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

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Abstract

Biodegradation rates of pharmaceutical active compounds (PhACs) in activated sludge systems are usually determined in lab-scale experiments where biomass is a key parameter. The latter is often addressed by lumped parameters such as total suspended solids (*TSS*). However, only active microorganisms mediate PhAC breakdown. In this context, this study focused on active heterotrophs (X_{bh}) that govern COD removal suggesting a potential determining factor for biological PhAC removal as well. The biodegradation of five polar PhACs was investigated in two wastewater treatment plants

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that differed clearly in size, operation and sludge retention time (SRT).

Results showed that fractions of X_{bh} / *TSS* differed significantly between the two sludges indicating that *TSS* does not reveal information about heterotrophic activity. Moreover, PhAC removal was clearly faster in presence of high amounts of heterotrophs and a low SRT. Pseudo first-order kinetics modified with X_{bh} was used to describe decreasing PhAC elimination with increasing SRT.

Keywords: Pharmaceuticals, Biodegradation, Activated Sludge, Active Heterotrophic Biomass, Sludge Retention Time

1. Introduction

The removal of pharmaceutically active compounds (PhAC) during wastewater treatment became 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 is easily accessible from WWTP routine measurements. Herewith, pseudo first-order reaction kinetics is proposed to describe PhAC removal being governed by biomass and the biodegradation rate constant k_{biol} (Maurer et al., 2007; Wick et al. 2009). However, a major drawback of utilizing *TSS* is that only a fraction of suspended solids can be considered as viable biomass while an inert fraction may also be present (Cronje et al., 2002). Only the viable fractions are responsible for removal processes

and hence rates should be expressed in terms of the active biomass. While this has been successfully achieved e.g. for COD and NH_4^+ transformation 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, recent research suggested slow growing specialized bacteria and broadened enzyme spectra to enhance PhAC removal. These bacteria are believed to be prevented from wash-out by growth in WWTPs that operate at above a critical sludge retention time (SRT) of 10 days (referred to 10 °C) (Clara et al. 2005). This concept corresponds to the implementation of nitrifiers in biological wastewater treatment and has been thereupon adopted for PhAC (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 SRT suggesting that heterotrophic bacteria being capable of degrading PhAC are present both at low and high SRTs. Moreover, biodegradation rates of aminopolycarboxylic acids 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 the SRT and the microbial community structure and thus 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 that governs COD removal suggesting a potential determining factor for biological PhAC removal as well. It aims at contributing to refine biodegradation rate estimation and at explaining variability of the latter between WWTPs. The interrelationship with operational process parameters such as SRT and hydraulic retention time (HRT) was investigated. A spectrum of five different hydrophilic pharmaceuticals was chosen that covers a variety of molecular structures containing heterocyclic and aromatic rings with different functional groups (Table 1). The selected substances carbamazepine (CBZ), diclofenac (DCF), sulfamethoxazole (SMX) and paracetamol (PCT) as well as caffeine (CAF) have been detected widely in concentrations up to the µg L^{-1} level in wastewater influents (Heberer, 2002; Zhang et al., 2008). They are also covering a range from persistent to easily biodegradable chemicals. Partitioning of the investigated compounds to biomass particles by adsorption is usually not significant in the overall elimination (Ternes et al., 2004).

PhAC biodegradation kinetics were determined in activated sludge from two Luxembourg WWTPs that differed largely in size, HRT, SRT and sludge loading 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. The question was raised whether pharmaceutical attenuation can be attributed to heterotrophic activity and therewith linked to process parameters.

➔ ***Table 1***

2. Materials and Methods

2.1 Sampling & Bioreactor

Activated sludge (2 x 20 L) were taken from the aerated tanks of the two Luxembourg WWTPs studied, Mamer and Boevange, in May 2009. Aliquots (2.4 L) were used for a given experiment in the respirometer. 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 probes from Hach-Lange, was automatically maintained between 3 and 6 mg O_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 * 10^{-3} min^{-1}).

2.2 Estimation of Active Heterotrophic Biomass

Activated sludge aliquots (2.4 L) were decanted into the respirometer the same day of sampling to estimate the active heterotrophic biomass content. The sludge was set to endogenous phase for 8-12 h before the start of the experiment to make sure that no residual substrate was present. Autotrophic microorganisms were inhibited during the

respirometry experiment by addition of N-allylthiourea (concentration c = 10 mg L^{-1}). The amount of active heterotrophic biomass X_{bh} was estimated from modeling simulations of the oxygen uptake rate (OUR) responses to defined spikes of sodium acetate (c = 60 g L^{-1}, volume added v = 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 ($COD_{theoretical}$ = 70.6 mg O_2 L^{-1}) and experimental COD of sodium acetate spikes. Default values were used for the decay rate (b_h = 0.62 d^{-1}). 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 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 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 mg O_2 L^{-1} thus avoiding nitrogen or phosphorus from becoming

limiting factors. This amount ensured that the synthetic primary substrate was permanently present in excess during the period of the biodegradation test (5-6 h) being 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.

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 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.

3. Results

3.1 WWTP Characterization

3.1.1. Layout & Operation

Activated sludges from two WWTPs were chosen for biodegradation experiments that differed largely in design and operation (Table 2). WWTP Mamer operates at full capacity with 20'300 population equivalents, a sludge load of 0.095 kg BOD kg TSS^{-1} d^{-1} and a low SRT of 6 days. In contrast, the sludge load in WWTP Boevange is 6 times lower. In this plant the SRT was found to be 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.

➔ ***Table 2***

3.1.2. Heterotrophic Biomass X_{bh}

The two investigated sludges showed significant differences regarding the amounts of active heterotrophic biomass (Table 3). It was found to be equal to 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) and varied only very little during the three week measurement period in both plants. 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 sludge loading. Their activity can be expected to adapt to the available substrates and thus to vary significantly between WWTPs (Lemmer et al., 1994). This is also suggested by

data of seven Luxembourg WWTPs revealing increased heterotrophic biomass activity at higher sludge loading (App. A).

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 resulting in more inert particles entering the reactor tanks than in Mamer and contributing 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 that hold various microorganisms together building flocs (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 were found to differ only slightly with Y_H = 0.69 ± 0.02 in WWTP Mamer and Y_H = 0.61 ± 0.04 in WWTP Boevange.

➔ ***Table 3***

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_{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. 1) to the measured data (n=11) by minimizing chi square with an optimization routine provided in the evaluation software Origin® (Additive) while holding the heterotrophic biomass and the initial concentration constant:

Pseudo first-order: $$\frac{\Delta c_t}{\Delta t} = -k_{biol} \cdot X_{bh} \cdot C_0 \quad (1)$$

where $\Delta C_t / \Delta t$ is the reaction rate [ng L^{-1} h^{-1}], C_t is the pharmaceutical concentration at time t [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 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 is remarkable 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 (Figure 1a-d) for identical experimental conditions. The removal of carbamazepine was not significant in both sludges considering the standard deviation of

three replicates (Figure 1e). 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. In sludge from WWTP Boevange its k_{biol} is close to that of sulfamethoxazole.

Suggesting heterotrophs to be governing the removal, 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. 1). As it can be seen from Table 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.

➔ ***Figure 1a.tif***

➔ ***Figure 1b.tif***

➔ ***Figure 1c.tif***

➔ ***Figure 1d.tif***

➔ ***Figure 1e.tif***

➔ ***Table 4***

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

$$f_{at} = f_i \left(\frac{1}{1 + f_h \cdot b_h \cdot SRT + \frac{f_{sup} \cdot (1 + b_h \cdot SRT)}{f_{cv} \cdot Y_{Hv} \cdot (1 - f_{sup} - f_{sus})}} \right) \quad (2)$$

where f_{at} = fraction of active heterotrophs in *TSS*, f_i = VSS/TSS ratio of activated sludge; f_h = endogenous residue fraction, b_h = heterotrophic decay rate (d^{-1}), *SRT* = sludge retention time [d], f_{cv} = COD/VSS ratio (mg COD mg VSS^{-1}), Y_{Hv} = yield coefficient (mg VSS mg COD^{-1}), f_{sup} = fraction of non biodegradable particulate COD, f_{sus} = fraction of non biodegradable soluble COD. The amount of X_{bh} can be estimated from:

$$X_{bh} = f_{at} \cdot TSS \qquad [g\ L^{-1}] \quad (3)$$

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 1a-e). By substituting Eq. 2 and 3 for X_{bh} in pseudo first order kinetics (Eq. 1), the PhAC elimination can be described as a function of the SRT given that X_{bh} is the determining factor:

$$\frac{\Delta C}{\Delta t} = k_{biol} \cdot \mathrm{f_i} \left(\frac{1}{1 + f_h \cdot b_h \cdot SRT + \frac{f_{sup} \cdot (1 + b_h \cdot SRT)}{f_{cv} \cdot Y_{Hv} \cdot (1 - f_{sup} - f_{sus})}} \right) \cdot TSS \cdot C_s \quad (4)$$

To apply this approach to the two selected WWTPs, typical wastewater characteristics for raw and settled wastewater were taken from Ekama & Wentzel (2008) (App. B). The heterotrophic decay rate b_h was corrected for temperature. 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 by using for instance the hydraulic residence time distributions as input. Biodegradation rate constants k_{biol} and TSS were taken from Table 3 and 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 2a-b. The inert fraction is complementary to f_{at} and therefore increases with increasing SRT. The model matches well the measured f_{at} of WWTP 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 since it is biologically persistent. 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.

