Fate of human pharmaceuticals in biological treatment systems treating concentrated wastewater under various environmental conditions

Msc thesis

Els Schuman

Supervisors:
Dr. K. Kujawa-Roeleveld
Dr. Ir. G. Zeeman

Sub-department of Environmental Technology
Wageningen University

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Els Schuman
Registration number: 850607-752-020

Thesis Environmental Technology
Course code: ETE-80436

Supervisors:
Dr. Ir. K. Kujawa-Roeleveld
Dr. Ir. G. Zeeman

Sub-department of Environmental Technology
Wageningen University

July 2008

Abstract

In source separated sanitation systems, urine and black water (faeces and urine) are collected separately to treat these waste streams in concentrated form. The urine and faeces contain the pharmaceuticals excreted from the human body. Via the effluent of wastewater treatment systems, these pharmaceuticals can enter the aquatic environment. In case of the application of urine on the field as fertilizer, they can be released into the soil.
The objective of this thesis was to investigate the fate of human pharmaceuticals in biological systems treating concentrated waste streams under various environmental conditions.
A selection of pharmaceuticals was made including as much as possible different biological and physical-chemical characteristics. The selected pharmaceuticals are: acetylsalicyl acid (ASA), bezafibrate (BZF), carbamazepine (CBZ), clofibric acid (CFA), diclofenac (DCF), fenofibrate (FNF) and metoprolol (MTP).

Batch tests were performed to determine the biodegradation rate of selected pharmaceuticals in relative high concentrations (as in black water/urine) at aerobic, anoxic and anaerobic conditions. Moreover, the aerobic and anoxic tests were performed at two temperatures: 10 and 20^{o}C. The experiments were performed at time periods of 2 to 30 days for aerobic and anoxic tests and of 30 to 77 days in anaerobic tests. In analyzing the samples both pharmaceutical concentrations in liquid and solid phase were analyzed to distinguish between sorption and biotransformation processes.

In the aerobic batch tests ASA, FNF, IBU, MTP removal followed pseudo first order reaction kinetics with highest conversion rates for ASA (k_{biol} 25.5-26.4 L/gTS/d) followed by FNF (k_{biol} 3.74-4.46 L/gTS/d), IBU (k_{biol} 0.874-1.07 L/gTS/d) and MTP (k_{biol} 0.569-0.691 L/gTS/d) at 20^{o}C. These values are lower than those reported in literature (Joss et al. 2006). A temperature decrease from 20 to 10^{o}C resulted in a (slightly) lower biodegradation rate. For BZF and DCF partial or no elimination was observed within 2 days at aerobic conditions. After 30 days, disappearance is observed of ≥ 90% for both compounds. CBZ and CFA were not at all biodegraded.
At anoxic conditions, the biodegradation rate was lower than under aerobic conditions. DCF, CBZ and CFA were not at all eliminated in the anoxic tests. Lower removal rates at anoxic conditions compared to aerobic conditions are in consistency with findings reported by Zwiener et al. (2002).
Anaerobic results showed only elimination of ASA, FNF and IBU although at a much slower rate. Abiotic processes could not be excluded in this observed elimination, because in the controls, without any sludge, the pharmaceutical concentration of these three also lowered after 30 days significantly.

Overall, the selected pharmaceuticals showed different biodegradation potentials. Three groups of pharmaceuticals can be distinguished. The first group includes the in this research selected pharmaceuticals, ASA, FNF and IBU. They are biodegradable under different redox conditions. The second group, containing MTP, BZF and DCF, can be biodegraded as well, but this depends on the prevailing environmental conditions. Aerobic conditions are most favourable, followed by anoxic and subsequently anaerobic conditions. The third group includes CBZ and CFA. The size and relevancy of this group is important to investigate since pharmaceuticals in this group are persistent to biodegradation under all tested conditions.

Key words: acetylsalicylic acid, bezafibrate, biodegradation, biological treatment, black water, carbamazepine, clofibric acid, diclofenac, fenofibrate, human pharmaceuticals, metoprolol, redox conditions, sorption, source separated sanitation, temperature, urine.

Table of contents

1 Introduction

1.1 Presence of pharmaceuticals in the environment

Over the last years, more and more pharmaceuticals are detected in the environment. In many parts of the world, and especially near or close to urban areas, pharmaceuticals have been detected in surface and groundwater. This is related to the increasing consumption of pharmaceuticals and the development of analytical tools with very low detection limits to determine these trace compounds in various environmental matrices. Pharmaceuticals enter the environment after consumption of drugs by humans and animals and subsequent excretion of drugs with the faeces and urine. Via waste water treatment plants (WWTP) (which in their current configuration are not able to remove these compounds efficiently) effluents or application of (animal) manure on fields pharmaceuticals end up in aquatic systems because WWTPs are not able to remove these compounds efficiently in their current configuration. A continuous input of pharmaceuticals to the environment, although in low concentrations, can and does yield effects for the environment.
The use of pharmaceuticals is likely to increase and therefore its important to analyze and optimize their biological removal in treatment plants or to introduce new measures such as for instance source separated sanitation concepts.

1.2 Use of human pharmaceuticals

The consumption of pharmaceuticals has been increasing over the last years. This trend is likely to continue in future due to e.g. the growth and the aging of the population. The increasing number of users of drugs over the last years is shown in table 1-1 for the Dutch situation. The pharmaceuticals are divided 14 classes based on their functional use.

Table 1-1: Classes of drugs and the number of users (x 1000) in the Netherlands from 2002-2006. Source: (CVZ 2006)

	2002	**2003**	**2004**	**2005**	**2006**
A Alimentary tract and metabolism	2910	3004	2769	2969	3441
B Blood and blood forming organs	1655	1663	1667	1673	1944
C Cardiovascular system	2676	2759	2910	2982	3630
D Dermatologicals	3421	3465	3193	3166	3484
G Genito urinary system and sex hormones	2774	2703	1419	1412	1594
H Systematic hormonal preparations	828	854	890	927	1043
J Antiinfectives for systematic use	3840	3826	3775	3945	4229
L Antineoplastic and immunomodulating agents	145	157	169	180	221
M Musculo-skeletal system	3403	3423	3322	3136	3369
N Nervous system	3584	3598	3345	3308	3555
P Antiparasitic agents, insecticides, repellents	144	148	161	162	170
R Respiratory system	3149	3064	3033	3099	3481
S Sensory organs	1785	1802	1759	1755	2137
V Various	34	37	40	43	60

The classes cardiovascular system and antiinfectives for systematic use are the classes with the highest number of users of pharmaceuticals in 2006. In the class of the cardiovascular system, the increase in number of users of is also high over 2002 to 2006.

1.3 Fate, occurrence and effects of pharmaceuticals in the environment

Pharmaceuticals can enter the environment in different ways. The main route of human drugs to the environment is via sewage and wastewater treatment plants (WWTPs) effluents. After consuming pharmaceuticals part of this will be excreted unchanged and other parts will be excreted in the form of conjugates (complex formation with sulphate or glucuronic acid) or as metabolites (degradation products). Via the sewage system the excreted pharmaceuticals end up in the waste water treatment plants. Here a part of the pharmaceuticals will be removed (degraded or absorbed) and another part released into the aquatic environment (see figure 1.1). Removal rates differ for each pharmaceutical and can range from zero to almost 99%. Drugs can also be released in the environment via other ways as shown in figure 1.1 (e.g. via application of animal manure containing veterinary drugs). This research focusses on the route as described above and highlighted in figure 1.1.

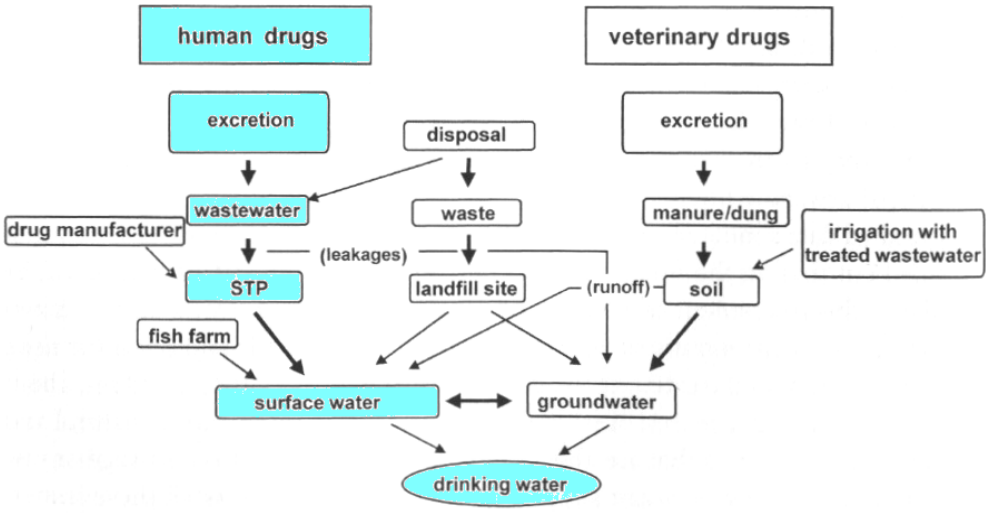


Figure 1-1: Various pathways of pharmaceuticals to enter the environment. Source: (Reemtsma 2006). Highlighted pathway is the main pathway this research focuses on.

In Fent et al (2006) an overview is given of WWTP influents and effluents concentration ranges of pharmaceuticals found in different researches (Table 1-2). The concentrations are dependant on several factors which can be different for each country, WWTP etc. This table illustrates the detection of pharmaceuticals in the µg/L range in the influent and effluent of WWTPs and their differences.

Table 1-2: Measured influent, effluent concentrations of pharmaceuticals selected for this research. Source: (Fent 2006) and Petrovic (2005)

Compound	Influent concentration µg/L)	Effluent concentration (µg/L)
Acetylsalicylic acid	3.2	0.6
Salicylic acid	57-330	0.05-3.6
Ibuprofen	2-38.7	0-4
Diclofenac	3.0	2.5
Carbamazepine	0.7-1.5	0.7-1.5
Metoprolol	-	0.08-0.73
Clofibric acid	0.15-1	0-0.88
Bezafibrate	0.42-5	0-0.84
Fenofibric acid	0.44	0.22-0.4

For some pharmaceuticals a lot of researches have been conducted to access their removal rates in WWTPs. Especially diclofenac, carbamazpine and ibuprofen have been extensively researched. Removal rates vary sometimes very much between different researches. Mainly this is caused by the conditions and configuration of the treatment plants.
The pharmaceuticals are released in the in surface water at relative low concentrations, in general in the range of ng/l up to low µg/l; and the pharmaceuticals will be further diluted when they come in contact with surface water. .
Because of the continuous input of pharmaceuticals even readily degradable pharmaceuticals are measured in rivers and other surface waters, especially near WWTPs effluents. Less easily degradable pharmaceuticals do also enter the sea. For example carbamazepine was detected in the North Sea (Weigel, Bester et al. 2001). Also in groundwater pharmaceuticals can enter. Multiple pharmaceuticals were measured in drinking water at low ng/L range (Versteegh and Dijkman 2007)). In figure 1-2 the occurrence of pharmaceuticals in small streams and rivers in Germany is reported (Ternes, 1998). The measured concentrations are in the low µg/L range.

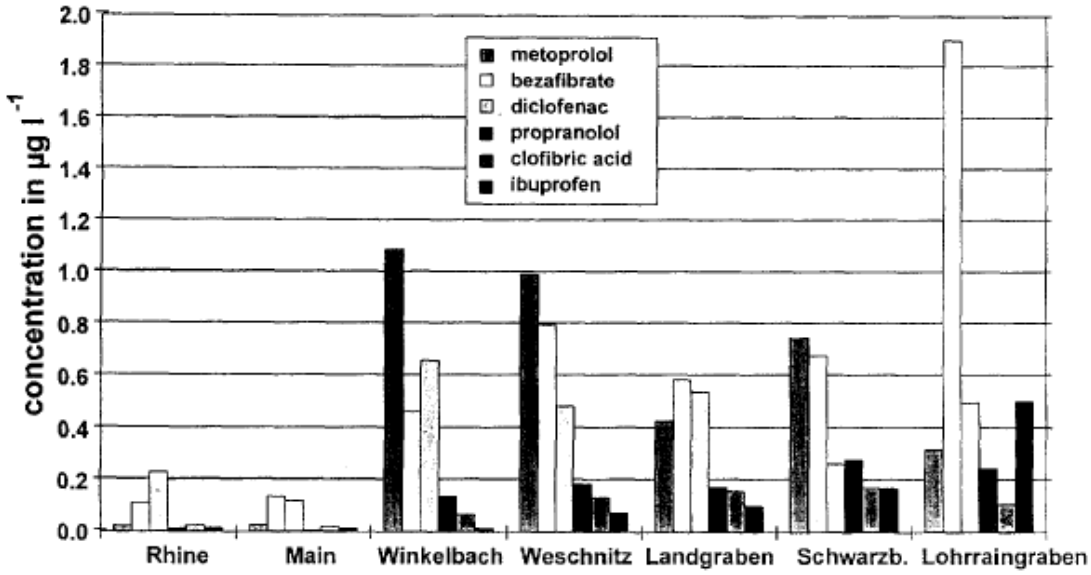


Figure 1-2: Concentrations of metoprolol, bezafibrate, diclofenac, propranolol, clofibric acid and ibuprofen in different rivers and brooks in Germany, from Ternes (1998).

It is difficult to determine the effects of pharmaceuticals in the environment. Some acute effects (effects in small time period) have been determined for aquatic organisms but these occur at pharmaceutical concentrations of several mg/l, and therefore they are not likely to occur at present situation. For instance, for ibuprofen a LC50 (96 hr) is determined at 173 mg/l for the bluegill sunfish (Hallingen et al., 1998).
Because of the low pharmaceutical concentrations in the environment but their continuous input, chronic effects of pharmaceuticals are much more likely. However, these effects are more difficult to predict because of the long time period is required before effects become clearly visible. Especially endocrine disruptors are known to disturb the functioning of organisms at very low concentrations. Some effects occurring at environmental realistic concentrations have yet been determined also for non-hormones. In the table below some examples of effects of pharmaceuticals selected in this study are given.

Table 1-3: Effects of ibuprofen, diclofenac and carbamazepine on various aquatic organisms at low pharmaceutical concentration (ng/l - µg/l).

Drug	Measured effect and concentration		Reference
	Effect	Concentration	
Ibuprofen	Significant decrease in activity of crustacean G. *pulex*	1 and 10 ng/L	De Lange et al., 2006
	Toxic effects to microbial communities	10 µg/l	Dorne, 2006
	Growth inhibition of duckweed L. minor, up to 25%	1,10,100 and 1000 µg/L	Pomati et al., 2004
Diclofenac	Subtle sub cellular effects in fish	1 µg/L	Fent et al., 2006
	Renal lesions and alteration of gills in fish	5 µg/L	Fent et al., 2006
Carbama-zepine	Slightly earlier maturation and reproduction of *Daphnia* and higher production of offspring	1 µg/L	Lürling et al., 2006

From literature reviews, which have been published on this topic, it becomes clear that many things about the chronic effects of pharmaceuticals on aquatic organisms are unknown. The type of effects that may occur are:

- Mixture effects (synergetic effects), different pharmaceuticals together can show unexpected effects based on single compound evaluations. An example is veramapil (a pharmaceutical used for the cardiovascular system), which increases the intercellular concentrations of other pharmaceuticals in organisms (Daughton 1999).
- Subtle effects, like for example genetic or behavioural changes are much more difficult to detect while they can have important consequences. At especially at low concentrations, pollutants can disturb the chemical signalling. (Lürling and Scheffer, 2007). Pharmaceuticals could also cause this and interfere in the information transfer between or within organisms. For estrogens this is already proven. At ng/l they disturb the endocrine system.
- Effects between and within species. Inter- and intraspecies differences like in males/females or between organisms of the different developmental stages may influence the toxicity of a pharmaceutical (Daughton and Ternes 1999).
- Effects based on the known mode of actions of pharmaceuticals. Similar targets of pharmaceuticals in humans and other organisms could indicate possible effects to these organisms (Fent 2006).
- Effects of metabolites. Pharmaceuticals may be degraded to metabolites which have bioactive and/or persistent properties. For example clofibric acid is the metabolite of clofibrate and is quite persistent and can be classified as harmful to aquatic organisms (Fent 2006).

1.4 Source separated sanitation concept

To reduce the pharmaceutical release into the environment measures have to be taken. In conventional sewage systems not all pharmaceuticals are removed. Additional treatment steps could be introduced for this purpose or the removal of pharmaceuticals can be integrated in the new source separated sanitation concept.
Source separated sanitation is considered as an option to reuse nutrients and clean water and to remove micro-pollutants like pharmaceuticals. In source separated wastewater collection, two domestic waste streams are separately collected. A concentrated black water stream consisting of urine and feaces and a low concentrated grey water stream including shower, bath, kitchen and laundry water is gathered (Zeeman 2006). These waste streams have both different characteristics. The greywater is low in COD concentration and nutrients. The black water contains an important part of the nutrients, pharmaceutical residues, pathogens and a large part of the COD in a relative small volume. This volume can be reduced even more by the use of low-flush systems. Moreover, the black water can be divided into two streams as well: a feaces and urine wastewater stream. This separate collection of faeces and urine, can be achieved when using no-mix toilets. Urine contains 75% of nitrogen and up to 50% of phosphorus originating from total household wastewater. Urine separation is therefore also an alternative for a better water pollution control with respect to nutrient removal and reuse (Ternes 2006).
By separate collection of the domestic wastewater streams, pollutants and pathogens present in black water are not mixed with the cleaner and high flow grey water. The high concentration of nutrients in black water enables a more easily recovery and reuse of nutrients compared to conventional wastewater. Also, source separation creates to opportunity to reuse water and save energy (Zeeman 2006).

The advantage of source separation in relation to pharmaceuticals is that as good as all excreted pharmaceuticals are in high concentrations, present in black water.
When also the urine and faeces are separately collected, about 70% of the pharmaceuticals excreted will be present in the urine (Ternes 2006) which originally is a very small waste stream (1.5 L/person/day). Treatment of urine without dilution can be more efficient (Ternes 2006).
The treatment strategy for black water is still being researched as well the biological as chemical treatment options. For instance in Sneek, the Netherlands, a demonstration plant has been built. The black water of 25 houses is collected separately. An anaerobic reactor treats the black water to reduce the COD concentration. To be able to reuse the nutrients the anaerobic reactor is followed by struvite precipitation. Autotrophic nitrogen removal is applied to reduce the nutrient concentration even further.
Two researches on source separated sanitation in which this thesis is part of are the SWITCH project and the project in Anderen.

1.4.1 EU SWITCH project

The SWITCH research programme is project co-funded by the European Commission to support research in the field of water management. Urban water management is encountering difficulties as well as from environmental perspectives as from economic ones. The SWITCH project is established to ‘catalyse change towards a more sustainable urban water management in the city of the future’ (SWITCH, 2008).
In this program collection and treatment of concentrated waste flows is regarded as a promising approach for new and cost effective alternatives in wastewater treatment since it can result in new local sources of water and the reuse of nutrients in agriculture. Since the decentralized, source separated systems require more developments before applicable on a

wider scale, these concepts will be researched within the SWITCH program (SWITCH, 2008).
The development of an advanced water treatment system for the removal of pharmaceuticals and hormones is part of this (SWITCH, 2008). This treatment system consists of biological (both anaerobic and aerobic) part and if necessary a physical-chemical treatment. The efficiency of both biological and physical-chemical treatments in the removal of pharmaceuticals has to be investigated.

1.4.2 Case study Anderen: "Using urine as fertilizer for energy crops"

Using urine as fertilizer for energy crops is a project in the Netherlands, location Anderen, about a separate collection and reuse of urine. The aim is to research the feasibility of the applying collected urine on the field as fertilizer. Studying the fate of pharmaceuticals in biological systems, especially in soil, is of importance since it gives information about whether biodegradation, sorption or leakage to groundwater of pharmaceuticals will take place.
STOWA (Stichting Toegepast Onderzoek Waterbeheer i.e. Foundation of Applied Research on Water Management) is the commissioner of this research. The separated collection of wastewater is getting increasingly attention of STOWA. According to STOWA, the separate collection of urine can give a positive effect to the energy use and water effluent of WWTPs. In the village Anderen it is tried to determine effective ways in processing the urine. 20 no-mix toilets will be installed in a centre and it is expected to obtain a yearly production of urine of 6 m3. The urine will be applied on the nearby fields.
The project is divided into two parts:

- research on the consequences of hormones and pharmaceuticals for soil and groundwater quality
- the application of the urine as fertilizer.

1.5 Research objectives

Although there is some research already done about the (bio)degradation of pharmaceuticals in WWTPs, hardly any research is carried out about the degradation of these compounds in source separated streams with significantly higher concentrations of these micro-pollutants.
In this research the biodegradability and sorption of pharmaceuticals present in source separated urine and black water waste streams was investigated, using sludge from various biological wastewater treatment processes.
Research objectives are:

- To determine the fate of pharmaceuticals in biological systems treating concentrated wastewater streams under various environmental conditions

- To predict the fate of pharmaceuticals in different biological treatment systems

1.6 Research boundaries

To determine biodegradation of pharmaceuticals, batch experiments were performed. In the batch experiments, the fate of pharmaceuticals in biological systems was researched by applying different redox potentials and temperatures and using activated and anaerobic sludges.

