Active heterotrophic biomass and sludge retention time (SRT) as determining factors for biodegradation kinetics of pharmaceuticals in activated sludge

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A R T I C L E   I N F O
Article history:
Received 28 February 2011
Received in revised form 8 May 2011
Accepted 13 May 2011
Available online 19 May 2011

Keywords:
Pharmaceuticals
Biodegradation
Activated sludge
Active heterotrophic biomass
Sludge retention time

A B S T R A C T
The present study investigates the biodegradation of pharmaceutically active compounds (PhACs) by active biomass in activated sludge. Active heterotrophs (Xbh) which are known to govern COD removal are suggested as a determining factor for biological PhAC removal as well. 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 Xbh and used to describe decreasing PhAC elimination with increasing SRT.

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1. 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 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 kbiodegradation. 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 NH4 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 both at 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 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 were selected as SRT and hydraulic retention time (HRT) was investigated. A PhAC removal capacity with operational process parameters such as variability of the latter between WWTPs. The interrelationship of refinement of biodegradation rate estimations and at explaining variability of biological PhAC removal as well. It aims at contributing to the mass which governs COD removal, suggesting a determining factor for the latter between WWTPs.

2. Methods

2.1. Sampling and bioreactor

Activated sludge aliquots (2.4 L) were decanted into the respirometer at the same day of sampling to estimate the active heterotrophic biomass content. The sludge was left for 8–12 h until it reached the endogenous phase before the experiment was started, to make sure that no residual substrate was present. Autotrophic microorganisms were inhibited during the respirometry experiment by addition of N-allythiourea (concentration c = 10 mg L⁻¹). The amount of active heterotrophic biomass Xₐ was estimated from modeling simulations of the oxygen uptake rate (OUR) responses to defined spikes of sodium acetate (c = 60 g L⁻¹, V_add = 2.5 ml) as presented in details elsewhere (Plattes et al., 2007; Vanrolleghem et al., 1999). Simulations were realized by use of the activated sludge model no. 1 (ASM1) within the wastewater treatment modeling software GPS-X from Hydromantis (Hamilton, Canada). Heterotrophic yields were calculated from theoretical (COD_theoretical = 76.0 mg O₂ L⁻¹) and experimental COD of sodium acetate spikes. Default values were used for the decay rate (b₀ = 0.62 d⁻¹, Henze et al., 2000). The growth of biomass during the experiment was negligible due to the small amounts of sodium acetate added. Subsequently, biodegradation tests described in the following section were performed with the same sludge.

2.2. Estimation of active heterotrophic biomass

Activated sludge aliquots (2.4 L) were decanted into the respirometer at the same day of sampling to estimate the active heterotrophic biomass content. The sludge was left for 8–12 h until it reached the endogenous phase before the experiment was started, to make sure that no residual substrate was present. Autotrophic microorganisms were inhibited during the respirometry experiment by addition of N-allythiourea (concentration c = 10 mg L⁻¹). The amount of active heterotrophic biomass Xₐ was estimated from modeling simulations of the oxygen uptake rate (OUR) responses to defined spikes of sodium acetate (c = 60 g L⁻¹, V_add = 2.5 ml) as presented in details elsewhere (Plattes et al., 2007; Vanrolleghem et al., 1999). Simulations were realized by use of the activated sludge model no. 1 (ASM1) within the wastewater treatment modeling software GPS-X from Hydromantis (Hamilton, Canada). Heterotrophic yields were calculated from theoretical (COD_theoretical = 76.0 mg O₂ L⁻¹) and experimental COD of sodium acetate spikes. Default values were used for the decay rate (b₀ = 0.62 d⁻¹, Henze et al., 2000). The growth of biomass during the experiment was negligible due to the small amounts of sodium acetate added. Subsequently, biodegradation tests described in the following section were performed with the same sludge.