➔ ***Figure 2a.tif***

➔ ***Figure 2b.tif***

Although WWTP Boevange has a clearly lower f_{at}, significantly higher removal performances are obtained. This is a result of the different HRT 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} resulting in even higher overall removal efficiencies. The SRT can be used as a proxy parameter for heterotrophic activity and PhAC removal capability. The eventual removal efficiency is nevertheless strongly influenced by the residence time, i.e. the HRT. Both SRT and HRT are governed by plant design and loading with very limited room of maneuver for tuning once the plant has been build. Our 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, the processing proxies can be used to estimate xenobiotic emission data

4. Discussion

Results suggested heterotrophs are 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 persistent carbamazepine. This is consistent with the fact that increasing heterotrophic biomass fractions are linked to decreasing SRTs and thus highest active fractions of X_{bh} occur at low SRTs. Microbial communities evolve according to the prevailing environmental conditions and depend therefore largely on the incoming wastewater composition also referred to as sludge loading. 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 much higher removal 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 an indicator 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 these 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 constantly eliminated up to 100 % independently of the SRT. The persistence of carbamazepine is also not linked to the sludge age. With regard to intermediate biodegradable substances, it remains unclear why reported enhanced removal at higher SRTs was only observed for some individual but not all compounds, e.g. for sulfamethoxazole (Schaar et al., 2010). No differences were found for sulfamethoxazole elimination at SRTs of 2 d and 17 d, respectively. These results are in conflict with the findings of this study where sulfamethoxazole was better removed at the lower SRT (6 d).

Apart from that, biodegradation potential for other substances (e.g. triclosan, bisphenol A) was also attributed to ammonia oxidizing bacteria besides heterotrophs (Roh et al. 2009). Hence, further investigation on a larger sample of sludges and substances is needed to better understand the role of heterotrophs for xenobiotic degradation capabilities.

5. 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 lower active biomass. In consideration of the HRT, the total removal of carbamazepine as well as caffeine and paracetamol was not affected by 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 potentials and to evaluate their impact as point sources for receiving waters.

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Appendix A

➔ ***Figure A.1.tif***

Appendix B

➔ ***Table B.1***

Table Captions

Table 1. Molecular Structures, physico-chemical properties and application of the selected compounds.

Table 2. General information on the investigated WWTPs.

Table 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.

Table 4. Pseudo first-order biodegradation rates constants of carbamazepine, diclofenac, sulfamethoxazole, paracetamol and caffeine; calculated using the amount of active heterotroph biomass; data points per fit: n = 11.

Table B.1. Wastewater parameters and mean hydraulic retention time (single pass) used for WWTP Boevange (raw wastewater) and WWTP Mamer (settled wastewater).

Table 1. Molecular Structures, physico-chemical properties and application of the selected compounds

	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	$2.16 * 10^4$	-	→***Figure Structure 1.tif***	Psychostimulant
Carbamazepine	298-46-4	2.45	236.28	112	1,2[c]	→***Figure Structure 2.tif***	Anti-epileptic drug
Diclofenac	15307-79-6	0.7[b]	296.16	2.37	16[c]	→***Figure Structure 3.tif***	Non-steroidal *anti-inflammatory* drug
Paracetamol	103-90-2	0.46	151.17	$1.4 * 10^4$	< 1[c]	→***Figure Structure 4.tif***	Non-steroidal *anti-inflammatory* drug
Sulfamethoxazole	723-46-6	0.89	253.28	610	260[d]	→***Figure Structure 5.tif***	Antibiotic

[a] SRC Database

[b] Jones et al., 2002

[c] Ternes et al., 2004

[d] Göbel et al., 2005

Table 2. General information on the investigated WWTPs

	Population Equivalents	SRT [d]	Average Flow[a] [m^3 h^{-1}]	HRT[b] [h]	Capacity Utilization [%]	Sludge Load[c] [kg BOD kg TSS^{-1} d^{-1}]	Treatment
WWTP Mamer	20'300	6	136 ± 54	16.7 ± 3.7	100	0.095 ± 0.022	Primary Clarifier / Denitrification / Aerated Tanks / Secondary Clarifier
WWTP Boevange	2'700	54	65 ± 6	58.4 ± 6.6	20	0.016 ± 0.005	Denitrification / Aerated Tanks / Secondary Clarifier

[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

Table 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.

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

Table 4. Pseudo first-order biodegradation rates constants of carbamazepine, diclofenac, sulfamethoxazole, paracetamol and caffeine; calculated using the amount of active heterotroph biomass; data points per fit: n = 11.

	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

[a] not significant

Table B.1. Wastewater parameters and mean hydraulic retention time (single pass) used for WWTP Boevange (raw wastewater) and WWTP Mamer (settled wastewater).

Parameter	Symbol	Unit	WWTP Boevange (raw wastewater)	WWTP Mamer (settled wastewater)
Pseudo first-order reaction constant	k_{biol}	h^{-1}	see Tab.4	see Tab.4
VSS/TSS ratio of activated sludge[a]	f_i	mg VSS mg TSS^{-1}	0.75	0.83
Endogeneous residue fraction[a]	f_h	-	0.2	0.2
Endogeneous respiration rate[a]	b_h	h^{-1}	0.1 (0.24) [b]	0.2 (0.24) [b]
COD/VSS ratio[a]	f_{cv}	mg COD mg VSS^{-1}	1.48	1.48
Yield coefficient[a]	Y_{Hv}	mg COD mg COD^{-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