There are thousands of pharmaceutically active compounds consumed. They cannot be investigated all. Therefore, a selection of 8 pharmaceuticals was made. The selection is based on the consumption rates, persistency, degradability, polarity and measured concentrations in the environment.
The 8 selected pharmaceuticals were:

- Acetylsalicylic acid (aspirin): analgesic drug and anti-thromboticum
- Bezafibrate: blood lipid lowering agent
- Carbamazepine: anti-epileptic drug
- Clofibric acid: metabolite of blood lipid lowering agents
- Diclofenac: analgesic and non steroidal anti-inflammatory drug (NSAID)
- Fenofibrate: blood lipid lowering agent
- Ibuprofen: analgesics and non steroidal anti-inflammatory drug (NSAID)
- Metoprolol: beta-blocker, used for heart diseases

In the next chapter a more elaborated description of the motivation of selection of these compounds and their characteristics is presented.
Moreover, the focus of the research was on the fate of the original pharmaceutical. Conjugates of pharmaceuticals (complexes) and metabolites were not researched.

1.7 Outline of the report

This report starts with the characteristics and motivation of the selected pharmaceuticals and the selection criteria, chapter 2. In chapter 3 the fate processes and the literature findings on the selected pharmaceuticals are reported. The materials and method applied in the biodegradation tests are described in chapter 4. This chapter is followed by results and discussion of the experiments as well as some implications for practice (chapter 5). Finally, conclusions and recommendations are given.

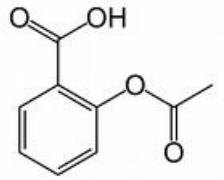
2 Characteristics of the selected pharmaceuticals

This chapter elaborates the motivation of selection the eight pharmaceuticals for this research: acetylsalicylic acid (aspirin), diclofenac, ibuprofen, carbamazepine, metoprolol, clofibric acid, bezafibrate and fenofibrate.
The selection criteria has been as following:

- high consumption rates in the Netherlands;
- representation of a variety of therapeutic classes;
- reported occurrence in the environment;
- reported eco-toxicity (acute and chronic);
- physical-chemical properties (hydrophobic / hydrophilic);
- susceptibility to biodegradation;
- availability of validated analytical methods

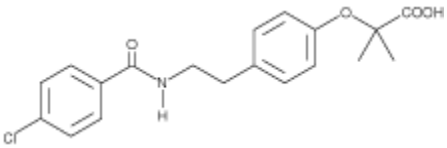
It was attempted to include as much as possible pharmaceuticals with different characteristics to obtain a good representation of pharmaceuticals released to the environment. In this way, one hopes to translate results of this study to other similar compounds.
A description of the characteristics of each selected pharmaceutical will follow now together with their structure formula.

2.1 Acetylsalicylic acid (ASA)



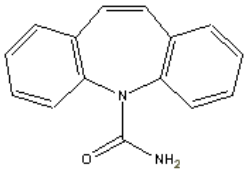
Acetylsalicylic acid (or aspirin) (ASA) is used for two functions. The most known one is probably the one of painkiller and anti-inflammatory agent. Aspirin is moreover a lot prescribed as inhibitor of platelet aggregation (to prevent heart attacks and blood clot formation) ((Wikipedia ,2008) and KNMP, 2006)).
Aspirin is consumed in high quantities in the Netherlands. It can be prescribed but can also sold over-the-counter. This makes the estimation of the consumption rate difficult.
When only the prescribed use of aspirin is taken into account, a consumption of 9.3 ton/year in 2006 is estimated (table 2-1) (CVZ 2006).
ASA is excreted via the urine. 1% is excreted in unchanged form. The other part is excreted as salicylic acid and metabolites of salicylic acid (KNMP 2006).
The high consumption of aspirin is also revealed in measurements of the influent concentrations in Waste Water Treatment Plants (WWTPs). About 3.2 ug/l of acetylsalicylic acid and 57-330 ug/l salicylic acid (the main metabolite) was measured in the research of (Fent 2006).
Moreover, aspirin is according to literature biodegradable (ECB, 2000). The biodegradation rate in the various environmental conditions, however, is not researched much. Besides the high consumption rate, aspirin is also selected because of its hydrophilic character. For hydrophilic pharmaceuticals, removal by sorption to sludge is not likely to play an important role.

2.2 Bezafibrate (BZF)



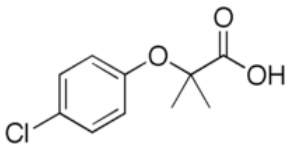
Bezafibrate is a fibrate used to treat diseases belonging to the cardiovascular system. Fibrates are used to treat hyperlipidemia. They help to lower the LDL cholesterol and triglyceride level in the blood and to increase HDL cholesterol level.
The consumption of bezafibrate was increasing over time. In 2006, the amount sold in the Netherlands is 0.88 ton/yr.
Excretion of bezafibrate occurs via the kidneys. About 50% is excreted in unchanged form. A 20% is excreted as glucuronide and the rest as other metabolites (KNMP 2006).
The influent and effluent concentrations of bezafibrate are reported in the range of 0.42-5 µg/l and 0-0.84 µg/l respectively (Fent, Weston et al. 2006). Bezafibrate is especially chosen because of its high low Kow value. Absorption to sludge is expected to be important compared with the more hydrophilic pharmaceuticals.

2.3 Carbamazepine (CBZ)



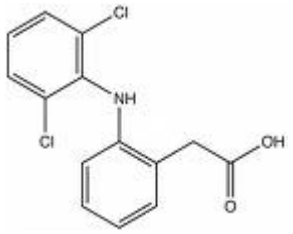
Carbamazepine is an anti-epileptic drug.. Although the consumption of carbamazepine is decreasing over the last 4 year, consumption in 2006 in the Netherlands is 8.6 ton/yr.
About 72% of carbamazepine and its metabolites are excreted via the urine. A 2% is excreted as the original compound. The other fraction consists of metabolites, among others the epoxide-metabolite and carbamazepine-diol (KNMP 2006).
The influent and effluent concentration in WWTPs are about the same and range from 0.7-1.5 µg/l ((Fent, Weston et al. 2006) indicating persistency to biodegradation.
The compound was detected in 44 rivers of the USA, in Canadian surface waters, Korean STPs effluents, in many surface waters in Europe and in the North Sea ((Han, Hur et al. 2006), (Jones 2001), (Fent, Weston et al. 2006) and (Weigel, Bester et al. 2001)). The highest mean concentration measured in a river is 1.2 µg/l ((Weigel, Bester et al. 2001)). Moreover, carbamazepine has been one of the substances which is detected most often in drinking water sources ((Versteegh and Dijkman 2007) in NL.
Carbamazepine is a non-acidic pharmaceutical with a moderate hydrophobic character.

2.4 Clofibric acid (CFA)



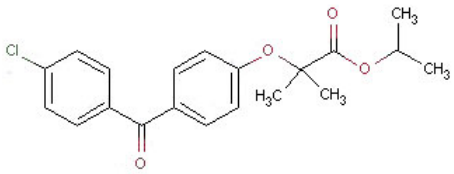
Clofibric acid (also named: clofibrin or chlorofibrinic acid) is the active metabolite of clofibrate, etofibrate and etofyllin clofibrate (Reemtsma 2006). They have the same function as bezafibrate: to treat hyperlipidemia.
The consumption of clofibrate in the Netherlands is quite low. In former days the consumption was higher. In other countries this might still be used in higher quantities.
The excretion fraction of clofibric acid after consumption of clofibrate is 40%, mostly via the urine.
Clofibric acid is measured in WWTP influents and effluents (Fent, Weston et al. 2006). The measured removal percentages range from 0 – 51 % ((Fent 2006). It is thus poorly degraded in WWTPs. The pollutant is detected in inland surface waters, in Guanabara Bay of Brazil (Stumpf, Ternes et al. 1999), ground water and in tap water (Heberer 1998).
Further, clofibric acid has a moderate hydrophobic character. It is selected mainly because of this character in combination with its poorly biodegradation rate.

2.5 Diclofenac (DCF)



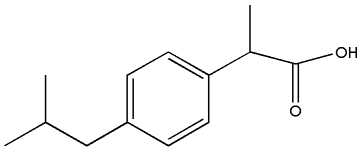
Diclofenac is used for analgesic, anti-inflammatory and anti-rheumatic purposes (KNMP 2006). Diclofenac can be sold over-the-counter like aspirin.
In 2006 diclofenac had about 1.5 million users in the Netherlands (only taking into account the prescribed use of diclofenac). But, the DDD (Daily Defined Doses) of diclofenac is quite low which results in a moderate consumption rate.
The majority of the excreted diclofenac is present in the urine. About 5% of the consumed pharmaceutical is present in unchanged form. The rest is excreted as metabolites. The most important metabolite is the 4-hydroxyderivate (KNMP 2006).
Observed concentration in WWTP effluents are in the range of 0.17 – 2.5 ug/l (Fent 2006). For diclofenac, some harmful effects at low concentrations has been observed. For example, a lowest observed effect concentration (LOEC) of only 1 ug/l for fish was determined (Triebskorn, Casper et al. 2004).
In addition, in Pakistan, India, Bangladesh and Nepal diclofenac has caused a severe decline of vultures in, after feeding themselves with domestic livestock and cattle, which were given diclofenac. All the dead vultures in which diclofenac is detected, have died because of problems related to renal failure. One of the side-effects of diclofenac is the occurrence of renal failure. (Oaks 2004).

2.6 Fenofibrate (FNF)



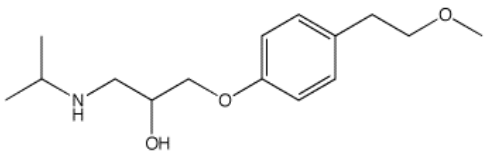
Fenofibrate is like bezafibrate and the parent compounds of clofibric acid a antihyperlipidemic drug.
Fenofibrate is a drugs which is not used in the Netherlands nowadays (KNMP 2006). But it is detected in drinking water samples in the Netherlands (Versteegh and Dijkman 2007). Likely it is consumed in other countries.
Fenofibrate is for 60-93% excreted via urine as metabolites, primarily as fenofibric acid and its glucuronate conjugate.
Not much is known about fate of fenofibrate in WWTPs.
For this research was mainly selected analogously to bezafibrate, for its hydrophobic character (exceptionally high Kow value).

2.7 Ibuprofen (IBU)



Ibuprofen is an analgesic, anti-inflammatory and anti-rheumatic drug as diclofenac. It can be sold over-the-counter or consumed by prescription.
The consumption of ibuprofen is high. It has over 1 million users and its amount sold each year is the second largest of all selected pharmaceuticals (table 2-1).
Excretion of ibuprofen takes place almost completely via the urine. About 1% is present as original compound (KNMP 2006).
The influent of ibuprofen in WWTPs ranges from 3 – 39 ug/l according to the results of several researches (Fent 2006). Ibuprofen can disappear in WWTPs for more than 90%. But still the presence of the compound is measured in surface waters and drinking water, likely because of the continuous input of the pharmaceutical. Mean concentrations measured are in the ranges of about 4 ng/l up to 10 µg/l in surface waters and WWTP effluents (Jones 2001).

2.8 Metoprolol (MTP)



In the Netherlands, a high number of prescribed drugs concern pharmaceuticals for heart diseases. Especially beta-blockers are a lot prescribed and within this category, by far metoprolol is used most (50% of the used beta-blockers concern metoprolol) (CVZ 2006). Metoprolol can be prescribed for several cardiovascular related diseases, especially hypertension.
In 2006 metoprolol was prescribed to 826.100 of users consuming metoprolol (table 2-2).
Excretion of metoprolol takes place mainly via the urine: a 5-30% is excreted in unchanged structure (KNMP 2006).
Effluent concentrations of municipal WWTP 0.73 µg/L metoprolol are reported by (Petrovic 2006).
Metoprolol is removed in WWTP for less than 10% according to Paxeus (2004). In Ternes (1998) a removal of 83% is reported. The biodegradability of metoprolol is therefore somewhat unclear and probably depends on process conditions.
Metoprolol has a hydrophilic character and is not acidic (in contrast to many other pharmaceuticals).
Metoprolol can have effects on the heart of invertebrates in the environment. In D. Magna metoprolol caused at low concentration acceleration of the heart beat (Fent, Weston et al. 2006).

The consumption figures of selected pharmaceuticals in the Netherlands and the physical-chemical and biological properties of the selected pharmaceuticals are summarized in table 2-1 and 2-2.

Table 2-1: Prescribed consumption of the selected pharmaceuticals in the Netherlands in 2006 (source: (CVZ 2006)).

Thera-peutic class	Pharmaceutical	Amount of users in NL (2006)	Fraction of users (%) within a class	Fraction of users (%) in the Netherlands	DDD (mg/p/d)	Amount of DDDs sold in NL in 2006	Amount sold (ton/yr)
B & N	Acetylsalicylic acid	656,950	34; 0.006 resp.	4.1	50; 3000 resp.	179,797,570	9.3
M	Ibuprofen	1,156,900	34	7.2	1200	23,627,700	28.4
M & S	Diclofenac	1,491,248	45; 2 resp.	9.3	100	52,189,600	5.2
C	Metoprolol	861,640	24	5.4	150	144,505,000	21.7
N	Carbamazepine	59,962	1.7	0.4	1000	8,570,600	8.6
C	Bezafibrate	3,222	0.1	0.02	600	532,670	0.3
C	Clofibrate	275	0.008	0.002	2000	31,590	0.1
C	Fenofibrate	0	0	0	200	0	0.0

B - Blood and blood forming organs, C - Cardiovascular system, M - Musculo-skeletal system, N - Nervous system and S - Sensory organs

Table 2-2: Summary of the physical-chemical and biological properties of selected pharmaceuticals. The biodegradation rate is based on the biodegradation constants measured in Joss et al. (2006).

Pharmaceutical	Physical-chemical character (based on log Kow)	Acidity	Biodegradability (based on kinetic constants)
Aspirin	hydrophilic	Acid	Moderate
Ibuprofen	moderately hydrophobic	Acid	High
Diclofenac	varying, depending on pH	Acid	Low
Metoprolol	hydrophilic	Neutral	Moderate
Carbamazepine	moderately hydrophobic	Neutral	Low
Clofibric acid	moderately hydrophobic	Acid	Moderate
Bezafibrate	hydrophobic	Acid	Moderate
Fenofibrate	hydrophobic	Acid	n.a.

The relevancy of the selected pharmaceuticals is confirmed by a research of the RIVM (Dutch Institute for Public Health and Environment) which has detected all the selected pharmaceuticals in drinking water sources in the Netherlands (Versteegh and Dijkman 2007).

3 Fate of selected pharmaceuticals in biological treatment systems: literature review

3.1 Removal mechanisms

Pharmaceuticals can be removed from the aqueous phase in a water treatment plant due to several processes. These are biodegradation, sorption, stripping to air and abiotic transformation. The processes and their relevancy for the selected pharmaceuticals will be discussed in this chapter.

3.1.1 Biodegradation

Biodegradation is a very important process in the transformation of organic pollutant in WWTP, (possibly) including pharmaceuticals. It can result in a energy gain for bacteria but it can also occur co-metabolically during degradation of other organic compounds. In the latter case, other energy sources have to be available.
Biodegradation in a WWTP can be partially or completely (mineralization). In case of the final products are carbon dioxide (CO2) and water (H20). Partial biodegradation results in the transformation of the pharmaceutical to metabolites (process also occurring in the human body). Metabolites can be persistent and therefore it is important to determine their fate in biological systems as well.
The biodegradation rate of a certain pharmaceutical can be described with a pseudo first order reaction (Ternes, 2006):

$$\frac{dC_i}{dt} = k_{biol,i} * SS * C_i$$

In where:
C_i total concentration of pharmaceutical *i* (µg/L)
t time (d)
$k_{biol,i}$ specific biological degradation rate constant of pharmaceutical *i* (L/gSS/d)
SS suspended solids concentrations (g/L)
This first order reaction shows that the degradation rate is proportional to the concentration of pharmaceuticals. The rate is also influenced by the suspended solids concentration, but this parameter is assumed to be constant in WWTPs or during batch tests where often biodegradability of compounds is being assessed. Therefore the reaction is pseudo first order.
Based on the specific biological degradation rate constants obtained from aerobic batch tests pharmaceutical compounds can be classified according to their removal due to biodegradation in conventional WWTP (Ternes, 2006):

k < 0.1 L/gSS/d: no removal (less than 20%)
0.1 < k < 10 L/gSS/d: partial removal (20-90%)
k > 10 L/gSS/d: more than 90% removal

In other sections of this chapter, the specific k-values of the selected pharmaceuticals will be further discussed.

3.1.2 Sorption

Sorption to sludge can be an important removal mechanism especially when a pharmaceutical is persistent and has a high sorption potential. Liphophilic properties and the electrostatic state is important for the amount of pharmaceutical that is sorbed to the sludge. Two different kinds of sorption mechanisms can take place: absorption and adsorption.

3.1.2.1 Absorption

Absorption is related to hydrophobic interactions of aliphatic and aromatic groups of a compound with the lipid fractions of the solids (Ternes and von Gunten 2005). The hydrophobic character of a compound can be indicated with the K_{ow} value. K_{ow} is the partition coefficient between octanol and water for a specific compound. The higher the log K_{ow} value, the more hydrophobic a substance is.
Three groups can be distinguished for their sorption behaviour based on the Log K_{ow} values (Jones 2005):

Log K_{ow} < 2.5	Low sorption potential
Log K_{ow} > 2.5 but < 4.0	Medium sorption potential
Log K_{ow} > 4.0	High sorption potential

The values of log K_{ow} of the eight selected pharmaceuticals are listed in table 3-1. Bezafibrate and fenofibrate are the most hydrophobic pharmaceuticals, with a log K_{ow} >4,0. From all the selected pharmaceuticals, removal due to absorption is expected be the most important for these two compounds. Aspirin will be least absorbed to sludge, this compound will stay in the water phase. Its log K_{ow} value is only 1.43 (Ternes 2006).

3.1.2.2 Adsorption

Adsorption is related to electrostatic interactions with the substance and the surface of micro-organisms. Because sludge is negatively charged, it will attract positively charged molecules and reject negatively charged molecules. Most of the selected pharmaceuticals are acidic and therefore at neutral pH, negatively charged. This decreases their adsorption affinity to the sludge. The pKa value indicates the acidity of a pharmaceutical. The lower this value, the more acidic a compound is. From selected compounds only metoprolol and carbamazepine are not acidic (see table 3-1). It increases their sorption affinity. However, their log K_{ow} value is quite low (1.9 and 2.7 resp.) which means that they are not hydrophobic and thus will not be absorped to a large extent.

Table 3-1: Physical-chemical properties of the selected pharmaceuticals. Source: Ternes (2006) and 1 (Van Beelen, 2007).

Pharmaceutical	Log Kow	pKa value at T = 20 ^{0}C
Aspirin	1.426	3.5
Bezafibrate	4.25	3.6
Carbamazepine	2.69	13.9
Clofibric acid	2.57	3.0
Diclofenac	0.7-4.5 depending on pH	4.15
Fenofibrate	5.19[1]	n.a.
Ibuprofen	3.481	4.5-5.2
Metoprolol	1.9	9.7

3.1.2.3 Solid-liquid partition coefficient

To determine the sorption of a pharmaceutical to sludge or other solids the solid-liquid partition coefficient, K_d, can be used, if available. This coefficient shows the overall sorption affinity of a compound and therefore it takes into account both adsorption and absorption processes. The solid-liquid partition coefficient is calculated with the following formula under

equilibrium conditions. The suspended solids concentration is required as well, since it influences the sorption too.

$C(i, sorbed) = K_{d,i} * SS * C(i, soluble)$

where:
C(i, sorbed) the particulate concentration of a compound *i (mg/L)*;
$K_{d,i}$ the sorption constant of a compound *i (L/kg SS)*;
SS suspended solids concentration in wastewater (kg $L^{-1}_{wastewater}$);
C(i, soluble) the soluble concentration of a compound i *(mg/L)*;;

The fraction of sorbed pharmaceutical related to the total pharmaceutical concentration can be described by the following:

$$\frac{C(i,sorbed)}{C(sorbed)+C(i,soluble)} = \frac{Kd,i*SS}{1+Kd,i*SS}$$

Sorption in conventional municipal WWTP can be neglected when K_d value < 500 L/kgSS (<10% sorption if sludge production between 200 - 400 gSS/m3 (Ternes and von Gunten 2005). However, if the biodegradability of a pharmaceutical is low, sorption can be the main removal mechanism if the compounds has sorption affinity.
For three of the 8 selected pharmaceuticals, the K_d value was determined by other studies (table 1-2). As it can be seen from this table the K_d value depends strongly on the characteristics of the sludge. With respect to secondary sludge, for none of the 4 pharmaceuticals sorption seems to be an important removal mechanism. Probably because these pharmaceuticals are not very hydrophobic and except for carbamazepine, they are also acidic.

Table 3-2: Partitioning coefficients of 4 pharmaceuticals for primary and secondary sludge (Ternes, Herrmann et al. 2004).

Compound	primary sludge Kd (L/kg SS)	Secondary sludge Kd (L/kg SS)
Diclofenac	459±32	16.0±3.1
Ibuprofen	- (< 20)	7.1±2.0
Clofibric acid	- (< 30)	4.8±2.5
Carbamazepine	- (< 20)	1.2±0.5

Considering the above, only a minor part of the pharmaceuticals will be absorbed. Sorption is likely to be most significant for fenofibrate and bezafibrate.

