

Biodegradability of human pharmaceutically active compounds (PhAC) in biological systems treating source separated wastewater streams

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Abstract

A biodegradability of eight selected pharmaceutically active compounds (PhAC) was assessed under various environmental conditions (red-ox, temperature, character of biological sludge). Selected PhACs were characterized by different physical-chemical-biological properties in order to be able to extend the results of this research to the broader group of environmentally relevant micro-pollutants. The selected compounds were: acetylsalicylic acid (ASA), bezafibrate (BZF), carbamazepine (CBZ), clofibric acid (CFA), diclofenac (DCF), fenofibrate (FNF) and metoprolol (MTP). Many PhAC can be biodegraded under aerobic conditions; the extent of biodegradation depends in many cases on the exposure time of a biomass to a given compound. Aerobic biodegradation is faster than anoxic degradation; elevating operational temperatures speed up the biodegradation processes, as expected. Under anaerobic conditions and relatively long retention times (HRT=30 d) some PhAC can be degraded (ASA, IBU, FNF) but at much lower rate than under aerobic or anoxic conditions. The anaerobic digestion process, is however not expected, to contribute significantly to elimination of majority of PhACs. Optimisation of process conditions for a (semi)persistent group of PhAC (CBZ, CLF, DCF) will only result in their partial (if any) biodegradation. For new sanitation concepts for source separated wastewater, where anaerobic digestion is applied as an efficient pre-treatment for a bulk of organic matter, and aerobic as a main treatment, addition of a physical or chemical polishing unit to eliminate persistent compounds (when demanded) will be unavoidable.

Keywords: biodegradability, biodegradation kinetics, red-ox conditions, representative pharmaceuticals, sorption,

1 Introduction

Human pharmaceutically active compounds (PhAC) are consumed in large quantities world wide; the consumption is in the range of tons per year per one PhAC per country, depending on its size. The expectations are that these amounts will only keep increasing because of a improving health care system and longer life expectations of the citizens. The PhACs comprise a large group of chemical compounds (more than 3000 registered in EU) having different therapeutic mode of action in a human body, different chemical-physical properties and susceptibility to degradation in biological systems. After administered by humans they are metabolized and excreted partially as a parent compound (usually a small fraction) and a number of inactive or active metabolites. Generally approximately 70% of PhAC forms are excreted with urine while 30% with faeces (Lienert 2007). Once excreted they enter a sewer and a sewage treatment plant (STP). In current STPs employing activated sludge process as a main treatment, PhAC are removed only partially (Ternes 2006). Discharged to surface water they form a threat to aquatic life and re-enter water cycle. Advanced post-treatment processes (e.g. oxidation techniques, tight membrane filtration, activated carbon sorption) are reported to be promising techniques to lower further the concentrations of emitted PhACs. They are however very expensive since large wastewater volumes need to be treated.

A new sanitation approach, applying separation of wastewater streams containing all (black water) or majority (urine) of PhACs and their target treatment, may enable to minimise the emission of human PhACs to the environment since it concerns a significantly smaller stream to be handled (sewage 200 L/p/day; urine 1.5 L/p/d when collected undiluted, black water 7.5 L/p/d when vacuum toilet is applied). Little is known on fate of PhAC when biologically treating very concentrated wastewater streams.

This study focus on fate of selected PhACs (Table 1) in biological wastewater treatment system. For a selection of test compounds a number of criteria were taken into account: consumption, occurrence in aquatic environment, differences in physical-chemical properties (e.g. polarity, hydrophobicity) and suspected biological degradability (persistent, biodegradable), potential eco-toxicological effects and availability of analytical methods, to mention the most important. An attempt is taken hereby to represent with this selection of eight compounds a broader group of PhACs.