[a] Ekama & Wentzel, 2008

[b] corrected for temperature, standard value at 20 °C in brackets

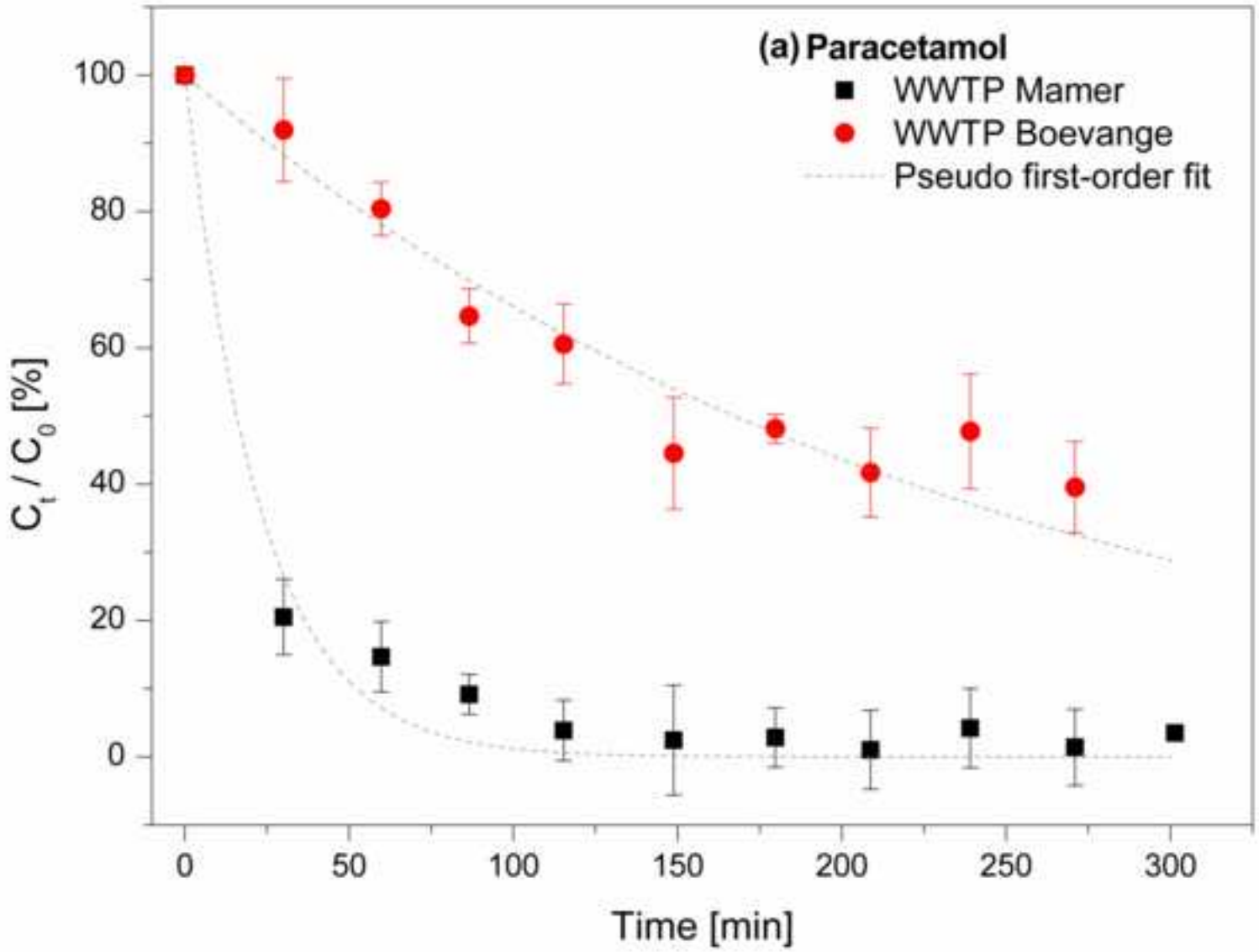
[c] calculated from flow through and tank volume of aerated treatment of daily average values of three weeks

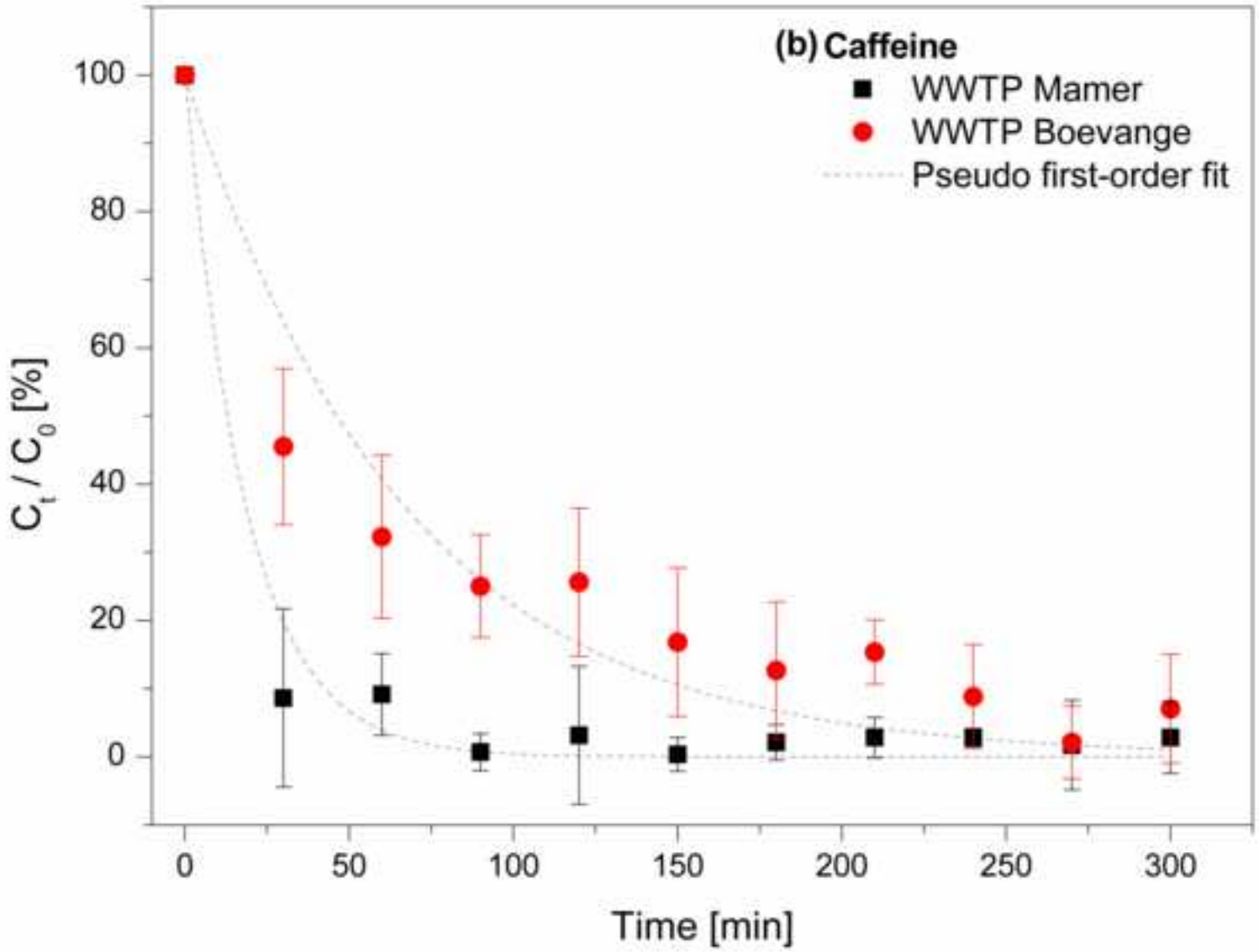
Figure Captions

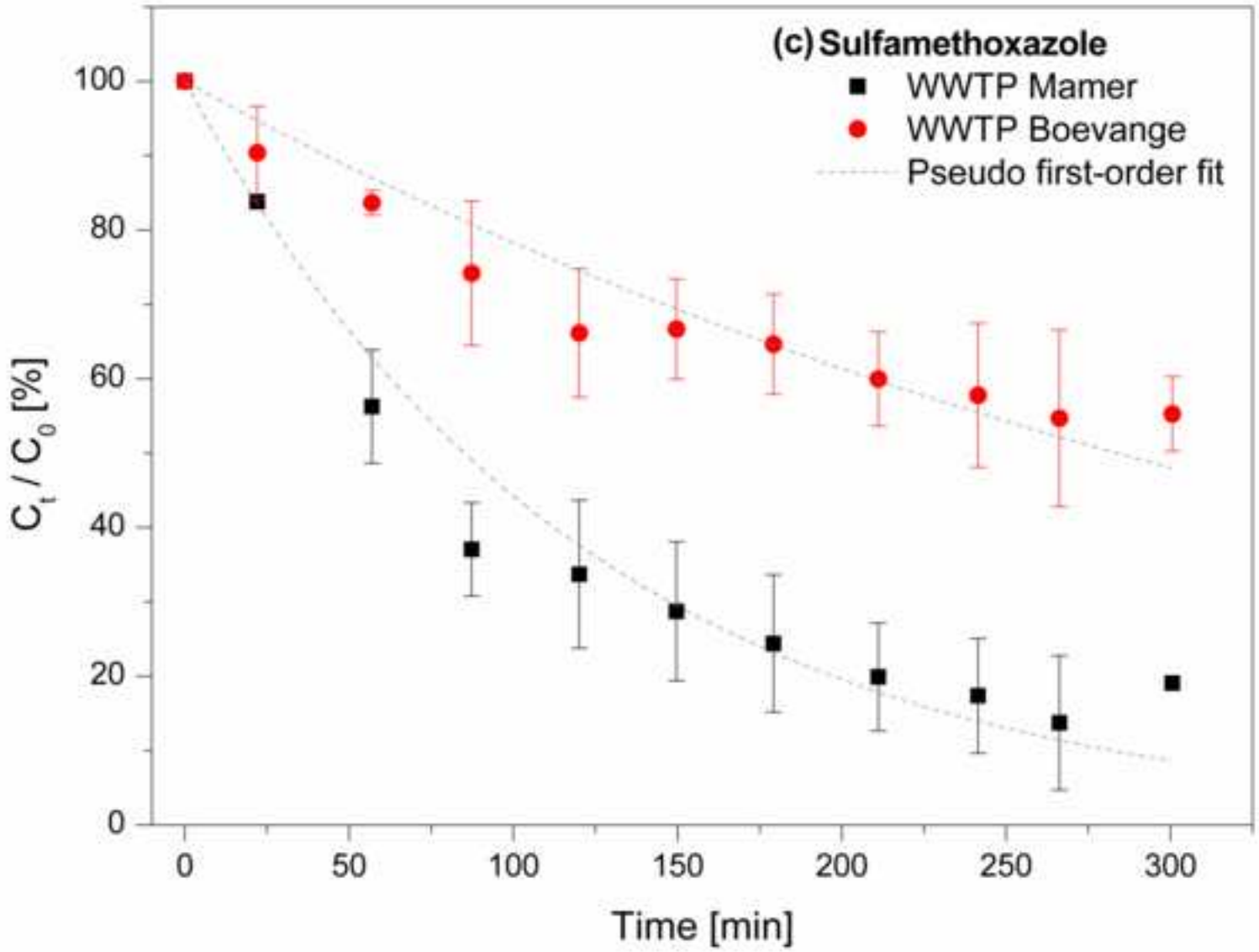
Figure 1a-e. Biodegradation and pseudo first-order fits of caffeine, paracetamol, sulfamethoxazole, diclofenac and carbamazepine; number of replicates n = 3; error bars indicate one standard deviation.

