efficiency can now be determined by estimating the reference load that actually corresponds to the effluent sample. This reference load is composed of load fractions of, in this case, four days (cf. Table 4.3 & 4.4) and can thus be calculated as the sum of the latter (see Appendix B for a mathematical description). It is then used in the mass

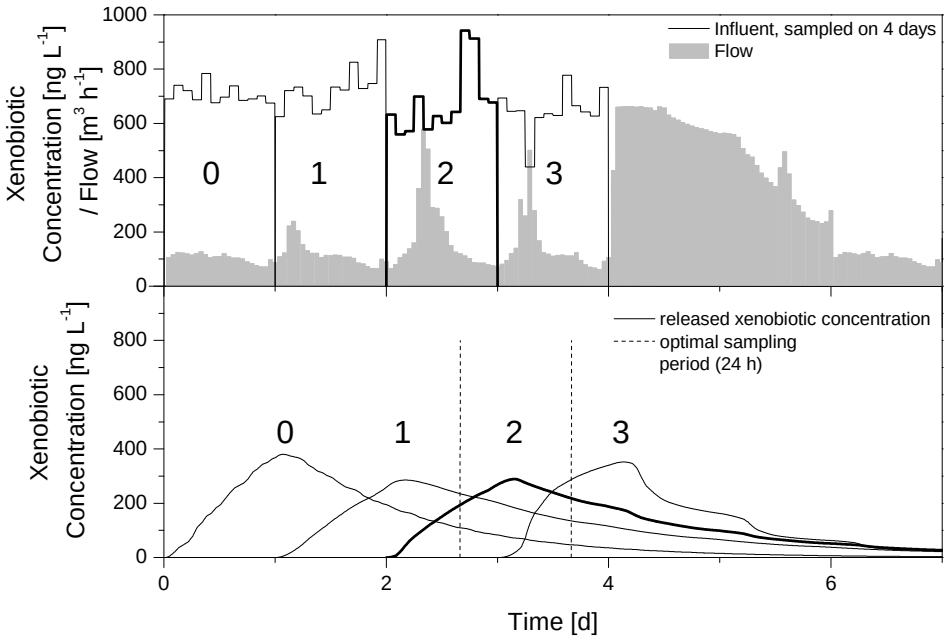

Figure 4.7: Sampling scenario 2: xenobiotic concentrations assumed to be sampled on four consecutive days in the influent (2-h composite samples; realistic influent variability of xenobiotic concentrations (diclofenac)) and one sampling day in the effluent shifted by the optimum offset (18 h from the beginning of influent day 2); measured flow values were used (hourly values); load fractions captured during the sampling period: 14.4 % of day 0, 22.4 % of day 1, 30.7 % of day 2 and 16.0 % of day 3.

Influent measurement day	Influent load fraction captured by effluent sampling[b] [%]	Proportion explained of the effluent load[c] [%]
day 0[a]	14.4	13.8
day 1	22.4	18.5
day 2 (optimum offset)	30.7	36.5
day 3	16.0	16.1
non-covered period	14.8	15.1

Table 4.4: Load fractions of the consecutive influent measurement days captured during the effluent sampling period (24 h, from Figure 4.7) and their proportion of the effluent load; the optimum outlet sampling period was calculated to start by an 18-h time shift from the beginning of day two. [a] referred to the influent load of each measurement day; [b] referred to the calculated effluent load (2.21 g d^{-1}); [c] comprises two days before day 0; loads were estimated as the average load of day 0-3.

balance calculations and compared to the measured and potentially degraded effluent load. In the case that less than 100 % of the relevant influent loads have been covered, the uncertainty of this non-sampled loading needs to be considered. This uncertainty decreases with decreasing contribution to the sampled effluent load. Details of this aspect are addressed in section "Uncertainty Analysis".

Expanding the influent measurement period to 3 or 4 days may be no financial issue with four 24-h composite samples but can become decisive when a higher temporal resolution is required e.g. with 8-h or 2-h composite samples. There, the cost-benefit ratio with regard to the reliability of the results should be assessed in advance.