Table 1 Selected PhACs for biodegradation tests

Acetylsalicylic acid, (ASA) painkiller, anti-inflammatory, antithrombotic $C_9H_8O_4$ $\log K_{ow}=1.426$, $pK_a=3.5$,	Metoprolol, (MTP) beta blocker $C_{15}H_{25}NO_3$ $\log K_{ow}=1.9$, $pK_a=9.7$
Diclofenac,(DCF) anti-inflammatory $C_{14}H_{11}Cl_2NO_2$ $\log K_{ow}=0.7-4.5$, $pK_a=4.15$, $k_{biol}<0.1$ L/gSS/d	Clofibrilic acid, (CFB) metabolite of cholesterol lowering agent, $C_{10}H_{11}ClO_3$ $\log K_{ow}=2.57$, $pK_a=3.0$, k_{biol} 0.3-0.8 L/gSS/d
Ibuprofen, (IBU) antiinflammatory $C_{13}H_{18}O_2$ $\log K_{ow}=3.481$, $pK_a=4.5-5.2$, $k_{biol}<21-35$ L/gSS/d	Bezafibrate, (BZF) cholesterol lowering agent, $C_{19}H_{20}ClNO_4$ $\log K_{ow}=4.25$, $pK_a=3.6$, k_{biol} 2.1-3.0 L/gSS/d
Carbamazepine , (CBZ) antiepileptic $C_{15}H_{12}N_2O$ $\log K_{ow}=2.69$, $pK_a=<1; 13.9$,	Fenofibrate, (FNF) cholesterol lowering agent, $C_{20}H_{21}ClO_4$ $\log K_{ow}=5.19$,

Fate of some of these compounds (e.g. CBZ, DCF, IBU) in wastewater treatment systems have been already described in literature to some extent, but very little in relation to source separation based wastewater treatment concepts. The major difference between two concepts is a significantly higher concentration of PhACs (lower mg/L range against $\mu\text{g/L}$ as measured in conventional STP) as well as macro-pollutants (bulk organics, nutrients) and different process configurations (anaerobic pre-treatment followed by aerobic main treatment and tertiary physical or chemical polishing, (Kujawa-Roeleveld and Zeeman 2006). Biodegradation potential was determined in batch tests under various red-ox conditions (aerobic, anoxic and anaerobic), at various temperatures using different types of sludge spiked with a mixture of PhACs.

2 Materials and methods

2.1 Chemicals, solutions

The PhACs were obtained from Sigma-Aldrich (Steinheim, Germany): ASA $\geq 99.0\%$ (CAS-nr: 50-78-2), BZF $\geq 98\%$ (CAS-nr: 41859-67-0), CBZ (CAS-nr: 298-46-4), CFB 97% (CAS-nr: 882-09-7), DCF (diclofenac sodium salt) (CAS-nr: 15307-79-6), FNF $\geq 99\%$ (CAS-nr 49562-28-9), IBU $\geq 98\%$ (GC) (CAS-nr: 15687-27-1) and MTP as Metoprolol (+)-tartrate salt $\geq 98\%$ (titration) (CAS-nr: 56392-17-7). Sodium nitrate (for anoxic tests) and chloroform (for sample preservation) (pro analysi) were obtained from Merck (Darmstadt, Germany). Methanol (for stock solution of PhACs) (HPLC-grade) was obtained from LAB SCAN (Dublin, Ireland).

A stock solution of PhACs was prepared in 50 ml of methanol. A 0.5 ml of this concentrated stock solution was spiked to the batches for obtaining the desired concentration of each of 8 PhAC. The required initial concentrations of the eight PhACs in the batch experiments were approximately 2; 0.3; 0.8; 0.9; 0.5; 0.8; 2 and 2 mg/L for ASA, DLC, IBU, CBZ, MTP, CFA, BZF and FNF respectively. Such relatively high concentrations are likely to occur in source separated sanitation concept where urine or concentrated black water are separately collected and treated. The calculation of the concentration is based on the Defined Daily Dose (DDD) (WHO 2006) and an excretion rate of a parent compound by humans being treated (KNMP 2006).

2.2 Sludge origin and characteristics

Activated sludge for aerobic and anoxic batch tests was obtained from municipal STP in Bennekom (the Netherlands) involving a biological moderately loaded (0.4 kg COD/m³/d) activated sludge treatment with nutrient removal. Activated sludge samples for the aerobic and anoxic biodegradation batch tests were collected at the end (effluent to sedimentation tank) of the aeration circuit. The sludge for the anaerobic biodegradation test originated from a demonstration-scale UASB septic tank treating concentrated black water (T=35°C) in Sneek (the Netherlands) (Zeeman 2007).