3.1.3 Vaporization

The percentage of a compound that is vaporized during wastewater treatment depends on Henry coefficient and the amount of air getting in contact with the treated wastewater. The K_{aw} is the water-air partitioning coefficient for a certain compound and defined as:

$$K_{aw} = \frac{C_{air}}{C_{water}} = \frac{H}{RT}$$

where:
K_{aw} = partitioning coefficient (-)
Cair = concentration of pollutant in air (mg/L)
Cwater = soluble concentration of pollutant (mg/L)
H = Henry's law constant (atm m3/mol)
R = gas constant (atm.m3/mol/K)

T = Temperature (K)

A partitioning coefficient between air and water of >3 * 10-3 is required for effects of stripping to air in a reactor with fine bubble aeration. (Ternes 2006). Table 3-3 shows that the Henry Law constant and the K_{aw} of pharmaceuticals are very low. As a result, vaporization is not regarded to be as a significant mechanism for removal of the pharmaceuticals.

Table 3-3: Henry's law constants and partitioning coefficients for selected pharmaceuticals. (Source: US National Library of Medicine), T = 25 ^{0}C.

Pharmaceutical	Acetyl-salicylic acid	Clofibric acid	Carba-mazepine	Diclofenac	Ibuprofen	Metoprolol
Henry's Law constant (atm.m^3/mol)	1.30E-09	2.19E-08	1.08E-10	4.73E-12	1.50E-07	1.40E-13
K_{aw} (-)	5.32E-08	8.96E-07	4.42E-09	1.93E-10	6.13E-06	5.73E-12

3.1.4 Abiotic transformation

Abiotic transformation may occur via the processes of hydrolysis and photolysis. Andreozzi (1998) has determined half-lives of carbamazepine, clofibric acid and diclofenac for photolysis. In a test with glas-disk reactors in a thermostatic bath at a temperature of 25 oC direct photolysis was analyzed in various seasons and at several latitudes (20 oN – 50 oN). During winter and 50oN latitude the half-lives of carbamazepine and clofibric acid were in the order of 100 days. Half-live of diclofenac was in the range of 5 days. In summer the t1/2 for DCF was lowered to approximately 0.5 d (Andreozzi 2003).
Another research showed the rapid degradation of diclofenac in the lake Greifensee (in Switserland). The removal of diclofenac in this lake was over 90% (inflow and outflow concentration of max. 370 ng/L and max. 12 ng/L resp.), most likely due to photodegradation (Buser, Poiger et al. 1998). A first order degradation rate was determined in a laboratory experiment with a half-live of less than 1 hr in autumn at a latitude of 47^{o}N (Buser, Poiger et al. 1998). Metabolites were not studied in this case, thus this elimination of diclofenac could result from the production of OH-diclofenac to a much more advanced degradation.
Photolysis of diclofenac in lakes can thus be significant. For WWTPs, this process is however not so relevant because there is (almost) no light in activated sludge tanks.

3.2 Observed removal of selected pharmaceuticals in different biological systems

3.2.1 Removal of selected pharmaceuticals in conventional municipal WWTPs

Removal of pharmaceuticals in conventional municipal WWTPs consisting in general of a preliminary treatment, a secondary biological treatment and a clarifier, was assessed for different treatment plants in different countries. Removal rates of the selected pharmaceuticals are listed in table 3-4. This removal includes biodegradation of the original pharmaceutical and sorption. These processes are not much researched separately during investigations of fate of pharmaceuticals in WWTPs. Further, the removal rates refer mostly to the disappearance of the original parent compound. Conjugates, which are cleaved in the WWTP, formation of metabolites or other degradation products are not taken into account. These all can lead to an over-/underestimated removal rate.
Joss (2006) proposed to classify biodegradability of pharmaceuticals in WWTP according to their biodegradation rate constants. The classification of the selected pharmaceuticals is presented in table 3-4 too.

Table 3-4: Removal of selected pharmaceuticals in municipal WWTP as reported in literature. na = not available; pharmaceuticals are listed in alphabetical order

Pharmaceutical	Classification of the removal based on kinetic degradation constants (Joss et al.2006)	Removal in municipal WWTP or pilot WWTP (%)	References
Aspirin	Partial (20-90%)		
Bezafibrate	Partial (20-90%)	0-97 , 83	(Strenn 2004), (Ternes 1998),
Carbamazepine	No removal (0-20%)	0-29	(Strenn 2004), (Miao 2005) (Ternes 1998),
Clofibric acid	Partial (20-90%)	0-51	(Ternes 1998), (Tauxe-Wuersch 2005), (Zwiener 2002)
Diclofenac	No removal (0-20%)	0- 69	(Kosjek, Heath et al. 2007) (Tauxe-Wuersch 2005), (Strenn 2004)
Ibuprofen	Removal of >90%	10- >90, 91	(Kosjek, Heath et al. 2007), (Strenn 2004), (Carballa and Carmen Garcıa-Jares 2004) (Tauxe-Wuersch 2005), (Ternes 1998)
Fenofibric acid	Partial (20-90%)	64	(Ternes 1998)
Metoprolol	n.a.	<10, 83	(Paxeus 2004), (Ternes 1998)

The observed removal rates differ a lot between various pharmaceuticals and sometimes also for one pharmaceutical.

Acetylsalicylic acid (aspirin) is expected to biodegrade well, because it easily hydrolyses to the metabolite salicylic acid. This is a naturally occurring compound. Aspirin is observed to be degraded to a large extent according to Joss et al. (2006).

Bezafibrate is partially removed in WWTPs. Ternes (1998) found a removal of 83%. In Strenn (2004) the removal varied between 0 and 97% depending on the conditions.

Removal of carbamazepine is low in all researches. The highest removal efficiencies were found by (Miao 2005) – 29% over different treatment units of the wastewater treatment plant in Canada. This treatment plant included an UV treatment step (unlike the other researched WWTPs). He studied also removal of metabolites of CBZ. No significant removal for its metabolites was detected. In other researches, removal of carbamazepine was lower than 10%.

Clofibric acid is poorly to moderately degraded in WWTPs. Removal percentages range from 0-51%. This range is quite large. The explanation for the high variation is unclear. Different configurations of the WWTPs or analytical methods could have caused these differences.

Diclofenac is a compound for which a high variation in removal rates has been identified as well. The exact grounds for this are unclear as well. According to Reemtsma (2006) sludge age is likely to play an part in this.

Ternes (1998) concluded that fenofibrate was completely removed from the aqueous phase in a municipal WWTP. The metabolite fenofibric acid was removed for 64% (Ternes 1998).

Ibuprofen was in some researches removed to a large extent. Some removal rates are however very low. The low removal rate of 10% in table 3-4, refers to winter conditions (Tauxe et al., 2005). In the same research a high removal rate of 79% was obtained in summer for a WWTP with a HRT of 16 h. Kosjek (2007) observed a removal of 91% for a pilot WWTP with a HRT of 48 h.

Metoprolol was well degraded in a research of Ternes (1998). Paxues observed a very low removal of metoprolol. More researches are not found for metoprolol.

Part of all the discrepancies between the observed removal rates can be explained by the different properties of the different WWTPs (like solid retention time (SRT), hydraulic retention times (HRT) and redox conditions), variation in climate (influencing operational temperature) and different configurations.

For carbamazepine, ibuprofen and diclofenac, the influence of temperature and sludge age on the removal efficiencies in different treatment systems was investigated (figure 3-1).
Because both temperature and sludge age are varying between the different systems, interpretations are difficult. For ibuprofen, the system operating at the highest temperature (21 ºC) also shows the highest removal. Further, there is a high variation in sludge age between the different systems, but the high variation in SRT turns out not to influence greatly the removal of all three pharmaceuticals. A MBR with a SRT of 75 d tends to remove less diclofenac than the ones operation with a SRT of 16 and 33 days.
Carbamazepine is not affected at all in all systems: there is no significant removal presented of this compound. This is in coherence with the other findings on carbamazepine.

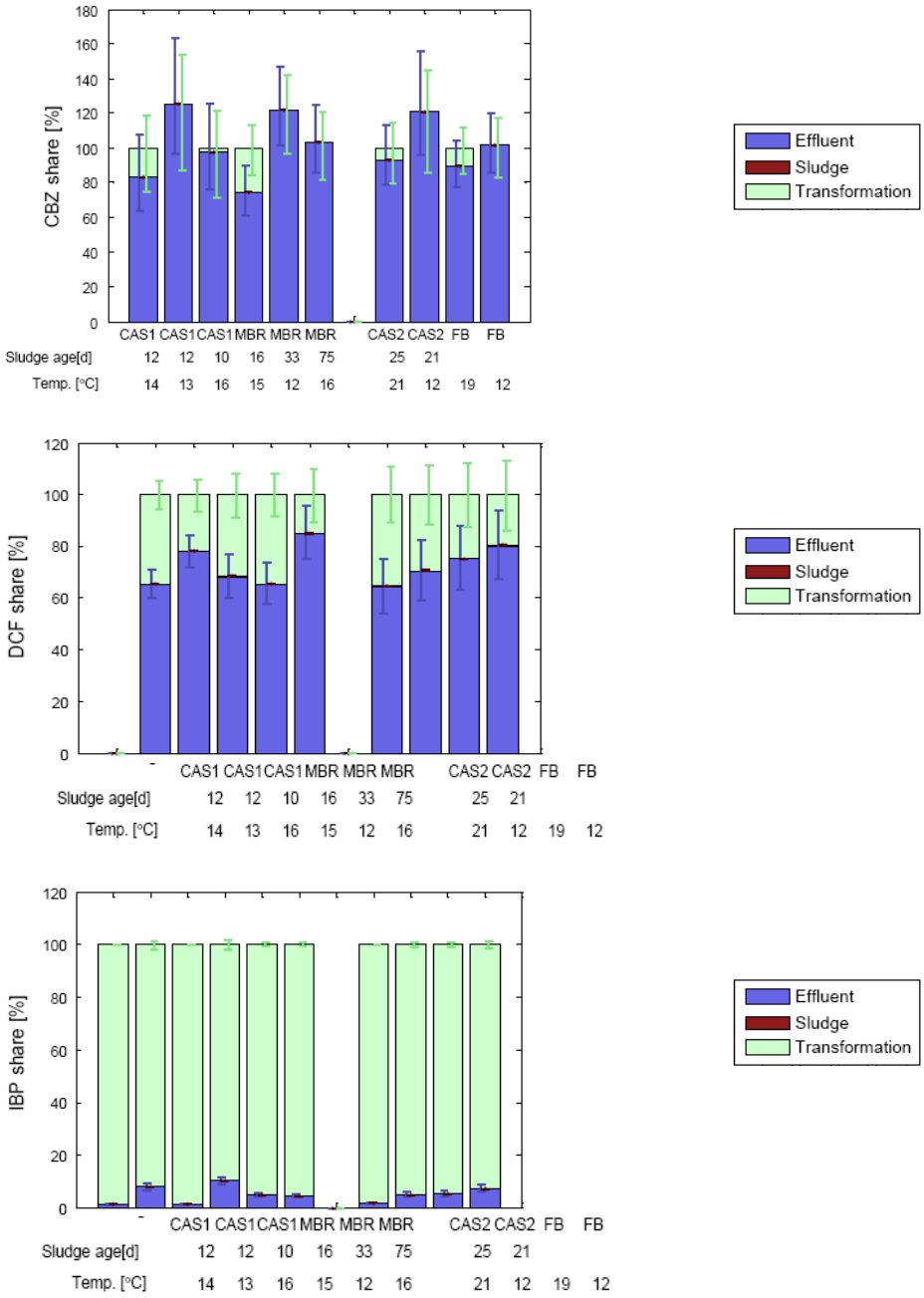


Figure 3-1: Removal of pharmaceutical compounds in full scale conventional activated sludge (CAS), pilot membrane bioreactor (MBR) and fixed bed (FB) WWTP systems; CBZ: carbamazepine, DCF: diclofenac, IBP: ibuprofen. On the y-axis the pharmaceutical amount normalized to the influent amount. (Joss 2005)

3.2.2 Removal in anaerobic digesters

Only few information is available on the fate of selected pharmaceuticals in anaerobic digestion systems. Two anaerobic pilot scale reactors digesting sewage sludge were applied to determine and assess the removal efficiencies of pharmaceuticals at different SRT (Carballa, Omil et al. 2007). The reactors were operated at mesophilic (37°C) and thermophilic (55°C) conditions. The suspended solids concentration was between 30-95 g/L. For ibuprofen a medium removal (+/- 40%) for both reactors was measured. The removal efficiency of diclofenac varied a lot between the different conditions. Removal was between

very low and quite high. An SRT of 10 d gave the highest removal efficiency for ibuprofen and diclofenac. For all the other compounds, the SRT had no significant influence on the biodegradation of pharmaceuticals. For carbamazepine, none to very low removal was observed. (table 3-5) Temperature had in general no effect on the removal between mesophilic and thermophilic anaerobic pilot reactors (Carballa, Omil et al. 2007).

Table 3-5: Removal of three of the selected pharmaceuticals during anaerobic digestion of sludge. SA: removal after sludge adaptation (Carballa et al, 2007).

Compound	Mesophilic	Thermophilic
Carbamazepine	No removal	No removal
Diclofenac	0-75% ; 69±10 SA	25-75%; 69±10 SA
Ibuprofen	41±15%	41±15%

Only the removal from the aqueous phase was investigated, therefore the fraction bounded to suspended solids is not known.

3.2.3 Removal in water and sediment

Fate of ibuprofen, carbamazepine and clofibric acid in water and sediment systems has been investigated by Ternes (2004). Table 3-5 includes the DT50 values of three pharmaceuticals. The DT50 is the time that is required to eliminate 50% of the pharmaceutical from the aqueous phase.

Table 3-6: Dissipation values for pharmaceuticals in water and in water + sediment. DT50 = the time required for 50% dissipation of the pharmaceutical concentration in aquous phase. (Ternes 2004).

Pharmaceutical	DT50 Water	DT50 Water/Sediment	Sorption
Ibuprofen	10 d	<20 d	Low
Carbamazepine	52 d	333 d	Unclear
Clofibric acid	82 d	119 d	Low

In aquatic systems ibuprofen is expected to be eliminated from the aqueous phase relatively fast because of the low DT50-value. Because sorption is expected to be low for ibuprofen, it is expected that at least part of the eliminated compound is biodegraded (Ternes 2004).
Carbamazepine and clofibric acid will be quite persistent in as well aerobic and anaerobic compartments of the water and sediment. Their half-live varies from 50 up to 333 days.

3.2.4 Removal of pharmaceuticals in source separated sanitation systems

In source separated sanitation, where black water (=toilet wastewater) and/or urine are separately collected and treated, the pharmaceutical concentration is generally significantly higher than in conventional sewage. Next to it, also other contaminants, organic matter and nutrients are present in high concentrations.

3.2.4.1 Urine

Removal of pharmaceuticals in urine using biological systems is an option although available literature is more focusing on the chemical removal alternatives (like ozonation and nanofiltration), for example in (Maurer, Pronk et al. 2006).

3.2.4.2 Black water

Fate of pharmaceuticals during biological treatment of black water has not been researched much either. Black water is usually (pre-)treated under anaerobic conditions. Therefore the fate of pharmaceuticals in this step might be comparable with the ones observed in anaerobic digesters of municipal sludge (see 3.2.2). On the other hand the pharmaceutical concentration in black water is much higher which can influence the removal rate.
The only information on the fate of human pharmaceuticals and human hormones in black water can be found in the PhD thesis of de Mes (2007) about the fate of the hormones estrogen (E1) and 17α- ethynyloestradiol (EE2) in biological treatment of black water. In this research, the fraction adsorbed to sludge was around 50% in batch test for both compounds present at a start concentration of 5 mg/l, but no degradation of EE2 was observed. For E1 a half-live was determined of 42 d. Under aerobic conditions the degradation was much higher ($t_{1/2}$<1 d) (de Mes 2006).
The hormones E1 and EE2 are semi-hydrophobic. Their log K_{ow} values vary from 3.43-4.1 (Ternes, 2006). Considering the log K_{ow} values of the selected pharmaceuticals in this research, the fraction bezafibrate and fenofibrate absorbed to suspended solids can be in the same order as the one that is observed for E1 and EE2 in de study of de Mes (2007).

3.3 Biodegradability of selected pharmaceuticals under various process conditions during laboratory batch tests

3.3.1 Aerobic conditions

The degradation of pharmaceuticals in batch tests was studied under aerobic conditions for many of the selected pharmaceuticals. Quintana (2005) researched the mineralization of the pharmaceuticals: bezafibrate, diclofenac and ibuprofen in batch tests. The batch test consisted of fresh sludge, a carbon source and pharmaceuticals, operating with activated sludge. Within a timeframe of 28 days, bezafibrate was 100% transformed and 30% was completely mineralized. Ibuprofen was for 96% mineralized and diclofenac was not mineralized at all.
Joss (2005) determined under aerobic conditions in batch tests the biodegradation reaction constants. Batch tests with sewage sludge from a conventional activated sludge treatment plant (CAS) and with sludge from a membrane bioreactor (MBR) were performed The CAS consisted of a nitrification, partial denitrification and chemical phosphorus removal step and the MBR of a nitrification, denitrification and biological phosphorus removal step. Pharmaceutical concentration were comparable with those measured in influent of conventional WWTPs (3 µg pharmaceutical /L). The calculated first order degradation constants, based on the outcomes of the batch tests are given in Table 3-7.

Table 3-7: Biological degradation constants from batch experiments using sludge from CAS and MBR. T = 17 ± 1^{0}C. Source: (Joss, Zabczynski et al. 2006).

Pharmaceutical	k_{biol} (L/gSS/d) for CAS	k_{biol} (L/gSS/d) for MBR
Acetylsalicylic acid	n.a.	n.a.
Bezafibrate	2.1-3.0	3.4-4.5
Clofibric acid	0.3-0.8	0.1-0.23
Carbamazepine	n.a.	n.a.
Diclofenac	<0.1	<0.1
Fenofibrate	n.a.	n.a.
Ibuprofen	21-35	9-22
Metoprolol	n.a.	n.a.

3.3.2 Anoxic conditions

Little is known about the biodegradation of pharmaceuticals under anoxic conditions. In general biodegradation of organic compounds under anoxic conditions proceed slower than under aerobic conditions.
Anoxic degradation has been described by Zwiener (2002) for ibuprofen. Under aerobic and anoxic conditions batch tests with sludge from municipal WWTP were performed to determine the degradation of ibuprofen. The test show a degradation of ibuprofen with 22% under anoxic conditions after 51 hr compared to 75% under aerobic conditions (Zwiener 2002).

3.3.3 Anaerobic conditions

Little information could be found about the biodegradation of pharmaceuticals at anaerobic conditions. Reaction rates are in general lower in anaerobic tests. For the selected pharmaceuticals the rate is also expected to be lower than those under aerobic and anoxic conditions.

3.4 Metabolites

During the transformation of pharmaceuticals metabolites are produced. This occurs as well as in the human body as during biotransformation in with biomass. This research does not focus on these metabolites but their biodegradability is of importance. The main known metabolites of the selected pharmaceuticals are described in this section and if available their biodegradability.

3.4.1.1 Ibuprofen

Three identified degradation products of ibuprofen are hydroxyl-ibuprofen (OH-Ibuprofen), carboxy-ibuprofen (CA-Ibuprofen) and carboxy-hydratropic acid (CA-AH) (Zwiener 2002) (figure 3-2). These metabolites are also known to be formed during the human metabolism of ibuprofen.

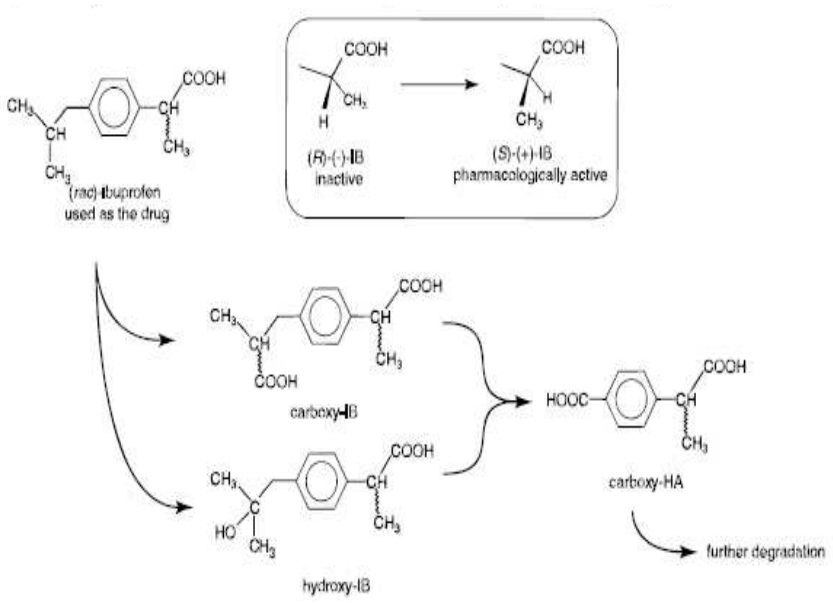


Figure 3-2: Part of the degradation path of ibuprofen. Source (Buser, Poiger et al. 1999).

The metabolites of ibuprofen were found to be removed efficiently. Only small amounts were detected in WWTPs effluents. In lakes, only ibuprofen is detected and none of its metabolites (Buser, Poiger et al. 1999) (Reemtsma 2006).
The metabolites of ibuprofen do not seem to be persistent and therefore not considered as problematic.