Biodegradation Scenarios

In both scenarios, the elimination was set to 0 % to calculate the fractions captured by effluent sampling. However, at full-scale, biodegradation takes place during biological treatment which reduces the influent load. Assuming that biodegradation is proportional to the concentration with a first-order rate constant k_{biol}, the relative fractions of the influent loads (and their ratios to each other) during the sampled effluent period remain constant, while only the absolute load changes. In this way, the calculation of the reference load is also valid for every biodegradation scenario. In the following example, biodegradation was simulated in scenario 2 for the three xenobiotics of different persistence and a surrogate with $k_{biol} = 0$ h^{-1} (Table 4.5). The resulting elimination efficiencies are associated with an error because not the full 100 % of the sampled load could be related to a sampled influent load. Therefore, it was assumed that the loads of the non-covered period preceding the influent sampling days had the same daily average load and varied with the same standard deviation (± 27.2 %) as the measured loads (day one and two). This would result in an uncertainty on the total elimination efficiency of ± 4 % for the surrogate, ± 3 % for a persistent, ± 2 % for a moderately biodegradable and ± 0 % for a biodegradable xenobiotic. The error decreases as the variation of the non-covered period has a comparatively lower impact on the mass balance for easily and moderately biodegradable xenobiotics.

Erroneous elimination efficiencies are obtained ranging from -14 to 16 % for both the surrogate and the persistent xenobiotic when using the conventional 1-1 day influent-effluent mass balancing approach for each sampling day (day 0-3) with average loads. The uncertainty of these values can be expected to increase considerably under more variable influent conditions. However, the apparent elimination efficiencies of moderately and easily biodegradable substances are well approaching the true efficiency. This is due to the fact that, relative to the influent load, variations of largely degraded effluent loads affect the mass balance to a lesser extent, as it was the case before for the variations of the non-covered period. Thus, the conventional approach is more robust to influent variations of

Xenobiotic	Degradation rate constant k_{biol} [h^{-1}]	Reference load [g d^{-1}]	Degraded load sampled in the effluent [g d^{-1}]	Elimination efficiency[a] [%]	Apparent elimination efficiency by conventional sampling scheme[b] [%]
Surrogate	0	2.21	2.21	0 ± 4[c]	-3; -14; 10; 1
Persistent	0.05	2.21	2.01	9 ± 3[c]	4; -6, 16; 9
Moderately biodegradable	0.5	2.21	1.09	51 ± 2[c]	48; 43; 55; 50
Easily biodegradable	5	2.21	0.08	96 ± 0[c]	96; 96; 97; 96

Table 4.5: Elimination efficiencies estimated from four biodegradation scenarios based on mass balance calculations by i) using the fractionated reference load and ii) comparing daily average loads of each influent sampling day (0-3) to the sampled effluent load; average loads are given in Table A.4.1 and section "Sampling Scenarios". [a] calculated using the reference load; [b] calculated by comparing the daily average load of each day (0-3) to the sampled effluent load; [c] error is caused by the assumed variation of the non-covered period preceding the influent sampling days (RSD = 27.2 %).

readily and moderately biodegradable xenobiotics. Further, there was only a low variation of the loads during the four days in the influent. Nonetheless, disregarding the variation preceding and during a sampling campaign would make it virtually impossible to estimate how reliable the obtained elimination efficiency value actually is.