2.3 Aerobic biodegradation experiment

In the aerobic biodegradation experiment a mixture of PhACs was spiked to a 1 L of activated sludge. The sludge was incubated and aerated at a constant temperature. Experiments were performed in duplicates at two temperatures of 20°C and at 10°C (AER-20, AER-10). The tests were carried out for 30 days. In the first two days an intensive sampling took place, referring to the typical HRT at a STP. After, two samples were taken, at 15 and 30 days, to see any adaptation of sludge or its changed behaviour when operating under stress conditions (no organic co-substrate added). Prior to start of the experiment the activated sludge was aerated for a few hours to bring it to endogenous conditions and the required temperature. The batches were aerated to keep a sufficient high oxygen level and ensure good mixing of the sludge and added substances. Water losses due to evaporation were compensated by addition of (Millipore) water.

2.4 Anoxic tests

In the anoxic biodegradation experiment a mixture of PhACs was spiked to the 1 L volume activated sludge subjected to anoxic conditions. The sludge was incubated at a constant temperature under oxygen free and nitrate rich conditions. Analogously to the aerobic tests the anoxic experiments were performed at 20°C and 10°C (ANOX-20 and ANOX-10), all in duplicates. The activated sludge was

sampled from the STP a day prior starting the experiment. The oxygen in a liquid phase was depleted by storing the sludge without aeration over night. To obtain and keep oxygen free conditions, the gas phase in the batches was flushed with nitrogen gas before the start of the experiment and after sampling. A nitrate solution was prepared to obtain an initial concentration of nitrate in the batches of 20-40 mg N-NO₃/L. Nitrate concentration in the liquid was followed in time. When denitrification was almost completed, an appropriate volume of NaNO₃ solution was added again to obtain a required N-NO₃ level in the remained volume. To assure a good mixing in the batches a shaker was used (85 rpm).

2.5 Anaerobic tests

In the anaerobic biodegradation experiment (AN-30) a mixture of PhACs was spiked to the 0.4 L volume of anaerobic (mesophilic) sludge. The batches (duplicate) were incubated under anaerobic conditions at constant temperature of 30 °C and shaken continuously. To ensure strictly anaerobic conditions, the bottles' content was flushed with nitrogen (10 s) prior to the start of the experiment. The bottles were capped. After each sampling, the gas phase of the bottles was flushed again with nitrogen.

During all batch experiments mixed liquor samples were taken; liquid and solid fraction were separated from each other to determine the concentration of the PhACs in both phases. From the declined total amount of considered compounds the biodegraded fraction was determined as well as process kinetics. Control tests containing a mixture of 8 selected PhACs in Millipore water to trace any possible interactions between PhACs themselves or (other) abiotic transformation were performed in parallel. The total solids and volatile solids (TS, VS) of the sludge were determined at the beginning of the test, after 2 days (except for AN-30) and after 30 days. Before the addition of PhACs, a sample was taken to determine the background concentration of PhACs in the activated sludge mixture. During the experiment pH, T and DO or ORP measurements were regularly performed. Bottles were covered with aluminium foil to prevent photolytic degradation.

2.6 Sampling

A 30 mL mixed liquor samples were taken with a syringe (AER) or sampling needle (ANOX, ANAE). By flushing with nitrogen, 30 ml of mixture was taken out of the bottle in the anoxic test. To the 30 ml of sludge mixture sample a 4-5 drops of chloroform were added. Subsequently, the samples were centrifuged for 10 min at 4000 rpm / 2800 rpf using the centrifuge Firlabo SW12R (with rotor type FACENSW12001) and IEC thermo CL31R (with rotor type AC 100.10A). After centrifuging, the solid (4 mL) and liquid phase (20mL) were separated. From the controls, samples of 20 ml liquid were taken. All samples were stored under -75 °C in disposable centrifuge tubes (PP-Test tubes 50ml, CELLSTAR). The series of samples were sent for analysis to RIVM (ARO-CRL) during and after finishing the experiment.