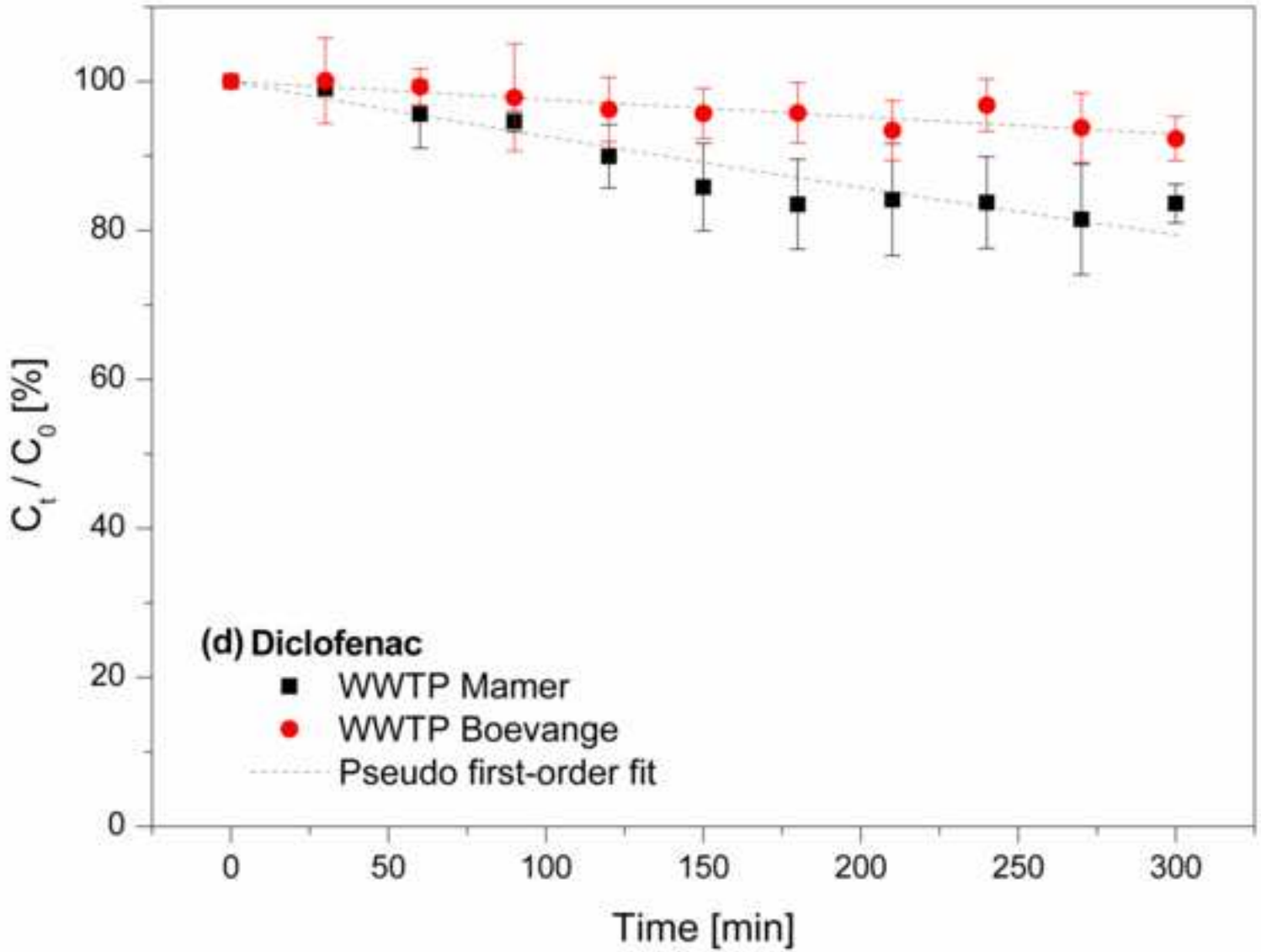
Figure 2a-b. Predicted active and inert heterotrophic fractions and elimination efficiencies of caffeine (CAF), paracetamol (PCT), sulfamethoxazole (SMX), diclofenac (DCF) and carbamazepine (CBZ) as a function of the sludge retention time in WWTP Mamer (a), HRT = 7.3 ± 3.5 h, and WWTP Boevange (b), HRT = 29.2 ± 2.7.

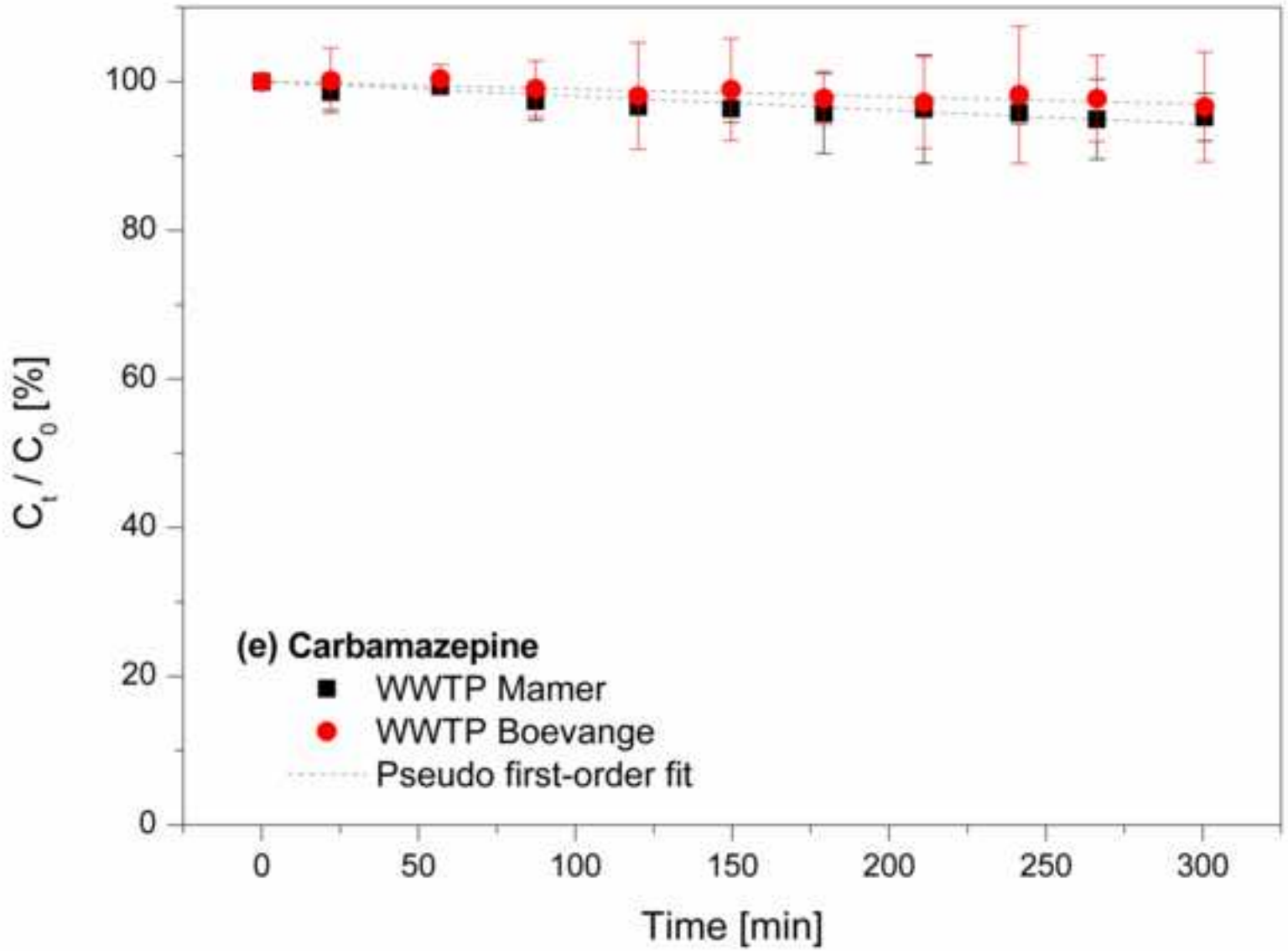
Figure A.1. Relationship between sludge load and the heterotrophic biomass in 7 Luxembourg WWTP; calculated by monthly average BOD values.