Uncertainty Analysis

Besides the uncertainty of the non-covered period, elimination efficiencies estimated with the proposed approach are additionally associated with the error introduced by discrete (24-h) composite samples, depending on the mode and frequency. Influent short-term variations are usually not captured by most sampling schemes and are therefore an error source leading to non-representative results for average loads (Ort et al., 2010b). Flow measurement errors (here assumed to be ± 10 %) affect also the accuracy of the reference load determined by the RTD approach. Ort & Gujer (2006) showed for a middle-sized catchment that a sampling interval of at least five minutes (time-proportional) was required to obtain a representative influent composite sample (2-h) with standard deviations lower than ± 20 %. These errors must be considered in order to reliably estimate the reference load. To this purpose, Monte Carlo simulations were used to investigate the propagated error of flow and concentration sampling on the estimated reference load. Simulation results showed

that fraction load estimates approximated a normal distribution with a relative standard deviation of ± 6.4 % and a range from -8.3 to 8.3 % (5 and 95 % percentile). As the total reference load is composed of multiple load fractions (in this case: the fractions of four days plus non-covered period), the total propagated error would be 14.3 % based on the standard deviation. The length of the sampling time span (12 h, 24 h, 36 h) in the effluent has no effect on this error assuming the same sampling mode and frequency.

These sampling errors as well as the conditions of the unknown days preceding the sampling campaign, which may be highly variable, consequently affect mass balances. The latter can only be closed here for the WWTP Mamer since the non-sampled period is assumed to have similar average loads and variability as the measured days. It nonetheless demonstrates clearly how daily influent variation can lead to misinterpretation when the sampling intervals are not accurately set and short-term data is used, in particular based on 24-h composite samples.

Conclusions

This study demonstrates that hydrodynamic characteristics are crucial for elimination, emission prediction and sampling of xenobiotics in municipal WWTPs. The hydraulic retention time is only of limited use since it does not reveal any information about mixing and distribution behavior. In order to tackle this issue, a residence time distribution approach linked with biodegradation kinetics was applied. This approach illustrated the problems encountered when trying to match influent loads with effluent loads. Depending on the flow regime, a 24-h xenobiotic influent load can expand significantly over more than one day when released in the effluent. It shows that a 24-h sampling period can cover only a small percentage of the corresponding influent load.

The optimal sampling setup for full-scale mass balancing at the WWTP Mamer was determined to be a coverage of four consecutive days in the inlet and a single day sampling at the outlet with an offset of 66 hours to the beginning of the inlet monitoring. The presented set-up would allow explaining the origin of more than 83 % of the incoming water under realistic conditions. This coverage can be calculated for every WWTP that should be monitored and it is advisable to use simulations for the planning and evaluation of a monitoring campaign with regard to calculation of the total elimination efficiencies. However, the number of consecutive influent sampling days must be selected plant-specifically. It is related to the prevailing mixing regime and thus requires calibration via tracer tests. We demonstrated that calibrating hydraulic models by wastewater conductivity can offer a cost-effective option compared to artificial tracers and should therefore be implemented in full-scale measurement campaigns. The uncertainty caused by non-covered periods preced-

ing the sampling days can be estimated from the average loads and standard deviation of the measurement days assuming them to be representative for dry weather conditions. In the Mamer plant, an accurate full-scale mass balance is only possible by high inlet coverage with monitoring. The sampling mode and frequency but also analytical errors can cause additional uncertainty. Sewer network and catchment structure as well as rainfall events greatly determine the variability of flow and xenobiotic concentrations and can lead to short-term variations in the range of minutes (Ort et al., 2010a,b). With regard to these aspects and that the origin of a sampled effluent water volume could not be explained to 100 %, this study reveals that elimination efficiencies of less than 15-20 % are probably impossible to track in full-scale investigations. The present paper raises the issue of mass balancing influent and effluent loads on the basis of short-term WWTP measurement campaigns. We showed that apparent negative elimination efficiencies can be caused by inadequate sampling strategies. Results illustrate the need to cover influent loads over several days and to consider the hydraulic characteristics in treatment plants. Hence, the accuracy of reported full-scale elimination efficiencies should be revised under these aspects.