2.7 Analysis of PhACs

All chemicals and reagents used in analytical method were of high purity quality. Liquid chromatography (LC): Waters Chromatography Acquity UPLC separation module; column: Acquity UPLC BEH C18 1.7 µm (100 * 2.1 mm ID) were applied. Column temperature was 65°C. The LC mobile phase consisted of a mixture of 0.1 percent acetic acid (solution A) and acetonitrile (100%). The gradient used was linear, started at 10% B and progressed to 30% B in 3 minutes after which it was increased to 100% B in 6 minutes. After 9 min the mobile phase was kept for 2 min at 100% B, then the percentage B was decreased to 10 percent in 0.01 minute. The mobile phase flow was set at 0.4 mL/min. The injection volume was 20 µL. Mass-spectrometer (MS) analysis was carried out on a

Waters-Micromass Ultima Platinum. Depending on a compound the measurement was carried out in positive or negative electrospray ionisation (ESI) mode. In case of co-eluting compounds the ionisation alternates between positive and negative. The following settings were used in positive ESI mode (MTP, CBZ, BZF, FNF): capillary voltage was 3.5 kV. Cone voltage was 35 V. RF lens 1: 15, aperture: 0.1 and RF lens 2: 0.3. Source temperature was 120°C and desolvation temperature: 325°C. The cone gas flow was 116 L/h and the desolvation gas flow was 701 L/h. LM1/HM1 resolution was 14, with ion energy: 0.8. LM2/HM2 resolution was 14.5, with ion energy: 1.0. For the collision cell the entrance was 7, with a CE gain of 2 and exit 0. Collision cell pressure 3.06×10^{-3} . In negative mode (ASA, CFA, DCF, IBU): capillary voltage was 1.2 kV. Cone voltage was 35 V. RF lens 1: 5, aperture: 0.5 and RF lens 2: 1.0. Source temperature was 120°C and desolvation temperature: 325°C. The cone gas flow was 116 L/h and the desolvation gas flow was 701 L/h. LM1/HM1 resolution was 14, with ion energy: 0. LM2/HM2 resolution was 14.5, with ion energy: 1.0. Collision cell pressure 3.06×10^{-3} . For the collision cell the entrance was 10, with a CE gain of 1 and exit 0.

Sample clean-up of the liquids was straight forward. The samples were 10 times diluted in LC-eluent A, after which they were vortexed for 10 seconds. Samples with lower concentrations were acidified with 2 µl 50% acetic acid. The samples were direct injected. Sample clean-up of the sludge was performed by a liquid liquid extraction. A portion of the sample (circa 0.5 gram) was weighted and 5 mL of acetonitrile was added. The samples were sonified by an ultrasonic finger for 20 s followed by rotating head over head for 10 min. After which the sample was centrifuged. The supernatant was transferred to a clean tube and evaporated under nitrogen at 55°C. The dried sample was reconstituted in 1 mL of eluents, followed by 10 min ultrasonification. To correct for losses due to sample storage and to correct for signal suppression due to matrix compounds were the calibration curves prepared in comprehensive blank materials for each corresponding experiment.

3 Results and discussion

3.1 Background concentrations of PhACs

The concentration of PhACs in the activated sludge mixture from the end of the activated sludge tank (=effluent) and sludge mixture from the UASB septic tank are shown in Figure 1. Except for ibuprofen all PhACs were detected in the activated sludge in the low µg/l range, confirming literature findings. DCF was present in relative high concentrations. The presence of majority of the selected compounds in the effluent of a conventional STP confirms their poor or incomplete removal. In the anaerobic sludge from the demonstration scale UASB septic tank the PhAC concentrations were much higher (up to 100 µg/L). This higher concentrations confirmed expectations; the digester treats at least 20x more concentrated wastewater than sewage and the expected removal efficiency under anaerobic conditions is low for majority of PhACs. All measured PhACs prevailed in the liquid phase.

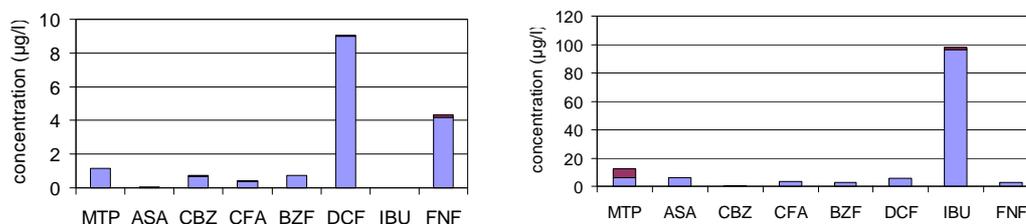


Figure 1 Background concentrations of selected PhACs in activated sludge sample from an effluent of the aeration tank of the municipal STP (left) and mesophilic anaerobic sludge from UASB septic tank treating concentrated black water (right).