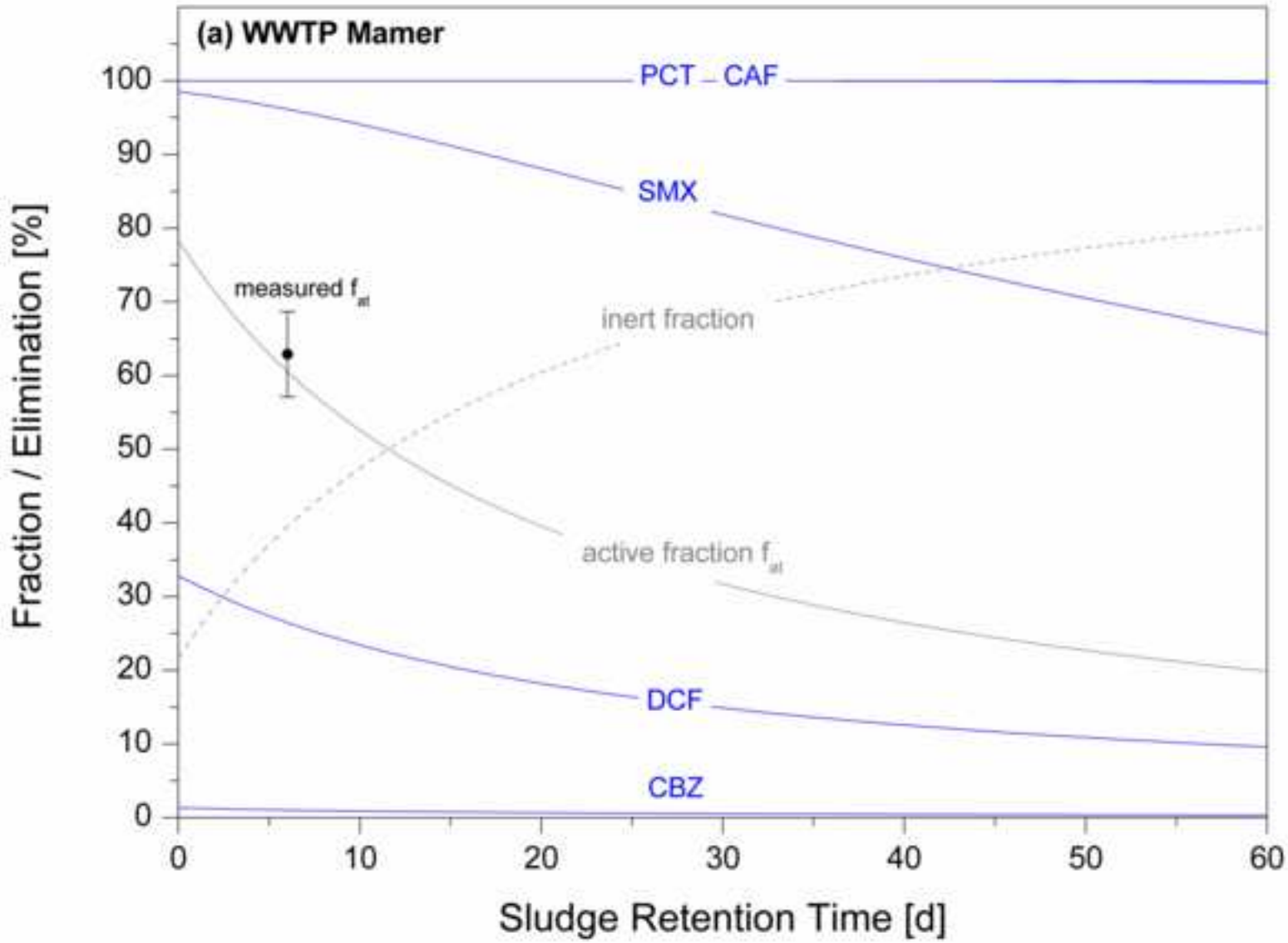

(a) Paracetamol
WWTP Mamer
WWTP Boevange
Pseudo first-order fit
C_t / C_0 [%]
100
80
60
40
20
0
0
50
100
150
200
250
300
Time [min]

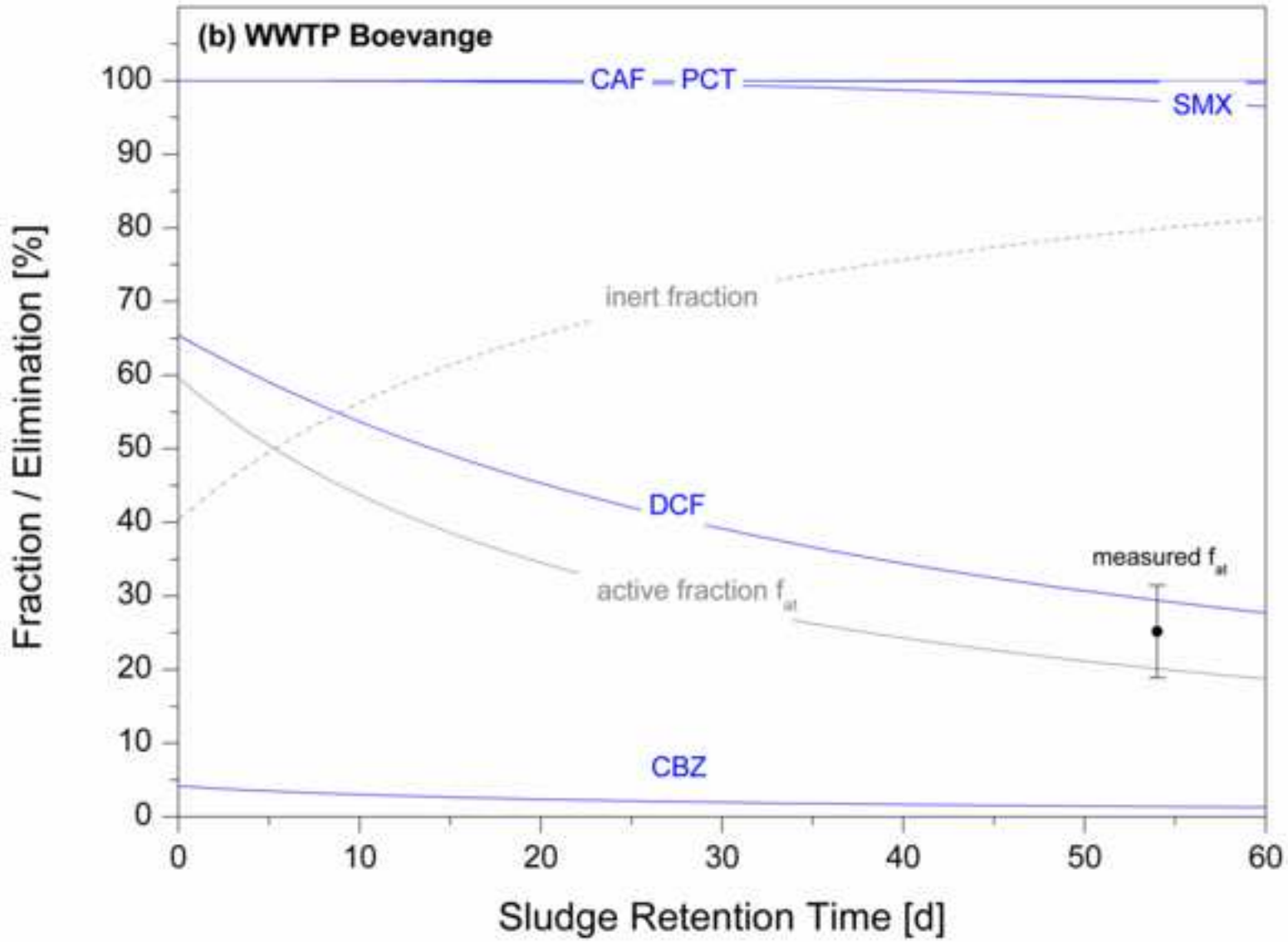

(b) Caffeine
WWTP Mamer
WWTP Boevange
Pseudo first-order fit
C_t / C_0 [%]
100
80
60
40
20
0
0
50
100
150
200
250
300
Time [min]