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Appendix A

Example Data

Example data introduced to scenario 2 consisted of i) the pharmaceutical diclofenac (time-proportional, 12 glass bottles, 2-h composite samples with 24 min aliquot sampling frequency in influents using ISCO 6700 autosampler units) analyzed on two independent days (28th May and 6th June 2009) as well as ii) hourly inflow data and tank volumes that were obtained from the plant operators (Water Syndicate SIDERO). The measurement error associated with the flow data is unknown and therefore assumed to be ± 10 %.

Xenobiotic Analysis

Samples were collected directly after a sampling cycle, stored at 4°C and analyzed within 24 h. Diclofenac was analyzed by use of a LC-MS/MS system consisting of a Finnigan TSQ Quantum Discovery MAX from Thermo with a Surveyor MS Pump Plus (flow rate of 200 µl min^{-1}), a Surveyor LC-Pump Plus (flow rate of 2 ml min^{-1}) and an autosampler HTC PAL from CTC Analytics. A 1 ml online enrichment method was used with an extraction column Hypersil Gold (20 x 2.1 mm, particle size 12 µm) from Thermo. A polar endcapped C_{18} column Gold aQ (100 x 2.1 mm, particle size 3 µm) served as chromatography column. The eluent was increased from 70:30 % H_2O/MeOH to 0:100 % within 22 min. Limits of quantification (LOQ) were found at 125 ng L^{-1} in influent samples.

Diclofenac	Day 1 [average ± stdev]	Day 2 [average ± stdev]
Concentration [ng L^{-1}] (n=12)	728 ± 39	678 ± 31
Flow [m^3 h^{-1}] (n=24)	111 ± 14	160 ± 19
Loads [g d^{-1}]	1.9 ± 0.4	2.4 ± 0.8

Table A.4.1: Measured average concentrations and loads of diclofenac in the influent of WWTP Mamer on two independent measurement days for scenario 1 and 2 (Figure 4.6 & 4.7); loads calculated based on hourly flow and two hourly concentration data; stdev = one standard deviation.

Appendix B

Estimating the Total Elimination Efficiency

The influent concentration and flow data of n consecutive measurement days can be used for model simulations in order to calculate the actual inlet reference load that corresponds to the load proportions f_n [-] captured by an effluent sampling period. This reference load can be determined as:

$$L_{ref} = \sum f_n \cdot L_{inf,meas,n} \tag{A.4.1}$$

where L_{ref} = reference load [ng d^{-1}], fn = fraction of the influent load of day n [-] on the effluent sampling period, $L_{inf,meas,n}$ = measured influent load of day n [ng d^{-1}].

Subsequently, the measured (potentially degraded) effluent load can be related to L_{ref} in order to calculate the actual elimination efficiency E in [%]:

$$E = \frac{L_{ref} - L_{eff,meas}}{L_{ref}} \tag{A.4.2}$$

where $L_{eff,meas}$ = measured effluent load on the chosen sampling period [g d^{-1}].

Chapter 5

5 Conclusions and Outlook

5.1 Conclusions

This thesis dealt with basic questions concerning xenobiotic breakdown in aerobic sludge systems and contributed to the understanding of the variability of micropollutant removal efficiencies between different wastewater treatment sites. Activated sludge was characterized by the combination of respirometry and micropollutant biodegradation experiments in order to examine the factors responsible for micropollutant cleavage. The OUR, which is inherently associated with the amount of X_{bh}, was found to correlate with first-order biodegradation kinetics of the aminopolycarboxylic acids NTA and DTPA in six activated sludges from different treatment sites. The OUR as a measure of substrate oxidation and microbial activity indicated that the removal of organic substrates is related to the breakdown of chelating agents.

In order to consider the amount of biomass present during aerobic treatment, recent research has proposed to include TSS as an additional constant co-factor into pseudo first-order kinetics. The assumption that TSS equals the amount of viable biomass and that all of it would be responsible for micropollutant removal is clearly in conflict with the hypothesis of specialized bacteria present only at high SRTs. In this study, TSS was replaced by X_{bh} assuming that the micropollutant removal is governed by the latter. Results suggest that there is no specialized bacteria (or enzyme spectrum) required for the biodegradation of the investigated compounds which use them as the sole source of carbon. It is most likely that co-metabolism based on non-specific enzymes is the governing process. Certainly, at a species level, it cannot be excluded that specialized bacteria were present. Nevertheless, results demonstrated that faster removal occurs at increased amounts of active heterotrophic biomass.