2.3. Biodegradation experiments

The pharmaceuticals carbamazepine, diclofenac, sulfamethoxazole, paracetamol and caffeine (purchased from Dr. Ehrenstorfer GmbH, Germany) were added as a mixed stock solution (c = 1.2 mg L⁻¹ in H₂O, V_add = 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_add = 21.2 ml) at a concentration of COD_theoretical = 736.8 mg O₂ 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

### Table 1

<table>
<thead>
<tr>
<th>CAS</th>
<th>Log Kow</th>
<th>Molecular weight (g mol⁻¹)</th>
<th>Water solubility (mg L⁻¹)</th>
<th>Kd secondary sludge (L kg TSS⁻¹)</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>-0.07</td>
<td>194.19</td>
<td>2.16 x 10⁴</td>
<td>–</td>
<td>Psychostimulant</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>2.45</td>
<td>236.28</td>
<td>112</td>
<td>1.2</td>
<td>Anti-epileptic drug</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.7</td>
<td>296.16</td>
<td>2.37</td>
<td>16</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>0.46</td>
<td>151.17</td>
<td>1.4 x 10⁴</td>
<td>&lt;1</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.89</td>
<td>253.28</td>
<td>610</td>
<td>250</td>
<td>Antibiotic</td>
</tr>
</tbody>
</table>

* SRC Database.
* Jones et al. (2002).
* Ternes et al. (2004).
* Göbel et al. (2005).
taken from the reactor every 30 min over a period of 5 h (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.

2.3.1. 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 end-capped C\(_{18}\) column Gold aQ (100 × 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\(_2\)O/MeOH to 0:100 within 22 min. Limits of quantification were determined experimentally and lay by 50 ng L\(^{-1}\) for all investigated substances.

3. Results and discussion

3.1. WWTP characterization

3.1.1. Layout and operation

Activated sludges from two WWTPs were chosen for biodegradation experiments that differed in size, design and operation (Table 2). WWTP Mamer operates at full capacity with 20,300 population equivalents, an organic loading rate of 0.095 kg BOD kg TSS\(^{-1}\) day\(^{-1}\) and a low SRT of 6 days. In contrast, the organic loading rate in WWTP Boevange is six 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.

3.1.2. Heterotrophic biomass X\(_{bh}\)

The two investigated sludges showed significant differences concerning the amounts of active heterotrophic biomass (Table 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 3 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\(_{st}\)) of X\(_{bh}/\text{TSS}\): 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\(_{st}\). 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 done in modeling approaches. Furthermore, heterotrophic yields differed only slightly with Y\(_{bh} = 0.69 ± 0.02\) mgCOD mgCOD\(^{-1}\) in WWTP Mamer and Y\(_{bh} = 0.61 ± 0.04\) mgCOD mgCOD\(^{-1}\) in WWTP Boevange.

3.2. PhAC biodegradation

3.2.1. Pseudo first-order kinetic parameter estimation

Pseudo first-order reaction kinetics Eq. (1) was applied to describe pharmaceutical removal in batch tests. Thereby, degradation kinetics was assumed to depend on the degradation rate constant k\(_{\text{biol}}\) and the amount of active heterotrophic biomass that is expected to be constant over the duration of the experiment. The biodegradation rate constant k\(_{\text{biol}}\) is derived from fitting the analytical solution of Eq. (1) to the measured data (n = 11) by minimizing chi square with an optimization routine provided in the evaluation software Origin\(^{\text{\textregistered}}\) (Additive) while holding the heterotrophic biomass and the initial concentration constant:

\[
\frac{\Delta C}{\Delta t} = -k_{\text{biol}} \cdot X_{bh} \cdot C_0
\]

where \(\Delta C/\Delta t\) is the reaction rate [ng L\(^{-1}\) h\(^{-1}\)], \(C_0\) is the dissolved pharmaceutical concentration at time t [ng L\(^{-1}\)], t is the time [h], \(k_{\text{biol}}\) is the degradation rate constant [Lg X\(_{bh}\) 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\(^{-1}\)].

3.2.2. 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 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 activated sludge from WWTP Mamer compared to sludge from WWTP Boevange.