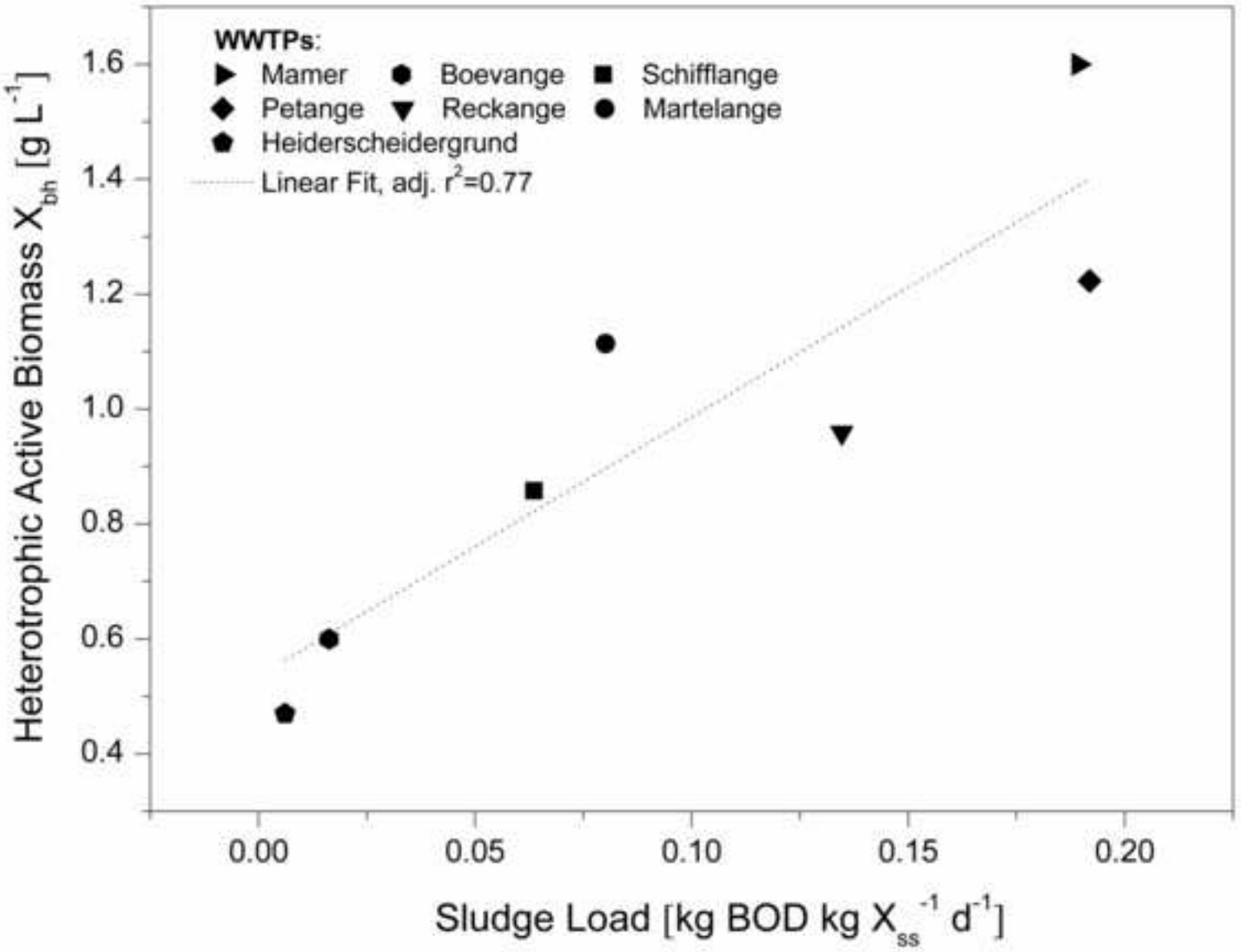

(c) Sulfamethoxazole
WWTP Mamer
WWTP Boevange
Pseudo first-order fit
C_t / C_0 [%]
Time [min]
0
20
40
60
80
100
0
50
100
150
200
250
300

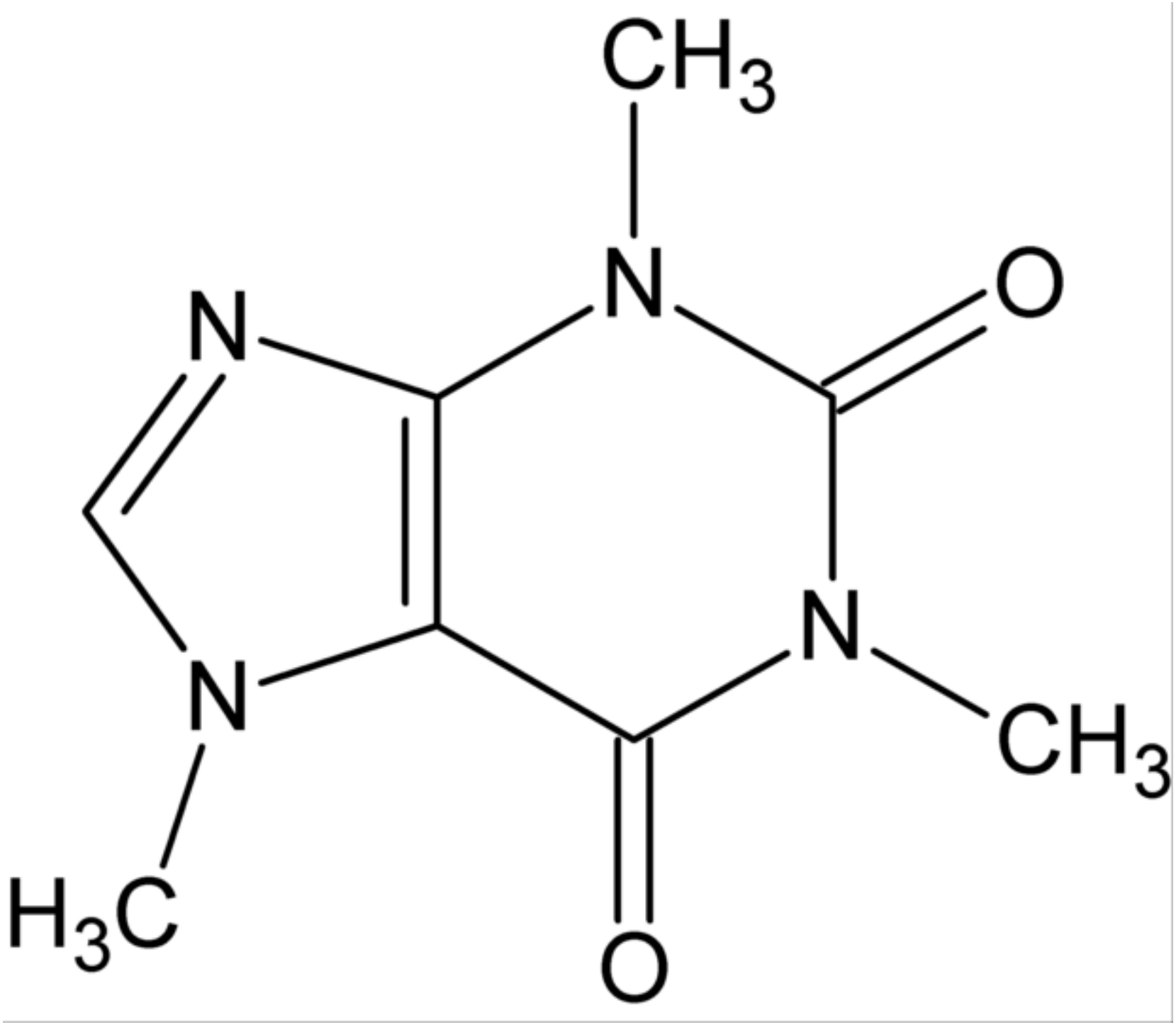

100
80
60
40
20
0
C_t / C_0 [%]
0
50
100
150
200
250
300
Time [min]
(d) Diclofenac
WWTP Mamer
WWTP Boevange
Pseudo first-order fit