A generalization beyond the investigated compounds is difficult due to the broad variety of anthropogenic engineered molecular structures of xenobiotics. Nonetheless, it could be shown that higher X_{bh} amounts promoted the removal of the selected pharmaceuticals sulfamethoxazole, diclofenac, paracetamol and caffeine. In contrast, the level of microbial activity had no effect on persistent substances such as EDTA and carbamazepine in all of the investigated plants. For three of the four investigated pharmaceuticals, degradation kinetics could be successfully normalized with X_{bh}. Nevertheless, co-factors other than heterotrophic biomass need to be investigated to explain the variability in degradation kinetics of paracetamol. Although the dependency of SRT and X_{bh} is well understood in wastewater engineering, there has been little cross-linking with micropollutant modeling yet. In this context, pseudo-first order reactions were modified to include the SRT that is dominated by heterotrophic growth activity. Modeling results showed that – for xenobiotics whose degradation correlates with microbial activity – faster removal occurred at low SRTs.

Concerning the prediction of total removal efficiencies these findings especially apply to intermediate biodegradable micropollutants. When considering the hydraulic contact time in WWTPs, the influent load of readily biodegradable micropollutants was found to be eliminated >95 %, independent of the SRT. The removal of carbamazepine was permanently <5 %. Therefore, increased attention should be given to intermediate biodegradable compounds in emission prediction of conventional WWTPs.

The leeway for optimization is however very limited once a plant has been built. HRT and SRT are determined by the hydraulic and organic loads, and optimization by variation of these parameters cannot be performed without deteriorating the effluent quality in terms of nutrient and carbon content. This should however be given priority in order to achieve a low nutrient loading and to avoid eutrophication in receiving waters, for which municipal WWTPs have originally been designed.

Hence, in most cases there is no alternative to the installation of additional treatment steps to cope with the issue of micropollution. From this perspective, the increased number of studies and recent developments dealing with advanced tertiary treatment technologies as cited before is fully understandable. As a consequence, research and results scrutinizing the fate of xenobiotics during conventional treatment ought to aim particularly at benchmarking removal potentials in order to reliably predict emission loads. It is however not possible to experimentally examine all WWTPs at a meso- or macro-scale for global concepts in a river network. State-of-the-art approaches rely on average values or efficiencies obtained from single measurement campaigns. Chapters 2 and 3 clearly showed that this can lead to biased estimations. Activated sludge properties and xenobiotic biodegradation kinetics varied to a large extend between different WWTPs and typical properties could not be derived. The need for identification and application of proxy parameters allowing to transfer and extrapolate biodegradation kinetics to different treatment sites is therefore apparent. Moreover, the presented results allude that our understanding of variable removal efficiencies needs refinement concerning responsible biodegradation factors at the enzyme level.

Chapter 4 highlighted that, besides variable activated sludge characteristics, inadequate sampling schemes can result in erroneous full-scale mass balances. This must therefore be considered as one of the main causes for the variability of WWTP elimination efficiencies. A residence time distribution approach clearly demonstrated that the "traditional" set-up of short-term measurement campaigns with 1-1 day influent-effluent mass balances results in erroneous calculated elimination efficiencies. Only in the exceptional case that influent conditions are stable and the variability of flow and concentration is very low, this approach would yield reliable values. However, if the influent variability is unknown, any assessment as to whether the obtained elimination efficiencies are representative or due to an artifact of biased sampling is impossible. Using the proposed set-up, the occurrence of commonly

reported negative mass balances could be unambiguously reproduced. The innovative character of the approach developed in this thesis allows to reliably estimate mass balances independently of transient flow and concentration conditions.