3.4.1.2 Acetylsalicylic acid

Acetylsalicylic acid can be transformed to salicylic acid (KNMP 2006). Other metabolites of acetylsalicylic acid are salicyluric acid and gentisic acid (Hansen, Jensen et al. 1998). Biodegradation of salicylic acid can also happen easily because it is produced by nature itself as well. Removal rates of 99% of salicylic acid are observed in WWTPs (Fent, Weston et al. 2006).

3.4.1.3 Diclofenac

Degradation products of diclofenac have been identified by Kosjek et al. (2007) in effluent of a pilot WWTP (with aerobic and anaerobic compartments). The products are given in Figure 3-3. Not much is known about their biodegradability.

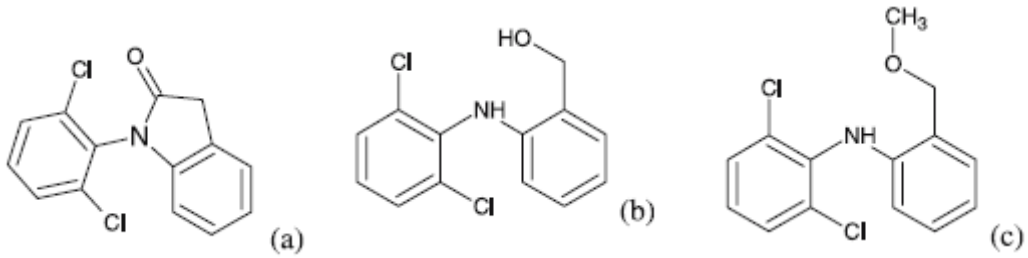


Figure 3-3: Degradation products of diclofenac. 2,6-dichlorophenyl-1,3-dihydro-2H-indol-2-one (a), 2-((2,6-dichlorophenyl)amino)benzyl alcohol (b) and 2-((2,6-dichlorophenyl)amino)benzyl alcohol methyl ether. Source: (Kosjek, Heath et al. 2007).

3.4.1.4 Carbamazepine

Carbamazepine is degraded in the human body into many metabolites. A 33 metabolites have been identified in urine. The main metabolites were investigated by Miao et al (2005) for their behavior in a conventional WWTP with UV as post treatment. These compounds are listed in Figure 3-4. The main metabolic pathway for carbamazepine degradation is oxidation to a expoxycarbamazepine (CBZ-EP) and the subsequent formation to CBZ-DiOH. (Miao 2005) and (Kitteringham, Davis et al. 1996).
At every sampled location in the wastewater treatment plant of Peterborough (UK?) the highest mean concentrations were for carbamazepine and for CBZ-DiOH. Other metabolites have been detected as well but in lower concentrations (Miao 2005).
In biosolids mainly the parent compound carbamazepine was present. Thus the metabolites are probably more polar than the carbamazepine itself.
Comparison of the metabolite concentrations of influent and effluent shows that the metabolites are not degraded. The effluent concentration is the highest for CBZ-DiOH followed by carbamazepine and other metabolites (Miao 2005).

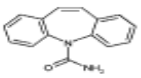
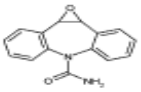
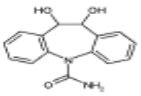
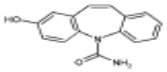
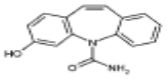
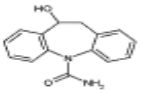
Structure	Analyte	Abbreviation [CASRN[a]]
	Carbamazepine	CBZ [298-46-4]
	10,11-dihydro-10,11-epoxycarbamazepine	CBZ-EP [36507-30-9]
	10,11-dihydro-10,11-dihydroxycarbamazepine	CBZ-DiOH [35079-97-1]
	2-hydroxycarbamazepine	CBZ-2OH [68011-66-5]
	3-hydroxycarbamazepine	CBZ-3OH [68011-67-6]
	10,11-dihydro-10-hydroxycarbamazepine	CBZ-10OH [29331-92-8]

Figure 3-4: Five main metabolites of carbamazepine (Miao 2005).

3.4.1.5 Metoprolol

The biodegradation products of metoprolol produced in WWTPs are unknown. In the metabolism of humans and animals however, the transformation of metoprolol is studied. A scheme of possible degradation pathways of metoprolol is given in
Figure 3-5.

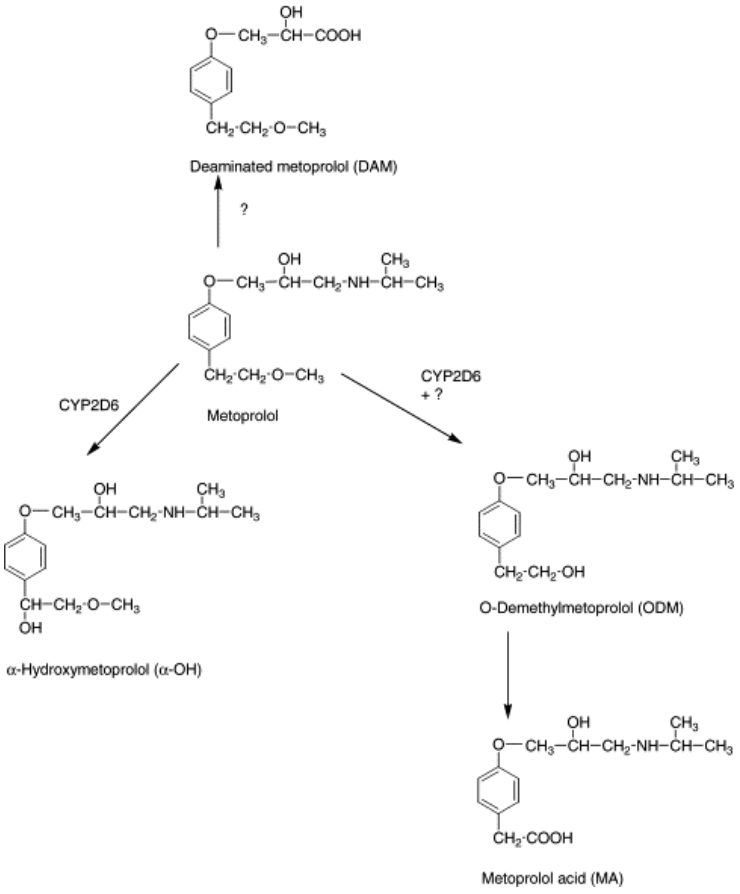


Figure 3-5: Pathways for metoprolol degradation (Fang, Semple et al. 2004).

Metabolites of metoprolol are, according to the figure 3-5: α-hydroxymetoprolol, O-demethylmetoprolol, metoprolol acid and deaminated metoprolol (Fang, Semple et al. 2004). The fate of the metabolites of metoprolol in WWTPs is not reported in literature.

3.4.1.6 Clofibric acid

Clofibric acid is a metabolite of three fibrates. Biodegradation products of clofibric acid and their behavior in environment were not found in literature.

3.4.1.7 Bezafibrate

The degradation of bezafibrate is not much researched either. A suggested metabolite is 4-chlorobenzoic acid. This compound can be mineralized completely. Formation and disappearance of this compound was detected in a bezafibrate aerobic biodegradation test with activated sludge (Quintana 2005).

3.4.1.8 Fenofibrate

Fenofibrate is in the human body easily hydrolyzed with esterases to fenofibric acid. This is the pharmaceutically active compound (Internet Drug Index 2008). The microbial metabolic pathway of fenofibric acid is not known.
Overall, for the selected pharmaceuticals much information is lacking about the fate of produced metabolites. For ibuprofen and carbamazepine most data is reported in literature.

3.5 Conclusions (knowledge gaps)

Biodegradation and sorption appear to be the main removal mechanisms of pharmaceuticals during biological treatment. With respect to biodegradation, it is important to distinguish between transformation and mineralization. More information is required on the concentration and properties of produced metabolites. Sorption is especially relevant for hydrophobic and persistent compounds. Vaporization and photolysis do not seem to be a significant factor in the removal of selected pharmaceuticals in a WWTP.
Removal rates have been determined for several pharmaceuticals in municipal WWTPs. However, not much distinction has been made between the different removal pathways. Therefore processes as sorption and degradation can not be quantified separately. Also, conjugates and metabolites which are present in WWTP are not often taken into account.
The fate of pharmaceuticals during biological treatment of concentrated waste streams, such as urine and black water has been hardly studied.
For some pharmaceuticals biodegradation kinetics under aerobic conditions have been determined. This is not yet the case for all of the selected pharmaceuticals. Next to this, information about degradation kinetics under anoxic and anaerobic conditions is lacking. Available data indicates lower degradation kinetics under anoxic conditions compared to aerobic situations.

4 Materials and Methods

To investigate the biodegradability of pharmaceuticals in concentrated wastewater, laboratory batch tests were performed. Because this research focuses on the case study Anderen, in which urine is separately collected, the utilized concentration of pharmaceuticals in the batch tests is mainly based on the expected calculated concentration of pharmaceuticals in urine. To determine the degradation of eight different pharmaceuticals under various conditions in wastewater treatment systems, five different experimental set-ups were designed varying redox potentials and temperatures. A summary of the performed experiments is given in table 4-1.
The analysis of pharmaceuticals in sludge samples is complex and it requires expertise. The samples have been analysed by the Dutch research institute RIVM.

Table 4-1: Summary of the different batch tests that were performed. The anaerobic and the aerobic test at 20ºC tests were performed twice.

	Aerobic	Anoxic	Anaerobic
Temperature 10 ºC	X	X	
Temperature 20 ºC	X	X	
Temperature 30 ºC			X

This chapter starts with calculations about the expected concentrations of pharmaceuticals in the urine and black water (section 4.1). Then, a description follows about the origin and characteristics of the chemicals and sludge (section 4.2 and 4.3). In section 4.4 the experimental set-ups are given. Section 4.5 elaborates on the method of analysis.

4.1 Predicted concentrations of pharmaceuticals in concentrated wastewater streams

4.1.1 Urine

Urine can be separately collected from faeces, as is also the case in project Anderen. The estimation of the pharmaceutical concentrations in urine involved some assumptions and uncertainties. The calculated concentrations of pharmaceuticals in human urine (UC) were calculated from the Daily Defined Doses (DDD) and excretion fraction of the original (parent) compound (eq. 4.1). The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults (WHO 2006). Real dosage can deviate from this value, because it is determined by the physician for each person individually. The excretion fraction is the fraction of pharmaceuticals which is after consumption excreted by the body. A high fraction of pharmaceuticals is excreted as metabolites or conjugates. In the calculation, only the excreted fraction of the free, original pharmaceutical is taken into account. Metabolites and conjugates are not included.

$$UC = \frac{DDD * E_f}{V_{urine}} \qquad \text{eq. 4.1}$$

Where:
DDD = Daily defined doses (mg/p)
V_{urine} = daily volume of urine produced (=1.5 L/p)
E_f = excreted fraction of the parent compound from the human body

The volume of the (undiluted) urine is estimated to be 1.5 L/person (Zeeman, 2003).

Finally the used concentrations in the batch tests were obtained by limiting the range in UC of eight pharmaceuticals. A maximum of 20 mg/l is applied..
From these values, eventually, 1/10th were taken. This outcome has been used in the batch tests (6th column of table 4-2). These steps were taken because of analytical reasons and to prevent any toxic effects of pharmaceuticals to the bacteria. Analytically, it is better to have the concentrations of pharmaceuticals in more or less the same range. Toxic effects of pharmaceuticals have been reported to be in the higher mg/l range (10-200 mg/l for effect concentrations of the pharmaceuticals to fish and lower organisms (Fent, 2006)). These steps are acceptable because in real-life situation urine will be diluted and not all medicines are used chronically (e.g. diclofenac, ibuprofen). The results of the calculations are shown in table 4-2.

Table 4-2: Defined Daily Doses (DDD), excretion fraction and predicted concentrations in undiluted urine for eight selected pharmaceuticals.

Pharmaceutical		DDD (mg)	Excretion fraction of original compound	Calculated UC (mg/L)	Calculated UC including user fraction (mg/L)	Concentration in batch tests (mg/L)
Aspirin	(ASA)	3000	0.01	20.00	0.82	2
Diclofenac	(DCF)	100	0.05	3.33	0.30	0.3
Ibuprofen	(IBU)	1200	0.01	8.00	0.57	0.8
Carbamazepine	(CBZ)	1000	0.02	13.33	0.05	0.9
Metoprolol	(MTP)	150	0.05	5.00	0.28	0.5
Clofibric acid	(CFA)	2000	0.06	80.00	0.001	0.8
Bezafibrate	(BZF)	600	0.5	200.00	0.04	2
Fenofibrate	(FNF)	200	0.14	18.67	0.00	2

The formula for UC assumes that everyone uses all eight pharmaceuticals. In other words, no user fractions are taken into account. Therefore the obtained values represent a worst case scenario.
Including user fractions could under estimate the pharmaceutical concentration. In situations where the urine from point sources is to be treated (hospital, nursing house, etc) where a group of people is concentrated using a lot and often the same medication, the fraction of users of the same pharmaceutical compound will be higher.
For project in Anderen, the user fraction can be different due to fact that the concerned residence is for disabled people. This target group could have its specific needs of medicines.
For comparison, table 4-2 also presents a calculated concentration in which the UC is multiplied with the user fraction in the Netherlands for each pharmaceutical (CVZ, 2006). The calculated UC differ quite some from these concentrations. The selected concentrations for the batch tests are slightly above these values.
Ternes (2006) proposed another formula to calculate a predicted urine concentration (PUC) for pharmaceuticals . It assumes also no dilution of the urine.

$$PUC = \frac{A * Ef}{P * U * 365} \qquad \text{eq. 4.2}$$

A = Amount of pharmaceutical sold in an specific area (ton/yr).
P = number of inhabitants in the specific area (person)
U = produced urine per capita per day (=1 L/person)

The equation takes already into account a user fraction of a given medicine. Escher et al. (2006) computed this PUC for ibuprofen, carbamazepine and diclofenac for German situation

and obtained concentrations of 2.7, 0.23 and 0.58 mg/L respectively. These values are in the same range as the concentrations computed for the batch tests.

4.1.2 Black water

For black water the concentration will be a lower than in the urine because of the use of more flush water. The dilution factor depends on the sanitation system in use. Vacuum toilets require 4.8-12 L of flush water a day. This means a dilution factor of 3-8. Low flush toilets (used in e.g. in boats) equipped with a wastewater tank, use about 3-6 L water per day, which a means a dilution of 2-4 times. Considering these dilution factors and the uncertainty in UC, it seems plausible that biodegradation kinetics in the batch tests will be valid for pharmaceuticals both urine and black water treatment.

4.2 Chemicals

The following pharmaceuticals were obtained from Sigma-Aldrich (Steinheim, Germany): acetylsalicylic acid ≥99.0% (CAS-nr: 50-78-2), bezafibrate ≥98% (CAS-nr: 41859-67-0), carbamazepine (CAS-nr: 298-46-4), clofibric acid 97% (CAS-nr: 882-09-7), diclofenac sodium salt (CAS-nr: 15307-79-6), fenofibrate ≥99% (CAS-nr 49562-28-9), ibuprofen ≥98% (GC) (CAS-nr: 15687-27-1) and (±)-Metoprolol (+)-tartrate salt ≥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 pharmaceutical stock solution) (HPLC-grade) was obtained from LAB SCAN (Dublin, Ireland).

4.3 Sludge origin and characteristics

Activated sludge was obtained from municipal wastewater treatment plant in Bennekom (the Netherlands). The WWTP consists of a primary treatment step, a biological treatment step (nitrification, denitrification and biological phosphorus removal) and a secondary sedimentation tank. The tank has a recycle to anaerobic and anoxic compartments. Activated sludge sample for the aerobic and anoxic biodegradation step was collected at the end of the biological treatment step. The sludge for one of the anaerobic test was from a pilot UASB reactor treating concentrated black water in Wetsus (Leeuwarden, the Netherlands). The sludge for the second anaerobic test was obtained from an UASB septic tank (UASB ST) treating concentrated black water in Sneek (the Netherlands). For details of the treatment plants from which the sludge samples were taken see Table 4-3.

Table 4-3: Characteristics of the WWTPs from which the sludge samples were taken. The black water used in the pilot reactor at Wetsus originates from the treatment plant in Sneek.

Treatment plant	Bennekom[1]	Sneek	Wetsus
Type of waste water treated	Combined domestic sewage	Concentrated black water (vacuum toilets)	Concentrated black water (vacuum toilets)
Average flow rate (m3/d)	3300	0.	0.0059
HRT (d)	2	35	8-9
SRT (d)	40	>1 yr	220
ORL (kg COD/m3/d)	0.396	0.4	0.07
Volume (of biological treatment tank) (m3)	5700	7	0.05
Temperature (^{o}C)	ambient	35	25

1Information is based on a publication about the characteristics of the WWTP in 2005 (Waterschap Vallei & Eem 2006)

The SRT of the anaerobic sludge in Sneek is not exactly known. The SRT will be high. Maximum once a year part of the sludge bed is removed. A characteristic of the UASB-ST is the accumulation and stabilization of sludge (Kujawa, 2005).

4.4 Experimental set-up

4.4.1 Aerobic batch tests

4.4.1.1 Introduction

In the aerobic biodegradation experiments a mixture pharmaceuticals was spiked to activated sludge. The sludge was aerated and incubated at a constant temperature. During the experiment samples of liquid and solid fraction were taken to analyze the concentration of the spiked pharmaceuticals in both phases in time. In this way it was attempted to assess the biodegraded fraction of pharmaceuticals.

4.4.1.2 Set-up of the experiment

Experiments were performed at two temperatures of 20^{o}C and at 10^{o}C.
Each test consisted of:

- 2 batch tests in where a mixture of 8 pharmaceuticals were spiked to activated sludge at time = 0 (duplicate).
- 2 batch tests in where a mixture of 8 pharmaceuticals was spiked to Millipore water (duplicate). This control was included to trace possible interactions between pharmaceuticals or (other) abiotic transformation.

Table 4-4 gives an overview of the volumes added to the different bottles. The experiment of 20^{o}C has been performed twice under the same conditions because the sampling method was improved after the first aerobic batch test at 20^{o}C (AER-20-1) (improved preservation of the samples and sampling of the solid phase). Moreover, the 2nd aerobic test at 20^{o}C (AER-20-2) and the aerobic test at 10oC (AER-10) were prolonged from 2 days to 30 days to observe any biodegradation of apparently persistent pharmaceuticals.

Table 4-4: Overview of the volumes of added media and solutions in the aerobic biodegradation experiment

Batch	Mixture of pharmaceuticals in methanol solution (ml)	Millipore Water (L)	Sludge (L)
Biodegradation test	0.5	0	1
Control	0.5	1	0

The activated sludge was taken from municipal WWTP in Bennekom. It was aerated a few hours prior to start of the experiment do deplete the carbon sources in the activated slugde, and brought to required temperature.
The total solids and volatile solids (TS, VS) of the sludge were determined at the beginning, after 2 days and after 30 days. Before the addition of pharmaceuticals, a sample was taken from the activated sludge to determine the background concentration of pharmaceuticals in the activated sludge mixture.
The batches were aerated to keep a sufficient high oxygen level and in this way also mixing of the sludge and added substances were achieved. During the experiment pH, T and O2 measurements were regularly performed. Especially at 20oC water evaporates easily. Water losses due to evaporation were compensated by addition of (Millipore) water. This addition was determined by the loss of weight of the batches.
Bottles were covered with aluminum folio to prevent photolytic degradation (if any).

4.4.1.3 Stock solution of pharmaceuticals

A mixture of pharmaceuticals was prepared in 50 ml of methanol. All pharmaceuticals were dissolvable in this solvent. A 0.5 ml of this concentrated stock solution was spiked to the batches for obtaining the desired concentration of each of 8 pharmaceuticals. The originally planned and expected concentrations (based on exact weights of the substances) of the eight pharmaceuticals in the batch experiments are given in Table 4-5.

Table 4-5: The planned and the expected concentrations based on exact weights of the substances added to the stock solution of the pharmaceuticals in all three aerobic batch tests.

Pharmaceutical	Intended concentration in the batches (mg/L)	Expected concentration AER-20-1 (mg/L)	Expected concentration AER-20-2 (mg/L)	Expected concentration AER-10 (mg/L)
ASA	2	2.058	2.005	2.004
DCF	0.3	0.298	0.321	0.327
IBU	0.8	0.808	0.819	0.818
CBZ	0.9	0.913	0.912	0.898
MTP	0.5	0.507	0.51	0.523
CFA	0.8	0.814	0.794	0.805
BZF	2	1.933	1.989	2.024
FNF	2	2.040	1.960	2.005

4.4.1.4 Sampling intervals

The duration of the experiments for determining biodegradation kinetics was set at 2 days. It refers to the maximum HRT in a conventional wastewater treatment plant.

Time intervals at which samples from the aerobic biodegradation test were taken were: t_0 = 0 h; t_1 = 0.5 h, t_2 = 1 h, t_3. = 3 h, t_4 = 20 h, t_5 = 48 h.

To assess a possible sludge adaptation or a utilization of (especially persistent) pharmaceutical compounds under stress conditions (no co-substrate supplied), the latter two experiments were prolonged to 30 days. During this period samples were taken in week 1, 2 and 4.

The controls were sampled at 0 h and t = 48 h in all tests. Controls of the second aerobic test at 20°C and the aerobic test at 10°C were sampled also at t=30 days.

4.4.2 Anoxic tests

4.4.2.1 Introduction

In the anoxic biodegradation experiment a mixture of pharmaceuticals was spiked to activated sludge. The sludge was incubated at a constant temperature under oxygen free and nitrate rich conditions. During the experiment samples of liquid and solid fraction were taken to analyze the concentration of the spiked pharmaceuticals in both phases over the course of the experiment.