3.2 Aerobic biodegradability

In AER-20 a fast decrease of ASA was observed; within 1 hour the concentration in the water phase was under the detection limit (d.l. = 0.005 µg/l). In AER-10 the concentration was lower than d.l. after 3 h (Figure 2). A fast decrease of FNF concentration was observed; at 20 °C the total concentration decreased to values under the detection limit. However, this was also observed in the controls. At 10°C a disappearance of FNF in the biodegradation test and in the control was observed as well. For this reason it is uncertain, which part of the FNF reduction was due to biological activity and which part was caused by abiotic reactions (sorption to materials used during the test, analysis and sampling). IBU was exponentially eliminated to concentrations under the detection limit within 2 days of the test. The disappearance rate of IBU was slower at 10°C as compared to 20°C.

MTP was eliminated exponentially but at slower rate as compared to IBU. At 20°C, this PhAC was eliminated to concentrations under the detection limit within 2 days, while at 10°C it was then still present. Prolongation of the test to 30 days, enabled to remove MTP below the detection limit. BZF was removed less efficiently. After 2 days of aerobic test at 20°C a 40% of BZF was removed. In the AER-10 the reduction of BZF was not significant. The difference between the tests was quite large. After 30 days the BZF in all three aerobic tests was under the detection limit. DCF was not eliminated in the first 2 days. In both tests, no significant decrease in DCF was measured within 48 hours. Remarkably after 30 days, DCF was transformed significantly, up to about 90% in both tests. This shows that DCF could be potentially eliminated in biological systems. No decrease in concentrations of CBZ and CFA was observed after 2 days nor after 30 days.

3.3 Anoxic biodegradability

The ANOX-10 was performed over a time period of 2 days while the ANOX-20 over 30 days. ASA was completely transformed in both tests, however this transformation was slower than in the aerobic tests. The transformation of ASA was faster at 20°C than at 10°C. At anoxic conditions FNF was eliminated relatively fast, like in the aerobic tests. Differences in transformation rates between different temperatures were not significant. The stability of the concentration of FNF in the controls in both anoxic tests at the first 2 days of the experiments was observed, contrary to aerobic tests. The stability of the FNF concentration in the controls in the first 2 days, show that the conversions of FNF had a biological character. IBU was removed, but at slower rate than under aerobic conditions. Differences between the anoxic degradation rate in relation to temperature were observed, with a higher rate at a temperature of 20°C, as expected. In contrary to aerobic tests, MTP was only eliminated to a small extent within 2 days. At 20°C, MTP continued to decrease in concentration after 48 hours to reach the d.l. after 30 d. In the ANOX-10 no significant removal of MTP was observed. BZF did not decrease in concentration in the ANOX-10 test, while in ANOX-20 a significant elimination of BZF was observed; after 1 month a concentration of BZF reached the d.l. Compared to the aerobic tests, a degradation rate in the ANOX-20 test was higher than this in the aerobic tests. At a temperature of 10°C, DCF concentration remained constant in time. In the ANOX-20, DCF seemed to be reduced to a certain extent after 48 hours but the samples taken after 30 days showed that the concentration of DCF was still in the same range. Both anoxic tests showed no decrease in concentration of CBZ and CFA.