Taking all this information into account, the accuracy of reported elimination efficiencies has to be contemplated. It is commonly found that elimination efficiencies based on influent-effluent comparison are used to evaluate the efficiency of new removal techniques such as membranes or ozonation steps, but also to assess the effects of process parameters such as the SRT. Referring to that, the reliability of removal efficiencies is inherently associated with the applied sampling scheme and therefore must be always seen in this context.

Future research should include hydraulic calibration of wastewater treatment tanks in order to adapt the individual sampling strategy to the given conditions. It was shown that this calibration can be carried out cost-effectively with distinct variation in wastewater conductivity caused e.g. by rainfall events. However, it must be taken into account that removal calculation derived from model simulations is only as accurate as the calibration itself. This, as well as the error propagation of discrete sampling, temporal resolution in terms of composite samples, analytical error and model uncertainty indicates that elimination efficiencies of less than 15-20 % are probably impossible to track in full-scale observations. The alternative consists in the investigation of biodegradation kinetics at lab-scale studies. These can then be extrapolated to full-scale with RTDs as the basis for the hydraulic contact time in the reactors. Indeed, this methodology overcomes sampling uncertainty, with the drawback of transferring lab-scale results to full-scale WWTPs.

5.2 Outlook

Hydraulic characterization of reactor tanks is well understood in environmental engineering and modeling. However, this knowledge should be made available to environmental chemists for future WWTP investigations. Since artificial tracer tests are cost- and work-intensive, recently attempts have been made to simplify these techniques by replacing the tracer substance with routine measurements such as temperature or, as in this thesis, by wastewater conductivity. These findings are promising for the replacement of tracer tests under given flow conditions. Further research is needed to make hydraulic characterization as well as individual sampling strategies a standard procedure for full-scale investigations.

The relationship between microbial activity and xenobiotic removal can be considered as a clear indication for co-metabolism. The latter is understood here as a process by which microorganisms do not gain any advantage in terms of energy or biomass synthesis. It is very likely that the (exo-) enzymes produced by bacteria are also capable of acting on functional groups of xenobiotics due to non-specificity. Xenobiotic molecules which exhibit

structural similarities and functional groups may follow biotransformation pathways analogous to classical organic and inorganic substrates. Compounds with structures different from primary substrates usually processed by a microbial community may be refractory to enzyme cleavage since the ready and abundant arsenal of suitable enzymes is lacking (Schwarzenbach et al., 2003). The enzymatic spectrum can be expected to quickly adapt to dominant substrates and structures in raw wastewaters which is however by definition not applying to micropollutants. Co-metabolism is a well-known phenomenon for decades that was observed in particular for halogenated aromatics and alkanes. Nevertheless, only few studies investigated co-metabolic oxidation processes for the "new" emerging micropollutants so far.

This study was not designed to go beyond sum parameters such as the OUR or X_{bh} and thus cannot provide any insight into the enzyme or species level. However, it raised the question of enzyme variability and differences between microbial community compositions for different activated sludges. While the formation and identification of metabolites received some attention, the question of governing co-metabolic biodegradation processes mediated by enzymes remained unanswered. The key for explaining the differences observed in the biodegradation potential of an activated sludge community must certainly be searched in this context which has not been realized to date. Furthermore, the effect of primary substrates present in raw wastewaters on co-metabolic degradation reactions has only been scarcely investigated. This inevitably leads to the question of how microbial communities evolve and adapt to incoming raw wastewater substrates. Understanding the system catchment – wastewater composition – microbial community – enzyme functionality – xenobiotic biodegradation will not only further refine the prediction of emissions but also help to understand biodegradation pathways and the occurrence of metabolites. The identification of responsible enzymes might also have great potential for novel developments where first attempts have just begun. Although the activated sludge process has been investigated for decades, xenobiotic fate and removal is still recognized as a great challenge by environmental scientists facing the ever-increasing variety of new emerging micropollutants.

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curriculumvitae

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