4.4.2.2 Set-up of the experiment

The anoxic experiments were performed at 20^{o}C and 10^{o}C (ANOX-20 and ANOX-10 respectivley). Each experiment consisted of:

- 2 batch experiments in where a mixture of 8 pharmaceuticals was spiked to activated sludge at time = 0 (duplicate).
- 2 batch experiments in where a mixture of 8 pharmaceuticals was spiked in water (duplicate). The control is included to trace possible interactions between pharmaceuticals and other abiotic transformation (such as hydrolysis).

Table 4-6 gives an overview of the volumes of the substances added to the different bottles.

Table 4-6: Overview of the volumes of added media and solutions in the anoxic biodegradation experiment

Batch	Mixture of pharmaceuticals in methanol solution (ml)	NaNO3 solution (ml)	Millipore Water (L)	Sludge (L)
Biodegradation test	0.5	0.5 (final [N-NO3: 20 mg/L)	0	0.5
Control	0.5	0.5 final [N-NO3: 20 mg/L)	0.5	0

The activated sludge was taken municipal WWTP in Bennekom. The oxygen in liquid and gas phase was depleted prior to start of the experiment by storing the sludge without aeration over night.
The TS/VS (total solids, volatile solids) of the sludge were determined at beginning and end (t=0 and t=2 d) of the experiment. Before the addition of pharmaceuticals, a sample was taken from the activated sludge, to determine the background concentration of pharmaceuticals in the activated sludge mixture.
To obtain and keep oxygen free conditions, the gas phase in the batches was flushed with nitrogen before the start of the experiment and after sampling.
A nitrate solution was prepared to be able to obtain an initial concentration of nitrate in the batches of 20 mg/l N-NO3. Nitrate concentration in the liquid was followed over time. When nitrate was almost denitrified, an appropriate volume of NaNO3 solution was added again to obtain again NO3- concentration in the remained volume of approximately 20 mg/L.
To assure a good mixing in the batches a shaker was used (85 rpm). The bottles were covered with aluminum folio to prevent photolytic degradation (if any). During the experiment redox potential, temperature and pH measurements were regularly performed.

4.4.2.3 Stock solution of pharmaceuticals

A mixture of pharmaceuticals was prepared in 50 ml of methanol like in the aerobic test. The planned and expected concentrations of the eight pharmaceuticals in the batch experiments are given in
Table **4-7**.

Table 4-7: The planned and the expected concentrations based on exact weights of the substances added to the stock solution of the pharmaceuticals in anoxic batch tests.

Pharmaceutical	Intended concentration in the batches (mg/L)	Expected concentration in ANOX-20 (mg/L)	Expected concentration in the ANOX-10 (mg/L)
ASA	2	2.004	2.012
DCF	0.3	0.324	0.384
IBU	0.8	0.826	0.838
CBZ	0.9	0.900	0.926
MTP	0.5	0,504	0.524
CFA	0.8	0.796	0.808
BZF	2	2.008	2.016
FNF	2	1.984	1.992

4.4.2.4 Sampling intervals

The duration of the experiments for determining biodegradation kinetics was set at 2 days. Time intervals at which samples from the anoxic biodegradation test were taken were: t_0 = 0 h; t_1 = 1 h, t_2. = 3 h, t_3 = 20 h, t_4 = 48 h.

The ANOX-20 was prolonged to 30 days. During this period samples were taken in week 1, 2 and 4. The controls were sampled at t=0 hr, t=48 hr for in tests and also at t=30 days in this batch test.

4.4.3 Anaerobic tests

4.4.3.1 Introduction

In the anaerobic biodegradation experiment a mixture of pharmaceuticals was spiked to anaerobic sludge. The batches were incubated under anaerobic conditions at constant temperature of 30^{o}C and were continuously shaken. Biodegradation of pharmaceuticals under anaerobic conditions is expected to be very low compared to aerobic and anoxic conditions. A relative high temperature of 30^{o}C is chosen for this test to observe the maximum biodegradation under the given condition. From the batches, liquid and solid samples were taken to analyze the concentration of the pharmaceuticals in both phases over time. The biodegraded fraction was assessed in this way as well as biodegradation kinetics.

4.4.3.2 Set-up of the experiment

The anaerobic experiment, performed at 30 ^{o}C, consisted of:

- A batch test with a mixture of pharmaceuticals spiked to anaerobic sludge (duplicate).
- A batch test with a mixture of pharmaceuticals spiked to water (duplicate). These controls were included to trace abiotic transformation and interactions between pharmaceuticals.

This experiment was repeated for the same reasons as in the aerobic test at 20^{o}C: improved sampling of solid phase and sample preservation after the first anaerobic test.
In Table 4-8 an overview of the volumes of the substances used for the both experiments.
In the first anaerobic test (ANAER-1) sludge was taken from the anaerobic pilot reactor treating concentrated black water (Leeuwarden, the Netherlands). The second test (ANAER-2) was performed with sludge from the anaerobic digester treating (the same) black water in Sneek (the Netherlands).

Table 4-8: Overview of the volumes of added media and solutions in the anaerobic experiments. For the dilution of biomass tap water was used, for the controls Millipore water.

Test	Batch	Mixture of pharmaceuticals in methanol solution (ml)	(Millipore) Water (mL)	Sludge (mL)	Total volume (mL)
ANAER-1	Biodegradation test	0.5	100	300	400
	Control	0.5	400	0	400
ANAER-2	Biodegradation test	0.5	0	500	500
	Control	0.5	500	0	500

The total solids and volatile solids (TS and VS) of the sludge were determined at the beginning and at the end of the experiment.
To ensure strictly anaerobic conditions, the anaerobic bottles were flushed with nitrogen (10 s) prior to the start of the experiment. The bottles were capped with covers which were equipped with ventilate to reduce the pressure caused by biogas production.
During the experiment pH, T and redox measurements were regularly performed.
Bottles were covered with aluminum folio to prevent photolytic degradation (if any). After sampling, the gas phase of the bottles was flushed with nitrogen.

4.4.3.3 Stock solution of pharmaceuticals

Pharmaceuticals stock solution was prepared in methanol like in the other batch tests. The planned and expected concentrations of the eight pharmaceuticals in the batch experiments are given in
Table **4-9**.

Table 4-9: The planned and the expected concentrations based on exact weights of the substances added to the stock solution of the pharmaceuticals in anaerobic batch tests.

Pharmaceutical	Intended concentration in the batches (mg/L)	Expected concentration in ANAER-1 (mg/L)	Expected concentration in ANAER-2 (mg/L)
ASA	2	2.000	2.040
DCF	0.3	0.300	0.448
IBU	0.8	0.765	0.818
CBZ	0.9	1.000	0.976
MTP	0.5	0.523	0.500
CFA	0.8	0.788	0.802
BZF	2	2.008	1.968
FNF	2	2.013	2.064

4.4.3.4 Sampling intervals

A period of about 30 days was used to determine biodegradation kinetics, representing the HRT in anaerobic digesters treating black water (e.g. Sneek) or WWTP's sludge. The time intervals at which samples were taken in the biodegradation test were: t_0 = 0 hr; t_1, = 3 hr; t_2 = 1 d; t_3 = 4 d; t_4 = 7 d; t_5 = 15 d; t_6 = 30 d.
From the controls only at the beginning and the end of experiment (t=0 and t = 30 d) liquid samples are taken. The first experiment was prolonged to 77 days. During this period, samples were taken in week 1, 2, 4, 6 and 8. Samples of controls were taken at t=0, t=30 and t=77.

4.5 Analytical method

4.5.1 Sampling

Samples were taken with a plastic syringe sampling 30 ml, in all tests. Extra in the anoxic tests was the use of a long sampling needle. In this way the closed system stayed close, preventing any oxygen inflow. To be able to take 30 ml of liquid from the anoxic batches, nitrogen was flushed in with a small needle.
To the samples, 4-5 drops of chloroform were added with a Pasteur pipette. This step was not taken in the first aerobic test at 20^{o}C (AER-20-1) and the first anaerobic test (ANAER-1).
Subsequently, samples were centrifuged for 10 min at 4000 rpm / 2800 rpf using the centrifuges FirlabO SW12R or IEC thermo CL31R.
After this, the solid (4 ml) and liquid phase (20ml) were separated. For the aerobic tests 20oC and the first anaerobic test, non-disposable centrifuge tubes were used. The solid phase had to be replaced therefore after centrifuging. Transferring solid was done with a drop of demi-water. In the first aerobic test and anaerobic test (AER-20-1 and ANAER-1), not all liquid from the sample was taken away in contrary to the other tests. In the anoxic tests and in the ANAER-2 centrifuging was done with disposable centrifuge tubes (PP-Test tubes 50ml, CELLSTAR). Also for the aerobic experiments these tubes were used during the extended time period (sampling period of t=15 and t=30). The use of disposable centrifuge tubes made the sampling of the solid phase more precise (no replacement of solid phase).
From the controls samples of 20 ml liquid were taken.

4.5.2 Sample preservation

Chloroform was added to stop microbial activity after sampling. In the samples from AER-20-1 activity of bacteria was observed. By addition of chloroform this was attempted to be prevented. An additional test was performed by RIVM on the effect on chloroform in the samples (see appendix). Chloroform was used upon the positive results of this tests when using it.
All samples were immediately stored in the freezer (-75 ^{o}C) until analyzed.

4.5.3 Analysis of pharmaceuticals

The analysis of the pharmaceuticals is carried out by the Dutch Research Institute RIVM (State Institute of Public Health and Environment), department ARO-CRL. The procedure of the analysis is as follows, provided by RIVM ARO-CRL:

4.5.3.1 Materials

All chemicals and reagents were of high purity quality. Besides standard laboratory equipment the system described below were used.

4.5.3.2 Apparatus

Liquid chromatography (LC): Waters Chromatography Acquity UPLC separation module. Column: Acquity UPLC BEH C18 1.7 µm (100 * 2.1 mm ID). Column temperature was 65℃.
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-1. The injection volume was 20 µl.
Mass-spectrometer (MS) analysis was carried out on a Waters-Micromass Ultima Platinum. Depending on 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: 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℃ and desolvation temperature: 325℃. The cone gas flow was 116 L hr-1 and the desolvation gas flow was 701 L hr-1. 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 3.06e-03. See table 1 for the measured MRM transitions.
In negative mode the following settings were used: 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℃ and desolvation temperature: 325℃. The cone gas flow was 116 L hr-1 and the desolvation gas flow was 701 L hr-1. LM1/HM1 resolution was 14, with ion energy: 0. LM2/HM2 resolution was 14.5, with ion energy: 1.0. Collision cell pressure 3.06e-03. For the collision cell the entrance was 10, with a CE gain of 1 and exit 0. See table 4-10 for the measured MRM transitions.

Table 4-10: Pharmaceuticals measured and their corresponding MRM's, retention time , ionisation mode and corresponding collision energy (V).

Compound	Retention time (min)	Ionisation mode	MRM	Dwell time (msec)	Collision energy (V)
Metoprolol	2.52	Positive	268.2>116.2	20	15
Acetylsalicylicacid	2.28	Negative	137.0>93.2	20	10
Carbamazepine	4.31	Positive	237.1>194.1	20	10
Clofibric acid	5.03	Negative	213.0>127.0	20	8
Bezafibrate	5.27	Positive	362.1>316.1	20	12
Diclofenac	6.03	Negative	294.0>250.0	20	8
Ibuprofen	6.23	Negative	205.0>161.1	20	5
Fenofibrate	7.66	Positive	361.1>233.0	20	10

4.5.3.3 Sample Clean-up

Sample clean-up of the liquids was straight forward. The samples were 10 times diluted in LC-eluens A after which they were vortexed for 10 seconds. For samples with lower concentrations the samples were acidified with 2µl 50% acetic acid. The samples were direct injected.
Sample clean-up of the soils was performed by a liquid liquid extraction. A portion of the sample (circa 0.5 gram) was weighted and five millilitres of acetonitrile was added. The samples were sonified by an ultrasonic finger for 20 seconds followed by rotating head over head for 10 minutes. After which the sample was centrifuged. The supernatant was transferred to a clean tube and evaporated under nitrogen at 55℃. The dried sample was reconstituted in one millilitre of eluens A followed by 10 minutes ultrasonification.

4.5.3.4 Calibration curves

To correct for losses due to sample storage and to correct for signal suppression due to matrix compounds were the calibration curves prepared in reprehensive blank materials for each corresponding experiment. In figure 4-1 a chromatogram is shown of a spiked sample containing a mixture of all the pharmaceuticals. Each trace represents the measured transition for the given compounds.

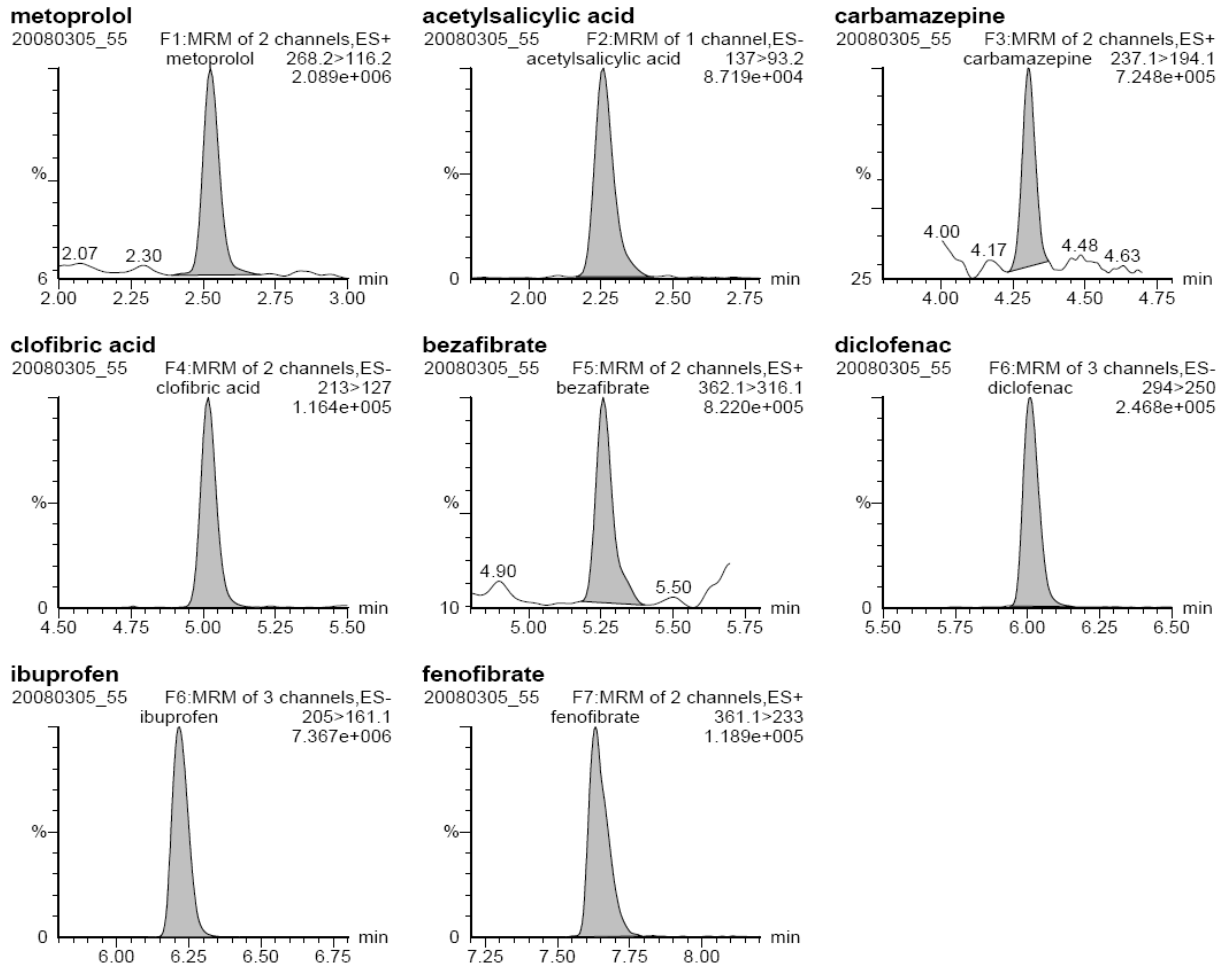


Figure 4-1: Reversed phase microbore LC-ESI MSMS profiles of an anaerobe sample spiked (5 ng/ml) with a mixture pharmaceuticals

4.5.4 Analysis of other parameters

VS and TS concentration are measured according to Standard Methods 2540 (Clescerl 1998).
The N-NO3 concentration is analyzed with Dr. de Lange method using kits with detection range from 5 to 35 mg/l N-NO3.
pH and O2 are measured with HACH HQd Field case. Oxidation reduction potential is measured with the ORP electrode.

4.6 Calculations

The equations used to assess the biodegradation and sorption of selected pharmaceuticals is given below. To calculate the degradation of a pharmaceutical the distinction was made between compounds present in the liquid- and solid phase.
The total concentration of pharmaceutical compound i in the batch tests at given time t was calculated using eq. 4.3 :

$$C_{t,i} = C_{l,i} + C_{s,i} = C_{l,i} + X_i TS \qquad \text{eq. 4.3}$$

where:
$C_{t,i}$ = the total concentration of pharmaceutical *i* (mg/L) at time = *t*
$C_{l,i}$ = pharmaceutical concentration in the liquid phase (mg/L)
$C_{s,i}$ = pharmaceutical concentration in the sludge phase (mg/L)
X_i = pharmaceutical concentration in the sludge (mg/g TS)
TS = sludge concentration (g TS/L)

Solid - water partition coefficient of a pharmaceutical i, Kd,i was calculated with the formula:

$$K_{d,i} = \frac{X_i}{C_{l,i}}$$ eq. 4.4

where:
$K_{d,i}$ = the sorption constant of a compound i (L/kg TS);

The biological degradation of pharmaceutical i is modeled as (pseudo) first order reaction.

$$\frac{dC_i}{dt} = k_{biol,i} * TS * C_i = k_i * C_i$$ eq. 4.5

where:
C_i = total concentration of pharmaceutical *i* (mg/L)
t = time (hr or d)
$k_{biol,}$i = specific biological degradation rate constant of pharmaceutical i (L/gTS/h or L/gTS/d)
k_i = biological degradation constant of pharmaceutical *i* (h^{-1} or d^{-1}).
TS = total solids concentrations (g/L)

The concentration of a pharmaceutical is proportional to the degradation rate as well as the concentration of biological sludge TS. This concentration is assumed constant during the batch test. Therefore, the reaction is called a pseudo first order reaction. The reaction constant $k_{biol,i}$ is expressed per g TS. It enables the comparison of the biodegradation kinetics in the batch tests with different suspended solids concentrations.
Integration of the first order reaction gives:

$$C_i(t) = C_i(0) * e^{-k_{biol,i} * TS * t}$$ eq. 4.6 and $$C_i(t) = C_i(0) \cdot e^{-k_i * t}$$ eq. 4.7

For difference in reaction rate at different temperature, the Arrhenius equation is used:

$k2 = k1 * e^{\kappa * (T2-T1)}$ eq. 4.8

where:
k_1 = specific reaction rate constant (L/gSS/d) at temperature T_1 (oC)
k_2 = the specific rate constant at a temperature T_2 (oC)
κ = the temperature coefficient (-).

For the mass balances of pharmaceuticals in water treatment systems eq. 4.9 and 4.10 are used:

$$\text{Influent concentration} = \frac{DDD * E_f}{V_{blackwater}}$$ eq. 4.9

$$\frac{dC}{dt} = Q_{in} * C_{i,in} - Q_{out} * C_{i,e} - k_{biol} * TS * C_{i,e} * V - TS / SRT * C_{i,e} * Kd * V$$ eq. 4.10

5 Results and discussion

Batch tests were performed in order to determine the biodegradation of the selected pharmaceuticals under various operational conditions. In total seven batch tests were performed. The background concentrations, the operational parameters and pharmaceutical concentration in the different batch tests will be presented and discussed. Further, implications of the results for especially systems dealing with concentrated wastewater streams such as urine and black water is described in this chapter.

5.1 Operational conditions batch tests

The operational parameters being controlled and monitored in all batch tests were temperature (T, ^{o}C), dissolved oxygen (DO, mg/L) or oxidation reduction potential (ORP, mV), volatile solids (VS, g/L)) and total solids (TS, g/L). The measurement procedures and equipment applied are described in chapter 4. The list of the controlled parameters and their values at different time intervals is given in table 5.1 for all tests.

Table 5-1: Operational conditions during all performed tests. DO= dissolved oxygen, VS= volatile solids, TS= total solids and ORP= oxidation reduction potential. Duplicates are specified with I and II.