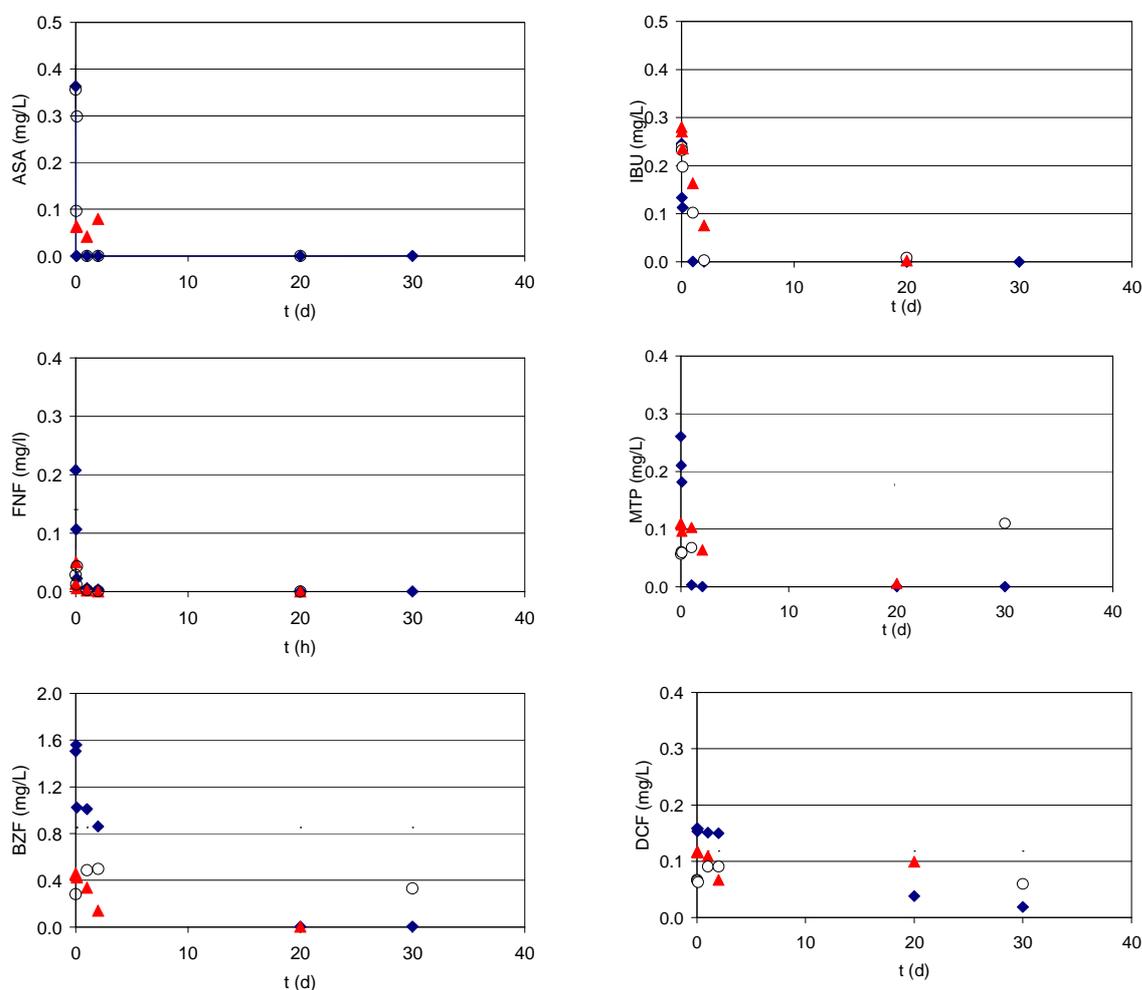
Comparing both, aerobic and anoxic tests, the PhACs, which showed to be (partly) degradable under aerobic conditions showed generally a lower degradation rate under anoxic conditions, with the exception of BZF in anoxic test at 20°C. Next to this, ASA, IBU, MTP and BZF showed a different degradation rate between the tests at 20° and 10°C. For MTP and BZF this temperature difference resulted in a small removal at 20°C and no significant removal at 10°C within 48 hours. After 30 days MTP, BZF, IBU, ASA and FNF decreased in concentration to under or close to the detection limit. In these anoxic tests the control concentrations stayed constant in the time interval in which PhAC

concentrations decreased. Elimination of PhACs in anoxic tests was therefore most likely a result of biotransformation processes.

3.4 Anaerobic tests

ASA, FNF and IBU were exponentially reduced in anaerobic tests, however with a much slower rate than under aerobic and anoxic conditions. Nevertheless, after 30 days, which could be a common HRT for anaerobic treatment of concentrated black water (STOWA 2005), the concentration of all three PhACs decreased by more than 90%. However, in the controls a sharp decrease in their concentrations was measured as well. Because of that the disappearance of a compound in the biodegradation tests, can not be fully assigned to biotransformation processes. Removal efficiency of IBU in anaerobic digesters was confirmed in literature and amounted to 26-56% (Carballa, Omil et al. 2007). However in their research also a removal of DCF was reported, of 59-79%, not found in this study. For CBZ no removal was observed, as in this study.