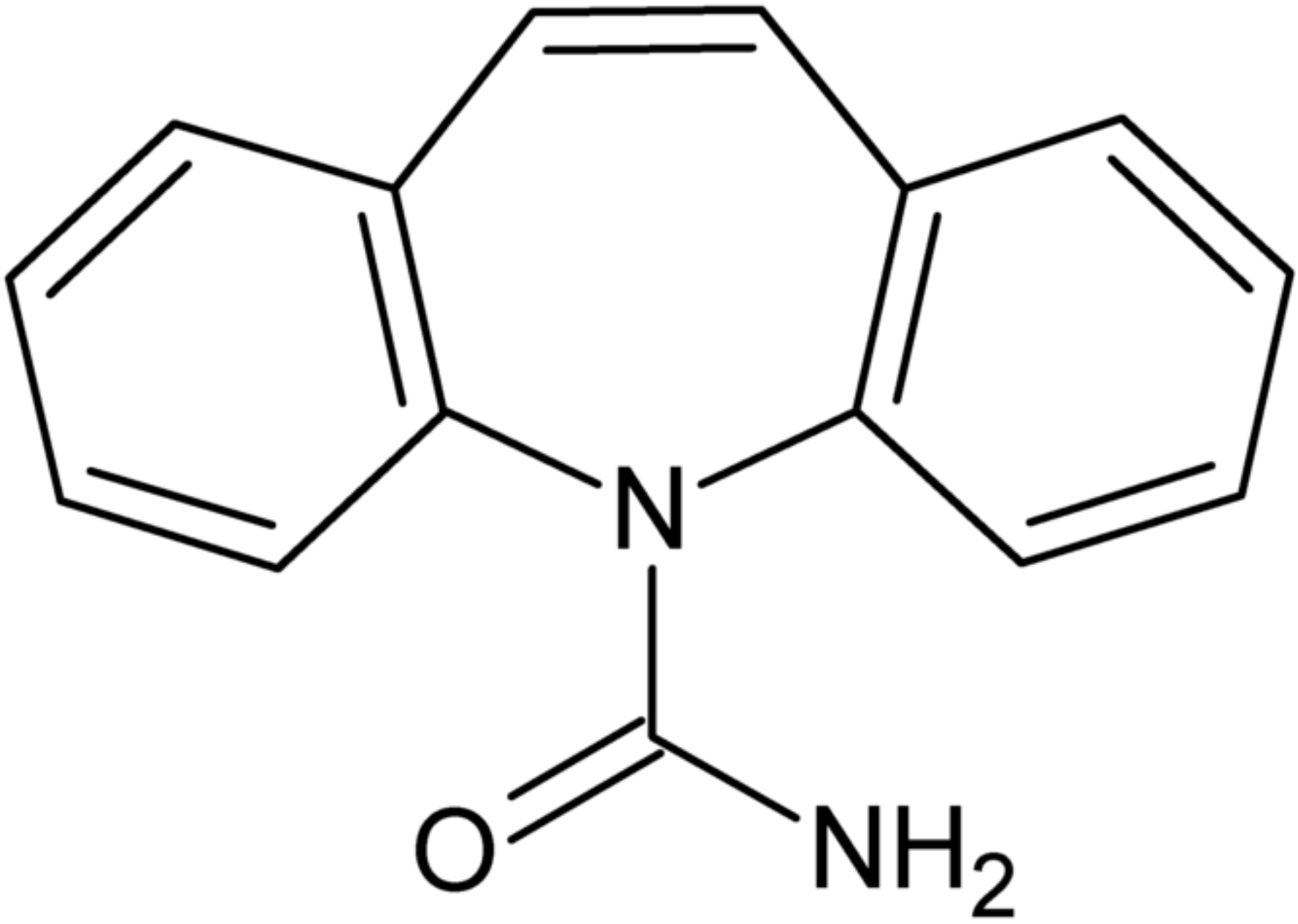

100
80
60
40
20
0
C_t / C_0 [%]
0
50
100
150
200
250
300
Time [min]
(e) Carbamazepine
WWTP Mamer
WWTP Boevange
Pseudo first-order fit

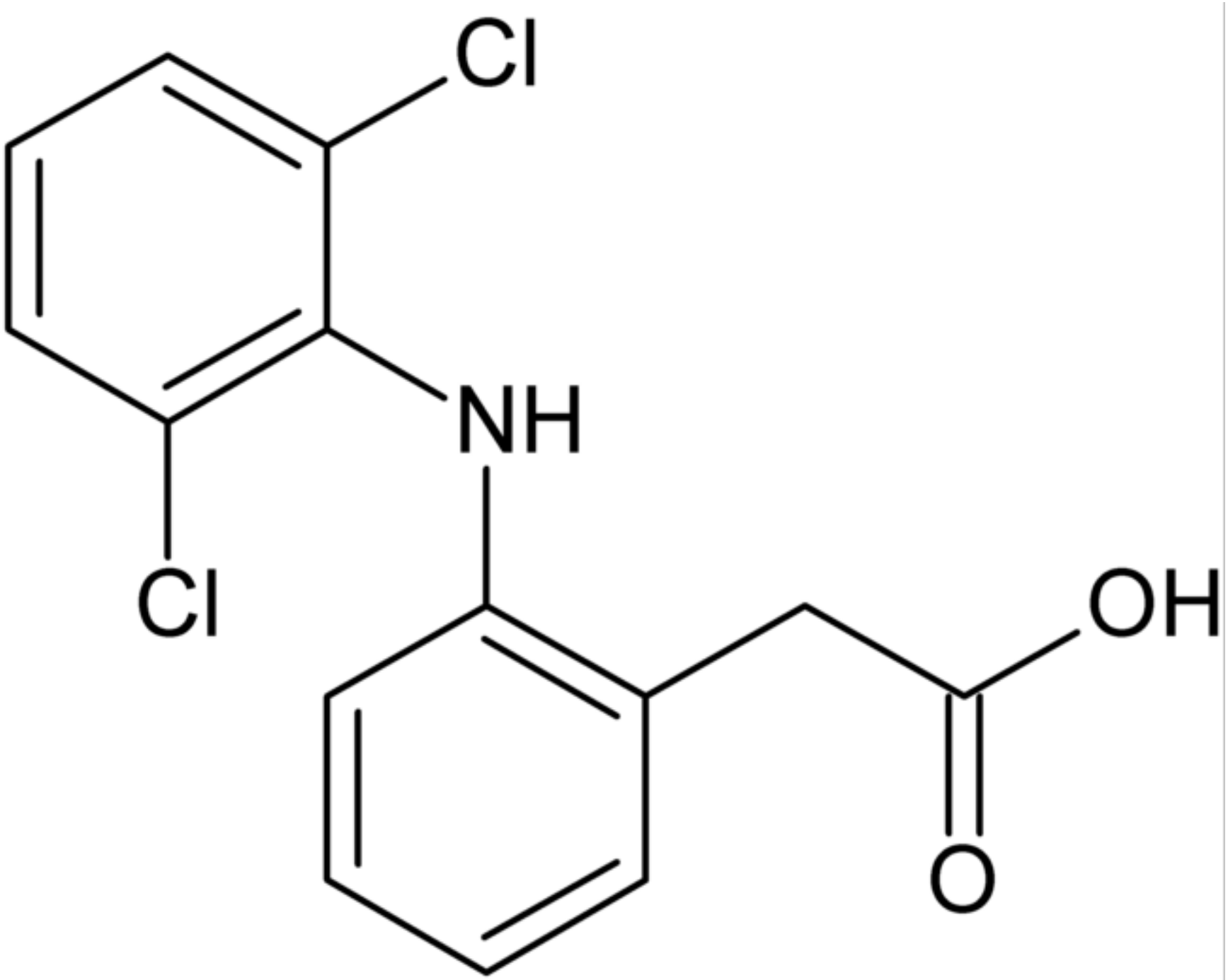

(a) WWTP Mamer
PCT – CAF
SMX
measured f_{at}
inert fraction
active fraction f_{at}
DCF
CBZ
Fraction / Elimination [%]
Sludge Retention Time [d]
0
10
20
30
40
50
60
70
80
90
100