Tests		Process conditions				
Aerobic 20^{o}C (AER-20-1)		T (^{0}C)	DO (mg/L)	pH	VS (g/L)	TS (g/L)
t = 1d	I II	18.0 18.0	8.49 8.75	8.3 8.5	2.967 2.967	3.992 3.992
t = 2d	I II	18.8 16.3	9.11 9.72	8.2 8.3	3.015 2.986	4.068 4.017
Aerobic 20^{o}C (AER-20-2)						
t = 0d	I II	17.0 18.0	8.08 9.00	7.7 8.0	3.830 3.830	4.955 4.955
t = 2d	I II	19.0 17.5	8.41 9.09	7.4 7.7	4.712 3.807	6.682 5.043
t =30d	I II	18.0 19.8	8.91 8.56	5.3 6.4	1.772 1.960	2.838 3.051
Aerobic 10^{o}C (AER-10)						
t = 0d	I II	10.2 10.0	10.61 10.96	7.3 7.4	3.801 3.801	4.782 4.782
t = 2d	I II	10.1 10.0	10.87 11.19	7.6 7.6	3.306 3.069	4.238 3.895
t =30	I II	12.8 11.9	8.85 9.45	5.8 5.6	2.722 2.533	3.733 3.432
Anoxic 20^{o}C (ANOX-20)			ORP (mV)			
t = 0d	I II	21.5 21.5	-146 -140	n.a.	3.718 3.718	4.769 4.769
t = 2d	I II	22.0 22.0	-93 -43	n.a	3.586 3.305	5.193 4.742

t =15d	I II	23.0 23.0	-180 -102	n.a		
t =30	I II	23.0 23.0	60 95	7.7 7.31	2.674 2.434	4.631 4.176
Anoxic 10^{o}C (ANOX-10)		T (^{0}C)	ORP (mV)	pH	VS (g/L)	TS (g/L)
t = 0d	I II	12.0 12.0	-80 -91		6.136	7.876
t = 2d	I II	13.8 13.9	-180 -183	8.04 7.91	5.821 5.823	7.979 7.944
t =15d	I II	13.2 12.0	68 79			
t =30d	I II	12.5 11.8	147 154	6.9 7.11	4.245 4.312	6.606 6.487
Anaerobic 30^{o}C (ANAER-1)						
t = 0d	I II				15.548 15.548	20.954 20.954
t = 77	I II	28.5	-358 -318	8.4 8.6	13.215 13.532	18.520 18.767
Anaerobic 30^{o}C (ANAER-2)						
t = 0d	I II	28.5 28.0	-325 -334	n.a.	7.275 7.275	12.264 12.264
t =15d	I II	29.5 29.0				
t =30d	I II	29.0 29.0	-5 -100	7.61 8.46	6.384 6.433	11.343 11.328

The aerobic tests targeted at 20^{o}C were performed at 18-19 ^{o}C. The lower temperature of a duplicate in the first aerobic test after 2 days is likely due to the addition of cold water (to compensate evaporation) just before sampling and measuring at t = 2 d.
The temperature of aerobic test (10^{o}C) were over the first 2 days around 10^{o}C, after this the temperature in the cooling system increased to 12^{o}C. Moreover, the cooling system has been broken for 1 week from t=18 to t=25 days. During this period temperature has not been controlled, which means the bottles were at ambient temperature.
The DO was quite high for all aerobic tests, close to saturated conditions. The pH was close to neutral or higher (max 8.3) at the start. After 30 days, the pH has became very low (no buffer was added to the medium) for the aerobic test at 20 and 10 ^{o}C both. Biological activity of the sludge is likely retarded at such this pH. The VS/TS concentrations first increased within 2 days this is unlikely and is rather an analytical error since no substrate was added. After 30 days the VS and TS concentration decreased significantly as no substrate was added (endogenous biodegradation).

The anoxic tests has been performed at slightly higher temperatures as originally planned: 12 (instead of 10) and 22-23 (instead of 20) ^{o}C. For the anoxic 10^{o}C test, the same cooling system was used as for the aerobic 10^{o}C test. In these tests the temperature was not controlled between day 3 and day 10 of the experiment.
The pH during the experiment was between 7 and 8. It did not decrease as significantly as in aerobic tests during the course of time.. A VS/TS concentrations decreased over the course of the anoxic experiments but not as significant as in the aerobic tests (anoxic substrate conversions rates are slower than aerobic ones).

The ORP indicated the presence of anoxic conditions in the first 2 days of both anoxic tests. After 15 days, oxygen diffused into the system because the redox potentials were higher and therefore condition was weak anoxic. Still denitrification can take place at these higher ORP (Hong, 1998).
The nitrate concentrations were at the start of the tests 40 mg/L N-NO3. A concentrated NaNO3 solution was added to supply nitrate to the sludge mixture, when their concentration became exhausted (denitrified). In the first 2 days the NO3-solution was added once to the batches in both anoxic tests after 24 hours. After about 15 days the NO3 concentration started to increase up to over the detection limit of 40 mg N/l (to about 70 mg N-NO3/L) in both anoxic tests in both anoxic tests. This could point out a decay of the sludge and the presence nitrification processes after 15 days.

The anaerobic experiments were performed at 29^{o}C instead of the targeted 30^{o}C .The initial pH was about 8 which is as expected from black water fed sludge (STOWA, 2005). The VS and TS in the anaerobic tests remained relatively constant.
The first experiment showed low redox potentials as expected under anaerobic conditions. The 2nd experiment also started with low redox potential. After 30 days the ORP had increased. This could be caused perhaps due to diffusion of some oxygen to the test bottles.

5.2 Background concentrations

To asses the contribution of the background concentration of the sludge to the total measured concentration in the batches, as well as to acquaint information on occurrence of selected compounds in effluent of wastewater treatment systems, the sludge used for the batch tests was analyzed for the presence of pharmaceuticals. The concentrations of pharmaceuticals in the effluent of the activated sludge treatment tank of municipal WWTP Bennekom are shown in figure 5.1.

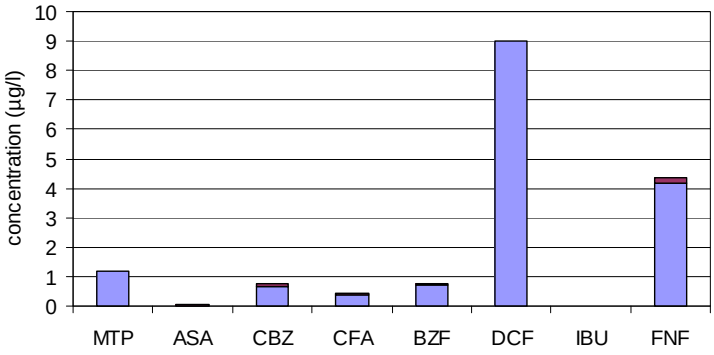


Figure 5-1: Background concentration of selected pharmaceuticals in activated sludge of municipal WWTP Bennekom. Presented data is obtained from three activated sludge samples taken at January and February 2008.

All pharmaceuticals were detected in the activated sludge, except for ibuprofen. Especially diclofenac was present in relative high concentration. Presence of fenofibrate is unexpected since this compound is officially not on the market in the Netherlands anymore. The detected pharmaceuticals were present in the low µg/l range, confirming literature findings (table 1-2).
The graphs show the presence of pharmaceuticals in the effluent of biological treatment system and therefore indicate the persistence or partial removal of the selected pharmaceuticals in WWTPs. The absence of IBU can indicate a low consumption of this pharmaceutical (which is not likely when looking to the consumption figures) or complete removal of this compound in the moderate loaded WWTP.

In the anaerobic sludge obtained from pilot-scale UASB and from the demonstration scale UASB septic tank – (chapter 4), the pharmaceutical concentrations were much higher (up to 150 µg/L). Ibuprofen, metoprolol, diclofenac and aspirin are present in the highest concentration. Figure 5.2 and 5.3 present the background concentrations for the pilot UASB and demonstration UASB-ST respectively. The feed of both reactors were originating from the same location: black water from the decentralized sanitation system in Sneek (the Netherlands). This higher concentrations confirmed expectations. The mentioned reactors treat concentrated black water and moreover, the expected removal efficiency of the anaerobic systems is lower.

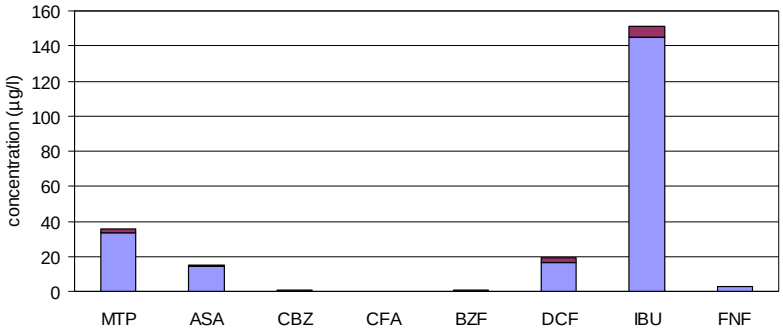


Figure 5-2: Background concentration of pharmaceuticals in anaerobic sludge sampled from pilot UASB reactor in Leeuwarden, the Netherlands.

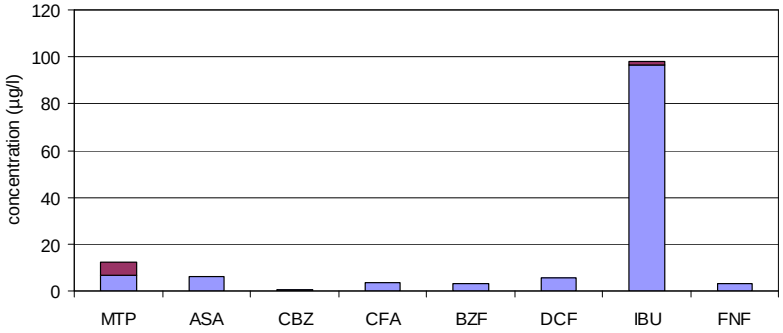


Figure 5-3: Background concentration of pharmaceuticals in anaerobic sludge obtained from UASB-ST in Sneek, the Netherlands.

In the graphs, the pharmaceutical concentration is presented for both water and liquid phase. All graphs show the prevailed pharmaceutical concentration in the water phase.

5.3 Biodegradation in aerobic batch tests

The aerobic batch tests were performed twice at 20°C and once at 10 °C. The difference between both tests at 20°C was the improved sampling method in the second test. Therefore, the focus is on the results of this test (AER-20-2). Results of the first test are in the appendix as well as all raw data. The experiments were run for 30 days. In the first 2 days the concentration of pharmaceuticals was frequently analyzed to determine the elimination rate during a maximum HRT in a conventional municipal WWTP (HRT=2 d). The sampling was continued up to 30 days (but less frequent) to determine whether persistent pharmaceuticals would be eliminated when bacteria are subjected to stress conditions (no other external carbon source added).

The results of the aerobic tests are given in the figures 5.4-5.12. The graphs show the total pharmaceutical concentration in the batch tests consisting of the sum of the concentration in the water and solid phase (so sorption to sludge is taken into account). Also the concentrations of pharmaceuticals in the controls (without sludge) are plotted in the graphs. The detection limit of the pharmaceutical concentration in the liquid phase was 0.005 µg/l and 0.005 ng/gTS the in the solid phase. The time scale of the graphs is 2 days for the pharmaceuticals which showed a relative fast decrease in concentration and 30 days for the other pharmaceuticals, if available. The fate of selected pharmaceuticals is discussed in order of the observed biodegradability.

In the first and the second aerobic test at 20°C (AER-20-1, AER-20-2) a fast decrease of acetylsalicylic acid (ASA) was detected. Within 1 hour, the concentration in the water phase was under the detection limit (0.005 µg/l) in the AER-20-2. In the test at 10°C (AER-10) the concentration was lower than the detection limit already after 3 hour (fig. 5.4). In the samples taken after 30 days of the AER-10 not only ASA was eliminated in the biodegradation test, but also in the controls. This could be the result of decomposition. Since the concentration in the controls stayed constant over the first 2 days, the fast elimination of ASA in the biodegradation tests was likely to be due to biological processes.
The initial concentration of ASA was expected to be 2.0 mg/l. (at t=0). This concentration was never obtained in the controls and the test bottles. Perhaps due to fast decomposition of ASA.

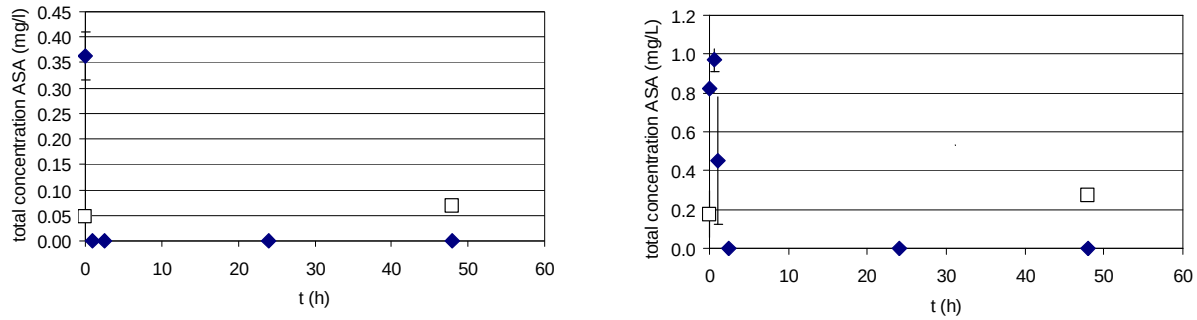


Figure 5-4: Total concentration of ASA in time in the aerobic batch test at 20° (AER-20-1, left) and at 10 °C (AER-10, right) (♦ with sludge, □ without sludge).

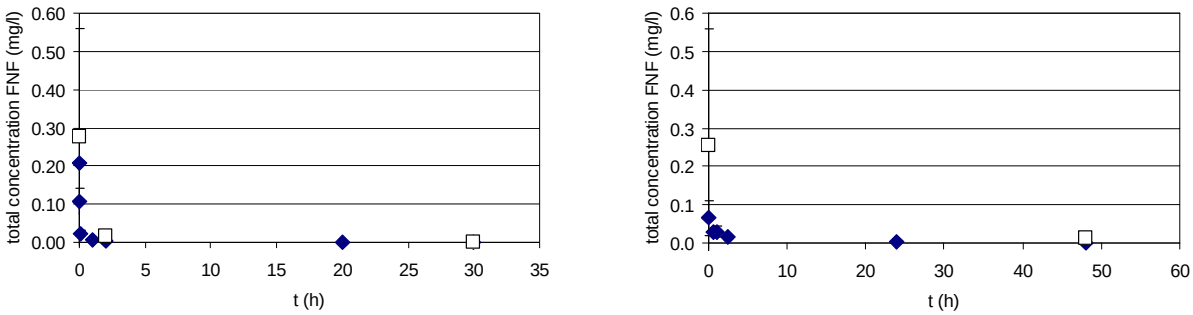


Figure 5-5: Total concentration of FNF in time in the aerobic batch test at 20^{o} (AER-20-1, left) and at 10 ^{o}C (AER-10, right) (♦ with sludge, □ without sludge).

The courses of fenofibrate (FNF) in aerobic batch tests are plotted in figure 5.5. A fast decrease in concentration was observed for this compound. Both tests at 20^{o}C gave comparable results. Within 2 days the total concentration decreased to values under the detection limit. However, this was also observed in the controls. At a temperature of 10^{o}C a disappearance of FNF in the biodegradation test and in the control was measured. 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.
The initial concentration of FNF was expected to be 2 mg/l. This concentration was not measured in any of the tests.
The cause of the disappearance of FNF in the controls could be conversion to fenofibric acid. Moreover, because FNF is very hydrophobic, absorbance to glassware and other used materials can also not be excluded.

The elimination of ibuprofen (IBU) is shown in figure 5-6. Within 2 days the pharmaceutical was effectively eliminated to concentrations under or close to the detection limit. The decrease in concentration followed an exponential trend. In AER-20-1, the IBU was transformed at the higher rate compared to AER-20-2 (the sludge could be more active at that time as taken in the warmer month). The disappearance rate of IBU was slower at 10^{o}C compared to both tests performed at 20^{o}C.
The biodegradation of IBU is according to literature. Removal rates of IBU of more than 90% in WWTPs are reported by e.g. (Kosjek, Heath et al. 2007) for a pilot WWTP with a HRT of 2 days.
The expected initial concentrations of IBU (0.9 mg/) was not completely confirmed in controls, but the values were close to it. In the test batches the initial concentration in the AER-10 was also close to the expected concentration. In the AER-20-2 there was a significant loss of IBU possibly due to sorption and insufficient extraction in the analytical method.

Metoprolol (MTP) was eliminated also exponentially. Compared to IBU the concentration decreased at a slower rate. In both tests at 20 ^{o}C, the pharmaceutical was eliminated to concentrations under the detection limit within 2 days. In the AER-10 50 µg/L was still present after 2 days. After 30 days, the concentration MTP was below detection limits also in AER-10 test. The expected initial concentration of 0.5 mg/l was confirmed in controls. In the tests with sludge approximately 50% could not be found, indicating a strong sorption and insufficient recovery during the analysis or an error in the measured liquid concentration. A very rapid biodegradation is not expected in case of MTP.

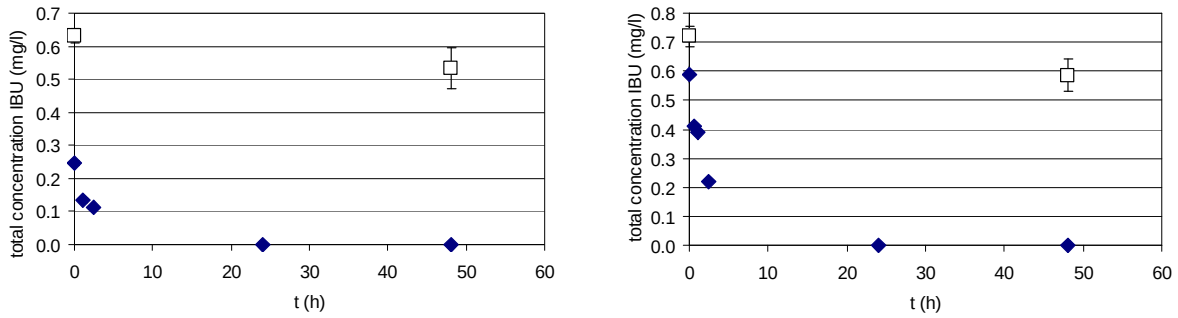


Figure 5-6: Total concentration of IBU in the aerobic test at 20°C (AER-20-2, left) and 10° (AER-10, right) (♦ with sludge, □ without sludge).

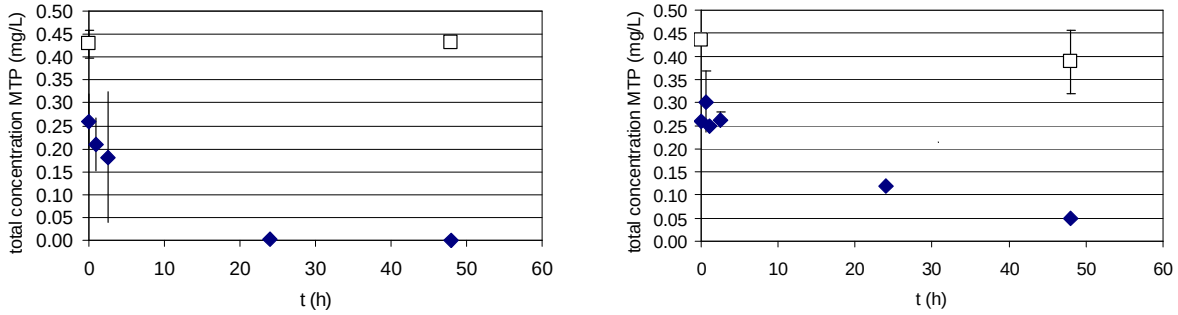


Figure 5-7: Concentration of MTP in the aerobic test at 20°C (AER-20-2, left) and 10° (AER-10, right), (♦ with sludge, □ without sludge).

The observed decrease in concentration of MTP is consistent with the reported removal rate of 83% in a municipal WWTP with aerobic biological treatment (Ternes 1998).

Bezafibrate (BZF) was removed less efficiently. The AER-20-1 and AER-20-2 tests showed a decrease in BZF concentration after 2 days of 15% and 40% respectively. These differences could be influenced by the different sampling method which was applied (addition of chloroform in AER-20-2). In the AER-10 test the decrease of BZF concentration was not significant. The difference between the tests was quite large. After 30 days the BZF in all aerobic tests was under the detection limit. This showed that BZF treated at 10°C can be biodegraded. Although, it should be mentioned that the batches were for some days above 10°C as is described in section 5.2. The concentration in the controls stayed more or less constant but the standard deviation of the concentration in the controls of AER-10 test is quite high.

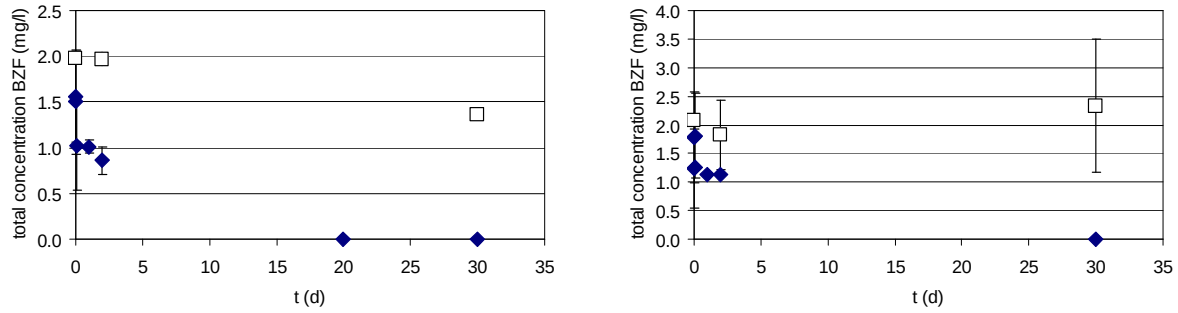


Figure 5-8: Concentration BZF in time in the aerobic test at 20 °C (AER-20-2, left) and at 10°C (AER-10, right), (♦ with sludge, □ without sludge).