For MTP, BZF, DCF, CBZ and CFA, no significant decrease in concentration was measured in the anaerobic test. Selected results of the batch tests, AER-20, ANOX-20 and AN-30 are presented in Figure 2.



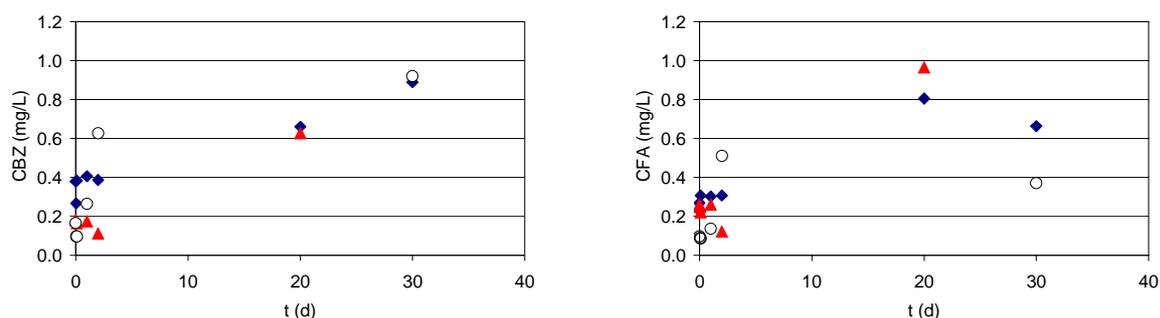


Figure 2 Course of PhACs in selected biodegradation tests; \blacklozenge AER-20; \blacktriangle ANOX-20; \circ AN-30.

3.5 Sorption

The fraction of the selected PhAC sorbed to the sludge was of a minor importance. For most of PhACs the concentration in the solid phase was <10%. For the non-acidic pharmaceuticals, MTP and CBZ, sorption was somewhat higher. The very hydrophobic but fast eliminated FNF was highly sorbed (up to 80% in anaerobic system).

3.6 Assessment of biodegradation kinetics

The results, in which exponential decrease of PhACs in the course of a given test was obtained, were used to calculate, the first order degradation rate constant (k , 1/d) and the pseudo first order reaction rate constant (k_{biol} , L/gTS/d) (Joss, 2006) (Table 2). The degradation rate constant of FNF and ASA was also determined, but not in all tests it is demonstrated that their removal is due to biological processes; certain values therefore may represent the disappearance rate as indicated in the table. The degradation kinetics in the aerobic, anoxic and anaerobic tests differed clearly. Comparing the aerobic and the anaerobic tests, the degradation rates were 20 to 200 higher for ASA, FNF and IBU. A difference between aerobic and anoxic tests was of a factor 2 to 4 for IBU and BZF. For other compounds, no exponential curve could be fitted, so no comparison could be made between the different rates.

The degradation kinetics under aerobic conditions are reported in literature for some of the selected PhAC (Joss 2006). In this research the k_{biol} – values were lower than these found in literature. The differences between these experiments and the experiments reported in literature is the concentration of PhACs. Also de Mes (2007) investigating biodegradability of elevated concentration of human estrogens found lower k_{biol} than reported in literature when working with lower concentrations. The activated sludge used in this research was not adapted to such high concentrations of PhACs. A continuous, pilot-plant experiment, where biomass would be exposed to higher PhAC concentrations could reveal possible enhancement of biodegradation due to adaptation of biomass. In contrast, the anaerobic sludge was already imposed for a longer period to elevated concentration of PhACs, but the anaerobic conditions do not favour the biodegradation of majority of PhAC compounds.