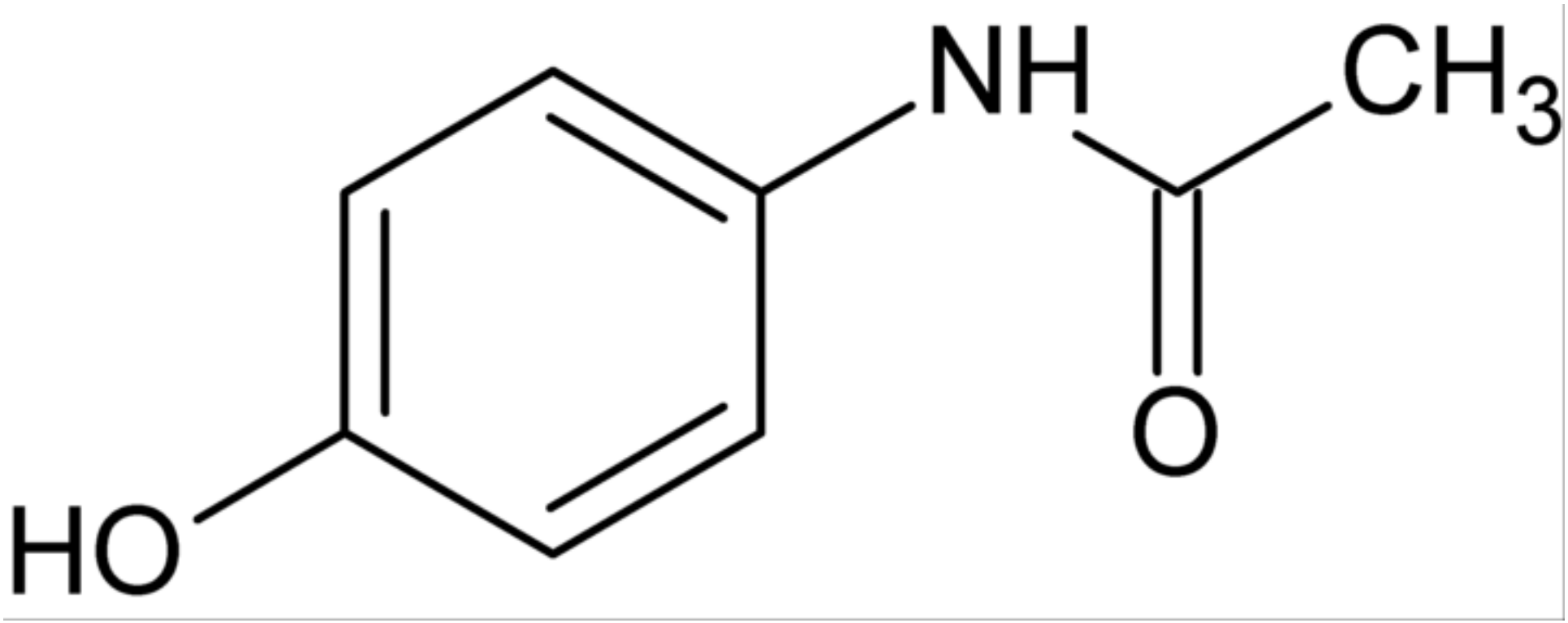

(b) WWTP Boevange
CAF
PCT
SMX
inert fraction
DCF
measured f_at
active fraction f_at
CBZ
Fraction / Elimination [%]
Sludge Retention Time [d]
0
10
20
30
40
50
60
70
80
90
100

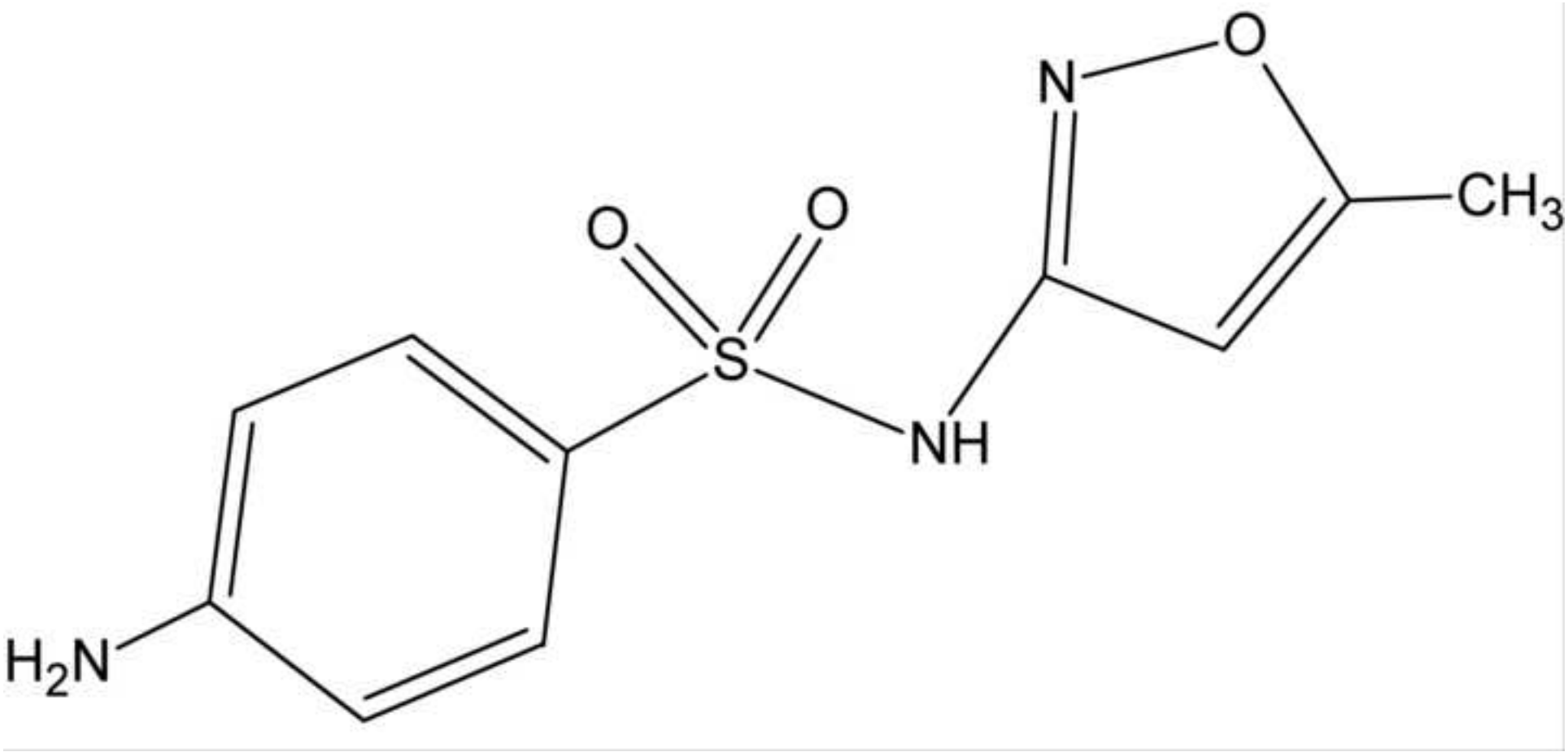

WWTPs:
Mamer
Boevange
Schifflange
Petange
Reckange
Martelange
Heiderscheidergrund
Linear Fit, adj. r²=0.77
Heterotrophic Active Biomass X_bh [g L⁻¹]
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.00
0.05
0.10
0.15
0.20
Sludge Load [kg BOD kg X_ss⁻¹ d⁻¹]


CH3
N
O
N
N
N
CH3
H3C
O


N
O
NH2


Cl
NH
Cl
OH
O


NH
CH3
O
HO


O
N
CH3
O
O
S
NH
H2N