Table 2

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Population equivalents</th>
<th>SRT (d)</th>
<th>Average flow(^a) (m(^3) h(^{-1}))</th>
<th>HRT(^b) (h)</th>
<th>Capacity utilization (%)</th>
<th>Sludge load(^c) (kg BOD kg TSS(^{-1}) day(^{-1}))</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP Mamer</td>
<td>20,300</td>
<td>6</td>
<td>136 ± 54</td>
<td>16.7 ± 3.7</td>
<td>100</td>
<td>0.095 ± 0.022</td>
<td>Primary Clarifier/Denitrification/Aerated Tanks/Secondary Clarifier</td>
</tr>
<tr>
<td>WWTP Boevange</td>
<td>2700</td>
<td>54</td>
<td>65 ± 6</td>
<td>58.4 ± 6.6</td>
<td>20</td>
<td>0.016 ± 0.005</td>
<td>Denitrification/Aerated Tanks/Secondary Clarifier</td>
</tr>
</tbody>
</table>

\(^a\) During dry weather conditions.

\(^b\) Calculated from flow through and tank volume; single pass.

\(^c\) Calculated from daily BOD and TSS values (n = 36).
(Fig. 1a–d) 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 $k_{\text{biol}}$ is close to that of sulfamethoxazole.

### Table 3

Estimation of active heterotrophic fractions $f_a$ 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.

<table>
<thead>
<tr>
<th>TSS (g L$^{-1}$)</th>
<th>$X_{bh}$ (g L$^{-1}$)</th>
<th>Fraction $f_a$ (%)</th>
<th>$Y_H$ (mgCOD mgCOD$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP Mamer</td>
<td>2.4 ± 0.3</td>
<td>1.5 ± 0.1</td>
<td>62.9 ± 5.8</td>
</tr>
<tr>
<td>WWTP Boevange</td>
<td>2.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>25.2 ± 6.3</td>
</tr>
</tbody>
</table>

### Table 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: $n = 11$.

<table>
<thead>
<tr>
<th>Degradation rate constant (L g$^{-1}$ X$^{-1}$ h$^{-1}$)</th>
<th>WWTP Mamer</th>
<th>$r^2$</th>
<th>WWTP Boevange</th>
<th>$r^2$</th>
<th>Ratio (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>0.007 ± 0.001$^a$</td>
<td>0.81</td>
<td>0.010 ± 0.001$^b$</td>
<td>0.78</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.029 ± 0.002</td>
<td>0.87</td>
<td>0.025 ± 0.002</td>
<td>0.82</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.307 ± 0.022</td>
<td>0.94</td>
<td>0.245 ± 0.014</td>
<td>0.89</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>1.654 ± 0.181</td>
<td>0.97</td>
<td>0.415 ± 0.034</td>
<td>0.89</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>Caffeine</td>
<td>2.030 ± 0.185</td>
<td>0.98</td>
<td>1.500 ± 0.147</td>
<td>0.92</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
</table>

$^a$ Not significant.
Suggesting heterotrophs to be governing, the \( k_{\text{biol}} \) of pseudo first-order kinetics would result in identical values in both sludges and hence in a ratio of 1 since \( k_{\text{biol}} \) is directly proportional to \( X_{\text{bh}} \) (Eq. (1)). As it can be seen from Table 4, the differences in xenobiotic degradation efficiency can be largely explained by \( X_{\text{bh}} \) for 4 of the 5 substances considered. In fact, the ratios of the kinetic rate constants \( k_{\text{biol, Mamer}}/k_{\text{biol, Boevange}} \) range around 1 except for paracetamol (ratio = 4.0 ± 0.8). As mentioned above, its \( k_{\text{biol}} \) is unexpectedly low in WWTP Boevange.

### 3.3. 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 \( X_{\text{bh}} \) are usually found at low SRTs. The relation between the active heterotrophic fraction \( f_{\text{at}} \) and SRT has been described as follows (Ekama and Wentzel, 2008):

\[
f_{\text{at}} = f_{\text{at}} \left( \frac{1 - f_{\text{at}} \cdot b_{\text{h}} \cdot \text{SRT}}{1 + f_{\text{at}} \cdot b_{\text{h}} \cdot \text{SRT} + f_{\text{sup/(1-SRT-fsup/fat)}}} \right)
\]

where \( f_{\text{at}} \) is the fraction of active heterotrophs in TSS, \( f_{\text{fi}} \) is the VSS/TSS ratio of activated sludge, \( b_{\text{h}} \) = endogenous residue fraction, \( b_{\text{e}} \) = heterotrophic decay rate (d⁻¹), \( \text{SRT} \) = sludge retention time [d], \( f_{\text{cu}} \) = COD/VSS ratio (mg COD mg VSS⁻¹), \( Y_{\text{hv}} \) = yield coefficient (mg VSS mg COD⁻¹), \( f_{\text{sup}} \) = fraction of non-biodegradable particulate COD, \( f_{\text{sus}} \) = fraction of non biodegradable soluble COD. The amount of \( X_{\text{bh}} \) in [g L⁻¹] can be estimated from:

\[
X_{\text{bh}} = f_{\text{at}} \cdot \text{TSS}
\]