For BZF the elimination after 2 days was expected to be higher since amongst others Ternes (1998) observed a removal of 83% in a municipal WWTP and Strenn (2004) reported a removal of >90% in lab experiments using a HRT of 2 days.

Diclofenac (DCF) was not eliminated in the first 2 days as it is shown in figure 5-9. In tests at different temperatures, 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 showed that DCF can be potentially eliminated in biological systems. The decrease in concentration after 30 days could be the result of a slow degradation rate, or the need for adaptation of the biomass before degradation of the specific compound could take place.
The fate of DCF in the controls was not consistent (in AER-20-2 decrease of DCF and in the AER-10 it remained stable). The causes of the decrease in AER-20-2 are, besides the possibility of measuring errors, unknown.

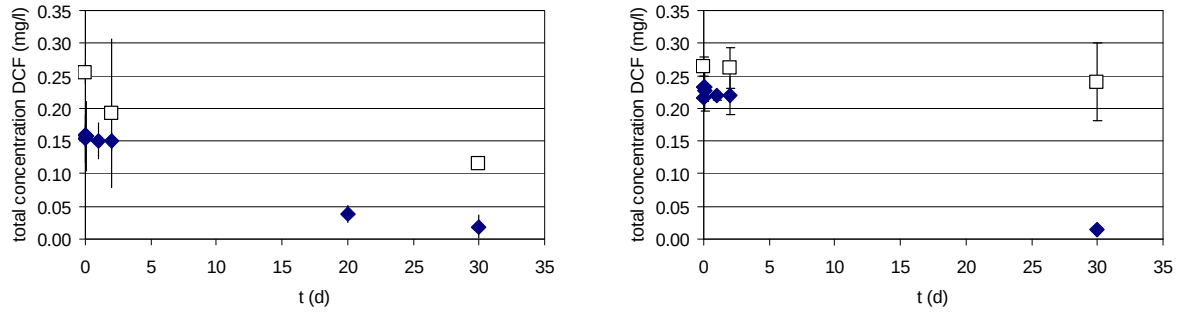


Figure 5-9: Concentration DCF in time in the aerobic test at 20 °C (AER-20-2, left) and at 10°C (AER-10, right) (♦ with sludge, □ without sludge).

For DCF different removal rates were reported in literature (see section 3.2.1). Tauxe et al. (2005) reported a removal of DCF of 0% in a municipal WWTP with a HRT of the biological treatment tank varying from 7 -16 h. Kosjek (2007) reported a removal of DCF in the water phase between 49-59% in a pilot WWTP run for 2 years with a HRT of maximum 2 days. Biodegradation of DCF is thus possible based on the observed findings but it is more difficult than for the pharmaceuticals described above.

The fate of carbamazepine (CBZ) at aerobic conditions is shown in figure 5-10. No decrease in concentration was observed after 2 days nor after 30 days. Moreover, the CBZ concentration during the period of 2-30 days was, according to the measurements, increasing. The increase could be caused by a fast sorption of CBZ in the beginning of the experiment and than its desorption due to aging (decay, changing of structure of activated sludge enabling a better extraction of considered compound in the analytical method) of the activated sludge.
Perhaps factors like concentration differences due to evaporation (although this is corrected for) or other errors in experimental set-up could be grounds for the increase in CBZ too.

The concentration of clofibric acid (CFA) during the 30 days lasting test is presented in figure 5-11. They are similar to those of CBZ. No significant decrease in concentration was observed over the entire duration of the test. The concentration CFA in the AER-20-2 increased slightly. In the AER-10 this was not observed.
This persistency of CBZ and CFA to biodegradation is in consistency with reported findings of Tauxe et al. (2005) and Strenn (2004) which found a removal of 0% for CFA and CBZ respectively during waste water treatment with activated sludge in several municipal WWTPs. For CFA however, also higher removal efficiencies, up to 51%, were reported by Ternes et al. (1998) (see also section 3.2.1).

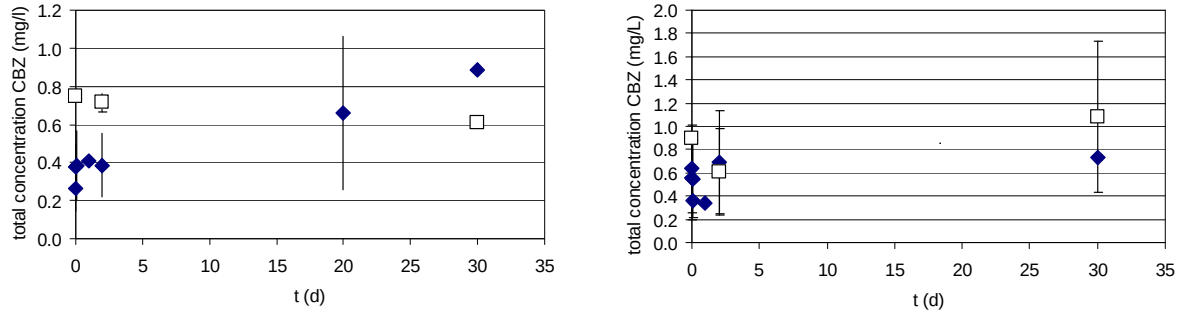


Figure 5-10: Concentration CBZ in time in the aerobic test at 20 °C (AER-20-2, left) and at 10°C (AER-10, right) (♦ with sludge, □ without sludge).

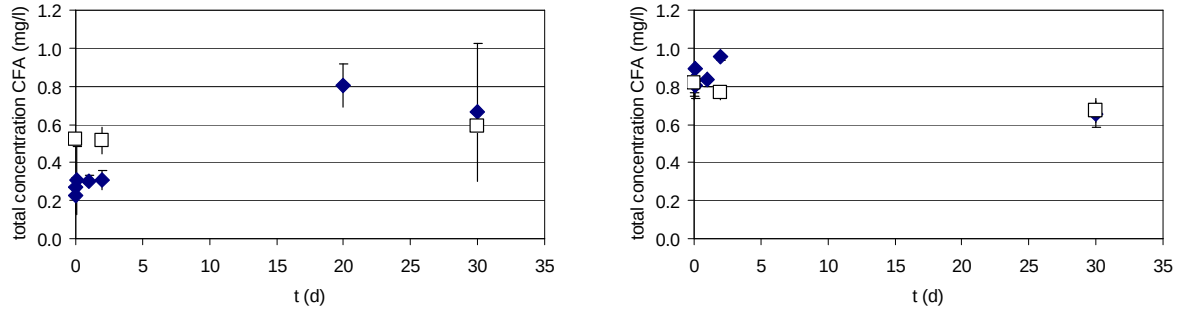


Figure 5-11: Concentration CFA in time in the aerobic test at 20 °C (AER-20-2, left) and at 10°C (AER-10, right) (♦ with sludge, □ without sludge).

Altogether, the aerobic tests showed a relative fast exponential decrease in concentration of ASA, FNF, IBU and MTP. The pharmaceuticals BZF and DCF are not or only to a limited extent eliminated within first 2 days of the test, after 30 days they were completely biodegraded. The absence of an easily biodegradable external carbon source could have influenced the biodegradability of pharmaceuticals positively, since for the bacteria no other source than pharmaceuticals and death organic material was available.
CBZ and CFA were not biodegraded at all in any aerobic test.

5.4 Biodegradation in anoxic batch tests

In figures 5-12 to 5-19, the results of the anoxic biodegradation tests performed at 10 and 20 °C are given. The batch test at 10 ℃ (ANOX-10) was performed over a time period of 2 days while the test at 20℃ (ANOX-20) was performed up t o 30 days.

ASA was biodegraded completely in both tests, within 48 hour ASA was under the detection limits. The decrease in concentration of ASA was faster at 20oC than at 10oC. The degradation rate in ANOX-20 and ANOX-10 was, however, slower than in the aerobic tests. Noteworthy was the initial concentration of ASA in the duplicates in the ANOX-10, which differed significantly from each other. One test started at 3.4 mg/L, the other at about 0.1 mg/L, while the expected concentration (amount presumably added) was 2 mg/L. The first duplicate subsequently showed an elimination of ASA to 0.044 mg/L (99% decrease in concentration). The second duplicate gave a lowest measured concentration of 0.039 mg/l (61% decrease in concentration) after 48 hours of the test. None of the controls resulted in the expected concentration of ASA of 2.0 mg/L. In the batches with sludge the initial concentration of ASA in ANOX-20 was very low and in ANOX-10 close to the expected one.

At anoxic conditions, FNF was degraded relatively fast like in the aerobic tests. Differences in degradation rates between different temperatures were not significant. The concentration of FNF in the controls in both anoxic tests at the first 2 days of the experiments stayed at a constant level. All other batch tests showed a decrease in FNF concentration in controls, but not in the anoxic batches, although the initial measured concentration was far from expected (0.1-0.2 mg/L against 2 mg/L respectively). Abiotic processes affected FNF less in the anoxic tests, apparently. The constant concentration of the FNF in the controls over the first 2 days, showed that the sludge played a role in the disappearance/degradation of FNF.

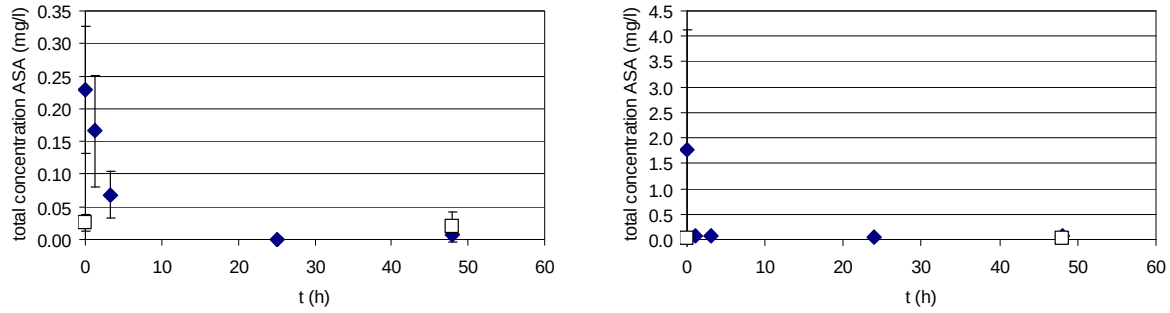


Figure 5-12: Total concentration of ASA in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

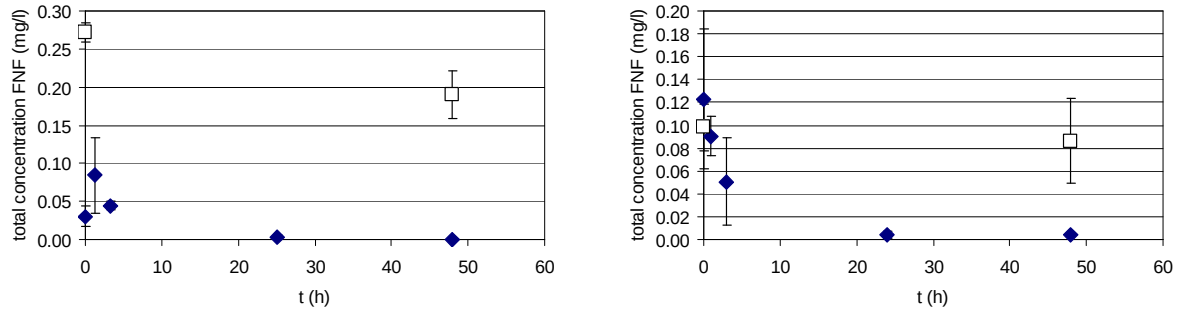


Figure 5-13: Total concentration of FNF in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

Ibuprofen was removed in the anoxic tests but slower than at higher oxidation-reduction potentials. Further, a large variation between the duplicates is observed (52% vs. 97% for B1 and B2 resp.). Both removal effiencies are higher than in literature. Zwiener (2002) reported 22% removal in anoxic batch test after 2 days for IBU.
Differences between the anoxic degradation rate in relation to temperature were observed between the ANOX-20 and ANOX-10 tests. A significant higher rate at a temperature of 20℃ was measured, as was expected.
The IBU concentration in the controls of the ANOX-20 stayed constant during the first 48 hours, but decreased significantly after 30 days. This could be the result of an error in the measurements or perhaps an unstable character of IBU at 20 °C in water while shaken. In such a case the elimination of IBU at 20°C could not be attributed to biodegradation only.
The initial concentration of IBU in both test batches was lower than expected like in the AER-20-2 test.
The overall removal rate of IBU under anoxic conditions might increase when applying a longer adaptation time for biomass. In the research of Suarez Martinez (2007) this was reported. In a completely mixed denitrifying reactor fed with an external carbon source and operating at a HRT of 1 day, the removal of IBU increased from 16% in the first 200 days and up to 75% at day 340. This can be related to the development of specific denitrifying biomass population in the denitrifying reactors (Suarez Martinez 2007).

In contrary to aerobic tests, metoprolol was only degraded to a small extent within 48 hours. At 20℃, MTP decreased in concentration up to about 40% after 48 hours. In the ANOX-10 no significant removal of MTP was observed. After 30 days, MTP concentration was under the detection limit in the ANOX-20. Again, the initial concentration in the test batches was lower than expected.

In the ANOX-20 a significant elimination of BZF was observed (about 70% reduction) after 2 days. After 30 days, the BZF concentration was close and under the detection limit in the ANOX-20 (duplicates). BZF was not decreased in concentration in the ANOX-10 test. Compared to the aerobic tests, the degradation rate in the ANOX-20 test was higher than in the aerobic tests. There is no clear explanation for this. It is unknown whether this concerns an analytical error or that BZF can be biodegraded faster under anoxic conditions. The latter could be possible since under anoxic conditions other, perhaps easier biodegradation pathways are used. The ANOX-10 showed similar results compared to AER-10: no significant removal of BZF within the first 2 days. The initial concentration in the ANOX test batches was much lower than expected, in contrary to the concentration in the controls. This possibly indicates a fast sorption of BZF onto the sludge and an insufficient recovery of BZF from sludge in the analytical method.

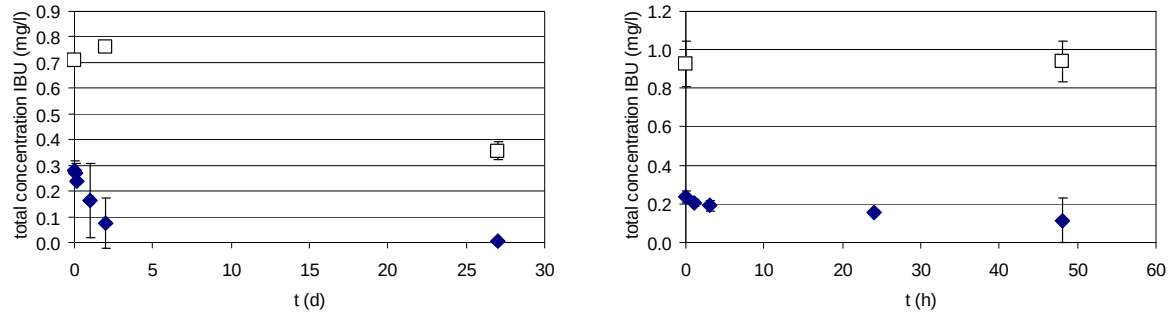


Figure 5-14: Total concentration of IBU in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

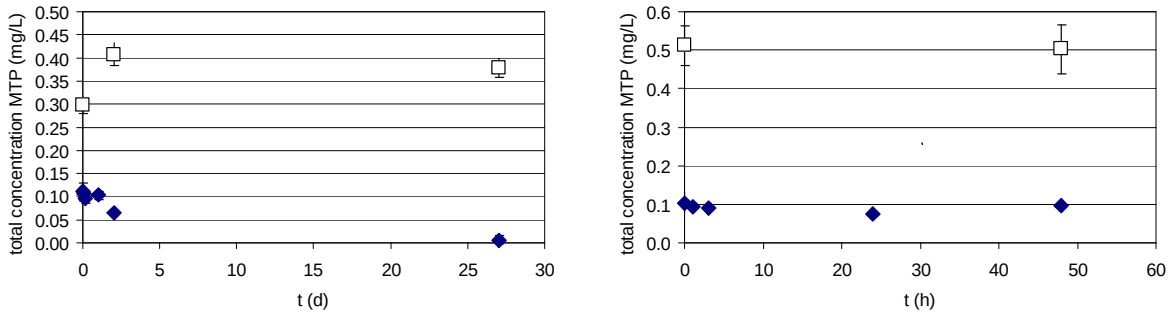


Figure 5-15: Total concentration of MTP in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

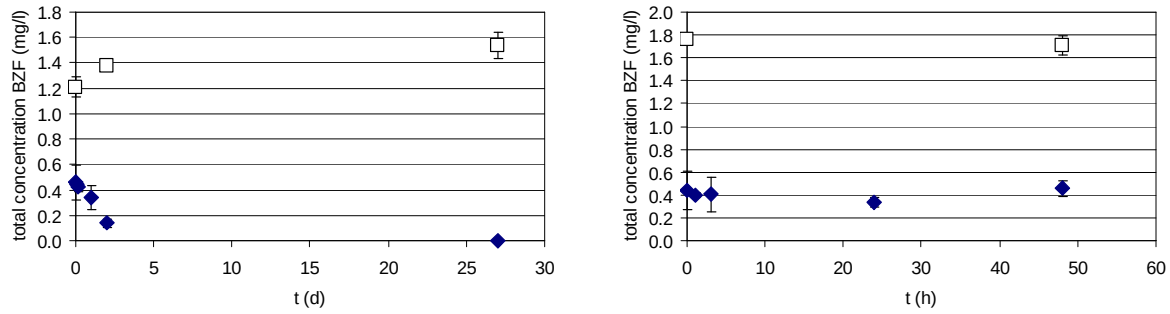


Figure 5-16: Total concentration of BZF in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

At a temperature of 10℃, DCF concentration remained constant in time. The graph of ANOX-20 shows that DCF concentration appeared to be reduced to a certain extent after 48 hours but the samples taken after 27 days showed that the concentration of DCF was still in the same range as before. This constant concentration over 27 days was in contrast to the aerobic tests. The controls confirmed the initial expected concentration of 0.3 mg/L, while in the tests this concentration was much lower. The latter would indicate again an error in the analysis of the solid or liquid phase concentration. Perhaps sorbed concentration was higher than measured.

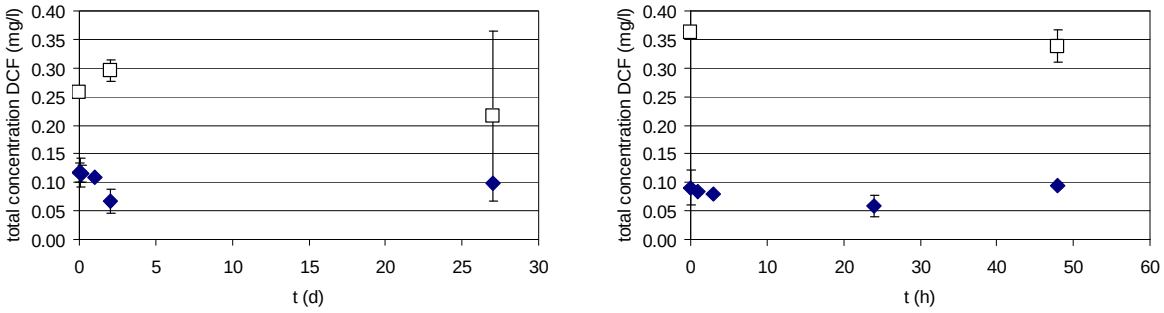


Figure 5-17: Total concentration of DCF in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

Both anoxic tests showed no decrease in concentration of CBZ, like in the aerobic tests. At the end of ANOX-20 test, the CBZ concentration measured was even increased.
For CFA the same was observed as for CBZ: no removal of the pharmaceutical under anoxic conditions and an increase in measured concentration after 1 month. No explanation is known for this other than an analytical error.

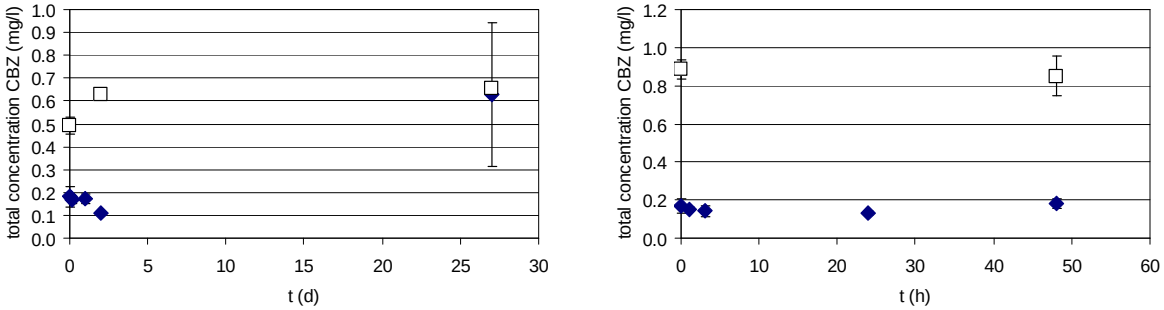


Figure 5-18: Total concentration of CBZ in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

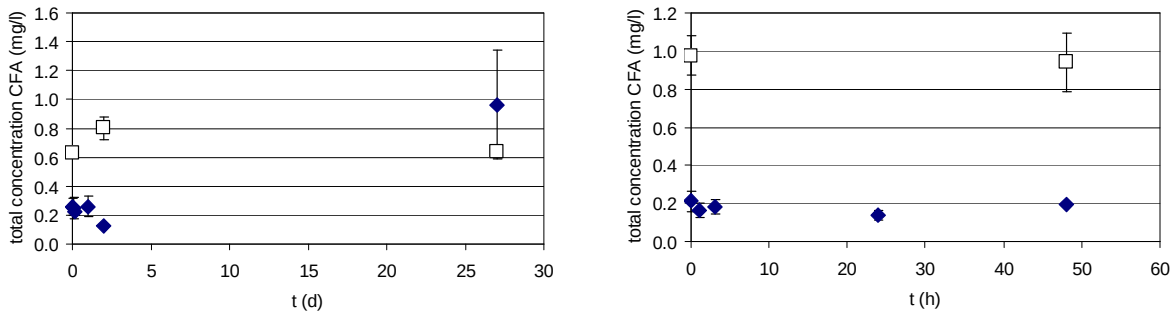


Figure 5-19: Total concentration of CFA in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

In addition, in the ANOX-20 it is observed that concentration after 48 hours is for all pharmaceuticals a little lower than the concentration after 24 hours, also for the persistent

pharmaceuticals. This might indicate a small analytical error at sampling time of 48 hours which could cause an overestimation of the biodegradation rate of the pharmaceuticals especially for IBU, MTP and BZF.