Table 2 The degradation rate constant k , its 95% confidence interval, the range of specific degradation rate constant k_{biol} based on the TS concentration and related the 95% confidence interval and the R^2 of the regression model for tested PhACs under various environmental conditions (assessed where possible)

PhAC	Test	k-value (1/d)	95% confidence interval of k	R^2	k_{biol} (L/gTS/d) range	k_{biol} (L/gSS/d) (Joss 2006)
ASA	AER-20	218	217 - 219	0.999	37.3 - 43.9	
	AER-10	74	72 - 76	0.830	15.9 - 17.5	n.a.
	AN-30	1.9	1.3 - 1.4	0.932	0.111 - 0.127*	
FNF	AER-20	22.0	21.8 - 22.3	0.960	3.74 - 4.46*	n.a.
	AN-30	0.38	0.36 - 0.40	0.930	0.031 - 0.035*	
IBU	AER-20	5.2	5.1 - 5.4	0.937	0.874 - 1.07	
	AER-10	4.4	4.3 - 4.6	0.900	0.952 - 1.06	9-35
	ANOX-10	0.9	0.8 - 0.9	0.903	0.103 - 0.119	(17±1°C)
MTP	AN-30	0.29	0.28 - 0.30	0.942	0.024 - 0.026	
	AER-20	3.38	3.3 - 3.5	0.954	0.569 - 0.691	
	AER-10	0.86	0.86 - 0.89	0.980	0.192 - 0.205	n.a.
BZF	AER-20	0.19	0.19 - 0.22	0.871	0.038 - 0.043	2.1-4.5
	ANOX-20	0.58	0.55 - 0.58	0.922	0.111 - 0.120	(17±1°C)

* the specific *disappearance* rate constant, since other abiotic processes could have played a role, n.a. not assessed

The biodegradation tests showed a potential for some of the selected PhACs to be bio-transformed, in some cases significantly. The degradation rates differed per compound and environmental conditions applied. The continuation of the aerobic and anoxic batch tests for 30 days provided more information. The, at the first sight, persistent DCF was eliminated for 90% in the aerobic tests after 1 month. The PhACs partially eliminated during 2 days, like MTP and BZF, were completely removed to levels under or close to the d.l. of 0.005 ug/l when the aerobic and anoxic test was prolonged to 30 days and biomass was subjected to stress conditions (no external substrate supplied).

It should be kept in mind that only the removal of the original PhAC was analyzed. Whether a given compound is mineralized or degraded and if the subsequent produced degradation product is biodegradable is at this moment unclear. Regarding IBU and ASA the produced metabolites are not likely to be persistent to biodegradation. According to results of (Quintana 2005) the metabolites of BZF are also degradable. The possible produced metabolite fenofibric acid of FNF can be transformed most likely too although not much is known about other metabolites produced. The biodegradability of metabolites of MTP and DCF are unknown.

Some compounds remain very persistent independent on the environmental conditions applied during the biological treatment (CBZ, CLF). If the objective of wastewater treatment will become once to eliminate all PhACs, the addition of advanced chemical or physical process units seems to be unavoidable to remove biologically persistent compounds.

4 CONCLUSIONS

- Biodegradation experiments were performed for eight selected PhACs under various red-ox conditions. The selection had a goal to include compounds of various physical-chemical-biological characteristics implying their different behaviour during biological wastewater treatment processes.

- The applied initial concentrations of PhACs were significantly higher than these at the conventional treatment plant (low mg/L range against low µg/L range). The reason was the applicability of the results to treatment concepts for concentrated black water or urine.
- A summary of biotransformation behaviour for all selected pharmaceuticals together with the influence of different environmental conditions is given in Table 3.

Table 3 Comparison of biotransformation rate of the selected PhACs at different environmental conditions. Biotransformability: +++ high, ++ good, + moderate, +/- only at HRTs > 2 days, - not biotransformed

	Aerobic 20°C	Aerobic 10°C	Anoxic 20°C	Anoxic 10°C	Anaerobic 30°C
ASA	+++	+++	++	++	+
FNF	+++	++	++	++	+
IBU	++	++	+	+	+
MTP	++	+	+	-	-
BZF	+/-	+/-	+	-	-
DCF	+/-	+/-	-	-	-
CBZ	-	-	-	-	-
CFA	-	-	-	-	-

- As certain PhACs remain persistent to biological degradation extension of biological treatment (anaerobic-anoxic-aerobic) of source separated wastewater with physical or chemical process units will be unavoidable when the intention is to stop the emission of PhAC to the environment.

Acknowledgements

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