With regard to the tested sludges, faster PhAC removal was observed for the activated sludge with the higher fraction of \( X_{\text{bh}} \) and lower SRT (Fig. 1a-d). By substituting Eqs. (2) and (3) for \( X_{\text{bh}} \) in pseudo first order kinetics Eq. (1), the PhAC elimination can be described as a function of the SRT given that \( X_{\text{bh}} \) is the determining factor:

\[
\frac{\Delta C}{\Delta t} = k_{\text{biol}} \cdot f_{\text{at}} \left( \frac{1 - f_{\text{at}} \cdot b_{\text{h}} \cdot \text{SRT}}{1 + f_{\text{at}} \cdot b_{\text{h}} \cdot \text{SRT} + f_{\text{sup/(1-SRT-fsup/fat)}}} \right) \cdot \text{TSS} \cdot C_0
\]

To apply this approach to the two selected WWTPs, typical wastewater characteristics for raw and settled wastewater were taken from Ekama and Wentzel (2008) (App. B). For elimination calculations, Eq. (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 \( k_{\text{biol}} \) and TSS were taken from Tables 3 and 4. A rate constant of \( k_{\text{biol}} = 0.001 \text{ L g}^{-1} \text{X}_{\text{bh}}^{-1} \text{ h}^{-1} \) was used for carbamazepine since its removal in the batch tests was not significantly different from zero. The biodegradation rate constants were assumed to be representative of full-scale plants.

Predicted elimination efficiencies and \( f_{\text{at}} \) as a function of the SRT can be seen in Fig. 2(a,b). The inert fraction is complementary to \( f_{\text{at}} \) and therefore increases with increasing SRT. The model matches well the measured \( f_{\text{at}} \) 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 carbamaz-
tent 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 pharmaceutical 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 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.

### 4. 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 SRT 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

This study was financed by The Luxembourg Research Fund (FNR) within AFR 07/017 and the DomesticPest Project funded by The Ministry of the Interior and the Greater Region, Luxembourg. We would like to thank the Water Syndicate SIDERO for their cooperation.

### Appendix A

See Table A.1

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### Table A.1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Unit</th>
<th>WWTP Boevange (raw wastewater)</th>
<th>WWTP Mamer (settled wastewater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudo first-order reaction constant</td>
<td>k_{biol}</td>
<td>h⁻¹</td>
<td>See Table 4</td>
<td>See Table 4</td>
</tr>
<tr>
<td>VSS/TSS ratio of activated sludge</td>
<td>f_s</td>
<td>mg VSS mg TSS⁻¹</td>
<td>0.75</td>
<td>0.83</td>
</tr>
<tr>
<td>Endogeneous residue fraction</td>
<td>f_e</td>
<td>–</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Endogeneous respiration rate</td>
<td>b</td>
<td>h⁻¹</td>
<td>0.1 (0.24)ᵇ</td>
<td>0.2 (0.24)ᵇ</td>
</tr>
<tr>
<td>COD/VSS ratio</td>
<td>f_v</td>
<td>mg COD mg VSS⁻¹</td>
<td>1.48</td>
<td>1.48</td>
</tr>
<tr>
<td>Yield coefficient</td>
<td>Y</td>
<td>mg COD mg COD⁻¹</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Non biodegradable particulate COD</td>
<td>f_{mp}</td>
<td>–</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Non biodegradable soluble COD</td>
<td>f_{ms}</td>
<td>–</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>Total suspended solids</td>
<td>TSS</td>
<td>g L⁻¹</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Hydraulic retention time</td>
<td>t</td>
<td>h</td>
<td>29.2 ± 2.7</td>
<td>7.3 ± 3.5</td>
</tr>
</tbody>
</table>

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.
References