To conclude, the pharmaceuticals showed a lower degradation rate under anoxic conditions compared to degradation at aerobic conditions, with the exception of BZF in anoxic test at 20℃. But for BZF this is likely due to an analytic al error. The lower biotransformation rate is as expected since the anoxic endogenous respiration rate is lower than the aerobic one (Kujawa-Roeleveld 2000).
ASA, IBU, MTP and BZF showed a higher degradation rate in the ANOX-20 compared to the ANOX-10. For MTP and BZF the temperature difference in the tests resulted in a small removal at 20^{o}C and no significant removal at 10^{o}C within 48 hours.
After 27 days MTP, BZF, IBU, ASA and FNF decreased in concentration to under or close to the detection limit. It should however be kept in mind that redox conditions increased up to about 80 mV (micro-aerobic conditions) in the ANOX-20; this increase of ORP could have influenced this degradation positively. The ORP is not likely to have affected the differences in both temperature tests over the first two days; the ORP of the 10 and 20^{o}C tests was similar. In addition, in all tests the initial concentration was lower than expected except for the controls, indicating possibly a higher sorption of pharmaceuticals to sludge and poor extraction of these pharmaceuticals in the analytical method or an error in the sampling procedure or in the analysis of the concentration in the liquid phase.

5.5 Biodegradation in anaerobic batch tests

The anaerobic experiments were performed twice at a temperature of 30℃. These tests are abbreviated with ANAER-1 and ANAER-2 respectively. The time period of the ANAER-2 was 30 days. The ANAER-1 was continued up to 77 days to observe any effect at a prolonged retention time of pharmaceuticals under stress conditions (no external organic substrate supplied). The results are in figure 5.20 to 5.27.

In the ANAER-1 the pharmaceutical concentration in the solid phase could not be determined. Therefore the concentration in the liquid phase is plotted in the graphs of ANAER-1.
Determining biodegradation rate of pharmaceuticals in the ANAER-1 was difficult. The liquid concentrations were even re-analyzed to obtain reliable data due to a difference in analytical method applied between samples of ANAER-1. Those new values are plotted in the graphs. These results showed that ASA and FNF were eliminated. However the concentration in the controls also decreased and the were below detection limits after duration of the experiment indicating the presence of other processes aside from biological degradation

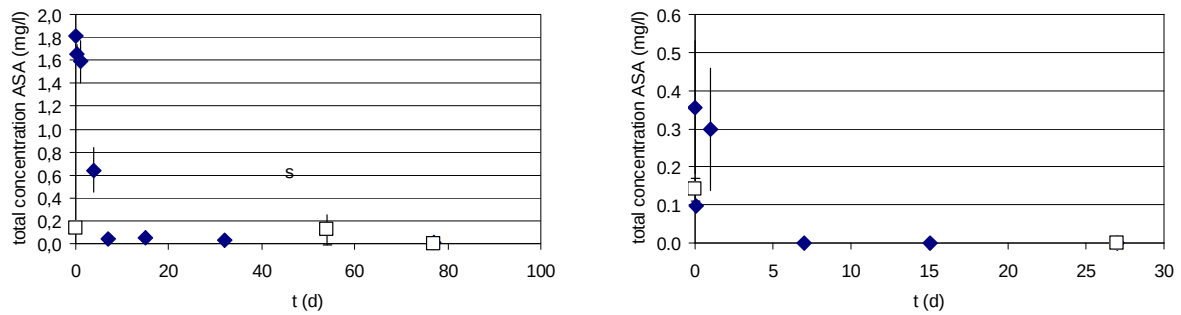


Figure 5-20: ASA concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

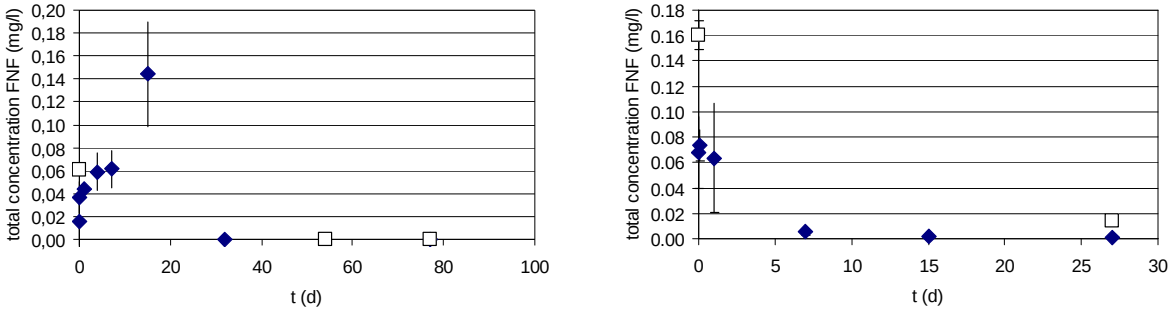


Figure 5-21: FNF concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

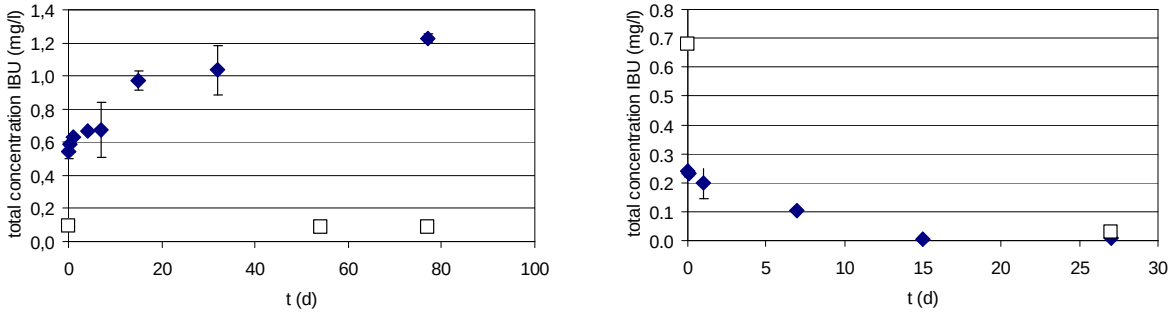


Figure 5-22: IBU concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

The initial concentration of ASA in the ANAER-1 was close to the expected concentration. The initial concentration of FNF was lower than expected.

In the ANAER-2, ASA and FNF were also eliminated. In this test also the concentration IBU decreased exponentially over time. The cause of the difference in the trend of IBU between both anaerobic tests is unknown. Removal efficiency of IBU in anaerobic digesters reported in literature was 26-56% (Carballa, 2007) with a SRT of 10-30 days. The anaerobic elimination of IBU is thus confirmed by literature.
In the ANAER-2 the control concentration decreased after 30 days with >99%, 95% and 90% for ASA, IBU and FNF respectively. The decrease in concentration in the biodegradation tests, can thus not be fully assigned to biodegradation processes.
The initial concentration of the batches with sludge were for ASA, FNF and IBU again lower than expected.

The biodegradation rates in both ANAER-1 and ANAER-2 were, compared to the aerobic and anoxic degradation rates, much lower. Nevertheless, after 30 days, which could be a common HRT for wastewater/sludge treated in anaerobic digesters, the concentration of all three pharmaceuticals decreased for more than 90%.

In case of ASA and FNF, the decrease in concentration and/or the very low start concentration in the controls was next to the anaerobic tests also measured in the aerobic and anoxic tests.
The decrease in concentration shows that, apparently, abiotic processes play also an important role in fate of ASA and FNF in biological systems. Hydrolysis can be an important process because both compounds can be very easily hydrolyzed in the human body to salicylic acid and fenofibric acid, respectively. For the hydrophobic FNF also absorption to materials in the batch tests (eg. glass walls, cups) and during sampling (syringe, centrifuge cups) and preservation (freezing) might play a role.

The much lower measured concentration can be due to a fast transformation or sorption of the substances. It could also be a matter of improper mixing at the start, but than the same phenomena should have been observed for all other pharmaceuticals, what was not the case.

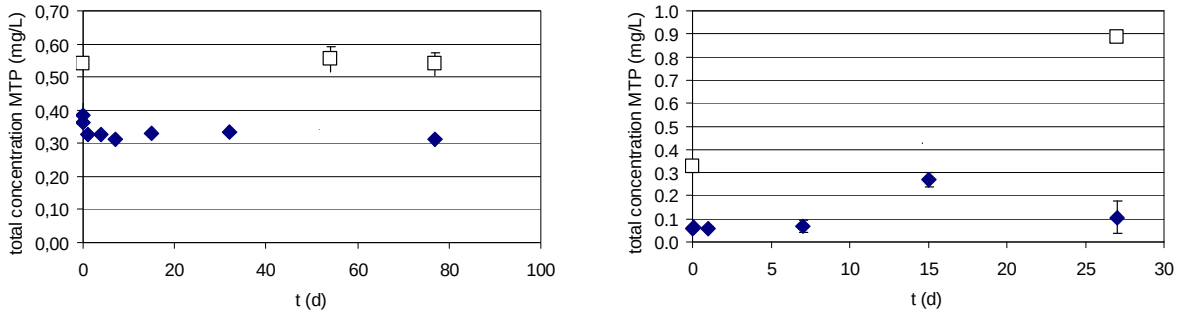


Figure 5-23: MTP concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

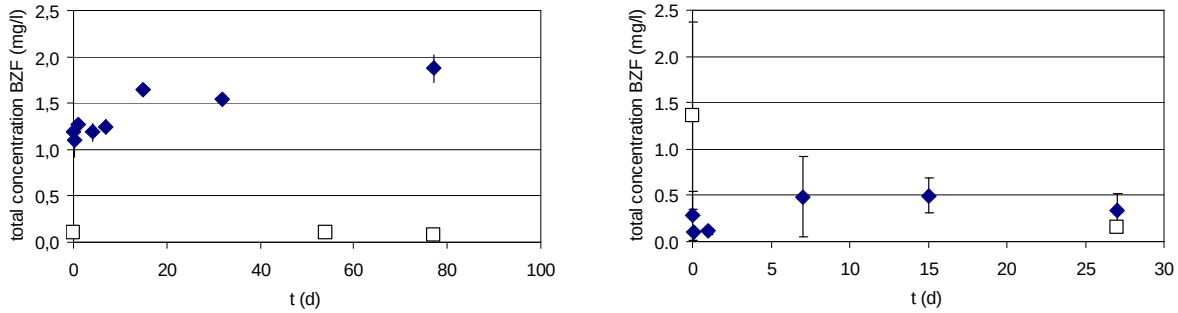


Figure 5-24: BZF concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

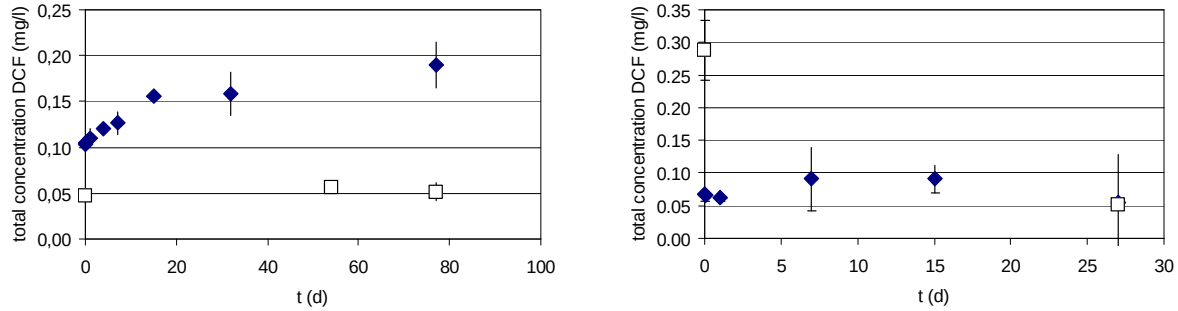


Figure 5-25: DCF concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

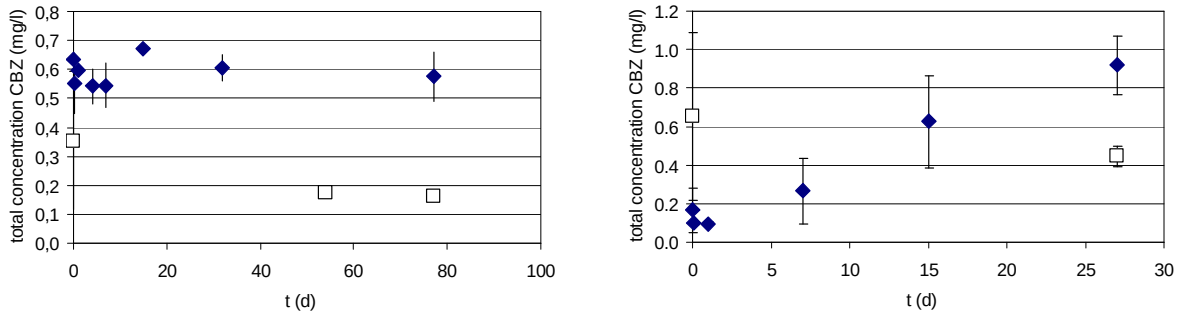


Figure 5-26: CBZ concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

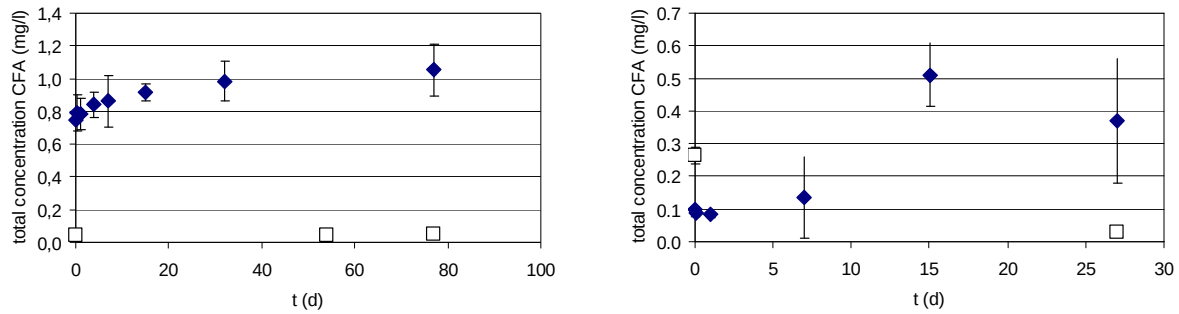


Figure 5-27:CFA concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

For MTP, BZF, DCF, CBZ and CFA, no significant decrease in concentration was measured in the ANAER-2 and ANAER-1. This is shown in figures 5-23 to 5-27. In ANAER-2, for DCF and BZF a slight decrease in controls was measured and a constant concentration in the batches with sludge.
For CBZ and CFA a high increase in concentration in the batches was measured in ANAER-2, which currently cannot be explained other than error in the experimental set-up or in the analytical procedure (possibly error in the sorption results).

Carballa (2007) reported a removal in anaerobic digesters with a SRT of 10-30 days of DCF of 59-79% in contrary to the results of this test. Perhaps this difference is partly due to removal by absorption to the suspended solids, which was high in concentration (30-95 g/l). The concentration sorbed to sludge was not analyzed in the study of Carballa (2007). Another factor could be a difference in sludge characteristics or the difference in DCF concentration. In case of CBZ, Carballa (2007) found, like in this research, no removal.

In the ANAER-2 the initial concentration of MTP, BZF, DCF, CBZ and CFA in the test batch were again lower than expected. In the ANAER-1 this was not the case, since the start concentrations in the batches with sludge were close the expected ones but not in most of the controls. This test was completely re-analyzed and therefore might be more precise. Again, it is put forward that in the ANAER-1 only the concentration in the liquid is presented.

In general, the anaerobic samples are more difficult to analyze than aerobic and anoxic samples. For example, because of the specific anaerobic sludge characteristics and high TS concentration the sludge, the solid phase in the anaerobic samples was less efficient separated from the water phase after centrifuging compared to the samples with activated sludge. An extraction of the compounds from the solid phase can be incomplete, while liquid phase can contain colloidal material, which makes it more difficult to analyze. This could have caused the increase in concentration of pharmaceuticals measured in ANAER-1 and ANAER-2 or the difference in expected and measured concentration at the start of some pharmaceuticals in ANAER-2.

Overall, the batch tests showed that at anaerobic conditions, the pharmaceuticals are not as efficient biodegraded than under aerobic and anoxic conditions.

5.6 Assessment of biodegradation kinetics

The biodegradation rates (biodegradation kinetics) of the pharmaceuticals are further asssed and quantified. The exponential decrease of pharmaceuticals over the course of the experiment was used to calculate with a first-order reaction rate biodegradation kinetics.
The degradation rate constant (k) and the specific biological degradation rate (k_{biol}) were according to the equations in section 4.7 calculated if a good exponential curve fitting could be made. The constants are given in table 5-2 together with the 95% confidence interval of k and the R^2 of the regression model.
With the 95% confidence interval the error in the calculated k is expressed. To calculate the 95% confidence interval for k_{biol}, this interval is divided by the both TS concentrations of the duplicates from a test.

Note that the kinetics presented are based on the measured concentration in the liquid and solid phase. The gaps in mass balances for some compounds (controls vs. tests), as uncertain, are not taken into account at this moment.

Carbamazepine, clofibric acid and diclofenac are not in the table 5-2 present. CBZ and CFA showed no (exponential) decrease in concentration in any test. Diclofenac was biodegraded in the aerobic tests, but after the first two days. There was not enough data to fit a curve.
For the other tested pharmaceuticals biodegradation kinetics were calculated at test conditions in which exponential decrease was observed. The curves fitted trough the measured concentrations over time with a too low R^2 (<0.85) were regarded as unreliable and therefore not given in table 5-2.

For the anoxic tests not many kinetics could be assessed. For IBU results in ANOX-20 this was because the trend in the two duplicates was too different. The decrease in IBU concentration in ANOX-10 was calculated as not significant. Only for BZF kinetics could be determined, but these could be a little overestimated as explained above (section 5.4).

The degradation rate constants of FNF and ASA were determined, but not in all tests it was demonstrated that this removal was due to biological processes. Therefore certain values are actually representing the disappearance rate as indicated in the table. Moreover, because of the fast decrease of ASA and FNF concentration in the aerobic tests, experimental curves could not be fitted. In this case, where possible, k-values for ASA were calculated based on assumption that the start concentration of ASA was 2 mg/l and that the first sample was taken 0.2 hour after the addition of pharmaceuticals. The obtained exponential trend is indicated with ‘best case scenario’. For FNF no proper fit could be obtained, also not when using ‘best case scenario’ method. For some other pharmaceuticals the start concentration was also lower than the initial expected concentration, but only for ASA and FNF a fast decrease in concentration within a few hours after the start of the experiment was observed. Therefore, only for ASA and FNF the ‘best case scenario’ methodology was applied.

Table 5-2: The biological degradation rate constant k, its 95% confidence interval, the R^2 of the regression model and the range of the specific degradation rate constant k_{biol} of pharmaceuticals.

Pharmaceutical	Test	k-value (1/d)	95% confidence interval of k		R^2	k_{biol} (L/gTS/d) range
ASA	AER-20-1	104	103	106	0.99	25.5 -26.4
ASA (best case scenario)**	AER-20-2	218	217	219	0.99	37 - 44
ASA	ANAER-1	0.48	0.42	0.53	0.95	0.021-0.027*
ASA	ANAER-2	1.9	1.3	1.4	0.93	0.11- 0.13*
FNF	ANAER-2	0.38	0.36	0.40	0.93	0.031- 0.035*
IBU	AER-20-1	5.6	5.4	5.9	0.98	1.5 - 1.4
IBU	AER-20-2	5.2	5.1	5.4	0.94	0.87 - 1.07
IBU	AER-10	4.4	4.3	4.6	0.90	0.95 - 1.06
IBU	ANAER-2	0.29	0.28	0.30	0.94	0.024 - 0.026*
MTP	AER-20-1	3.5	3.4	3.6	0.96	0.84 - 0.89
MTP	AER-20-2	3.4	3.3	3.5	0.95	0.57 - 0.69
MTP	AER-10	0.86	0.86	0.89	0.98	0.19 - 0.21
BZF	AER-20-1	0.24	0.22	0.24	0.96	0.054 - 0.060
BZF	AER-20-2	0.19	0.19	0.22	0.87	0.038 - 0.043
BZF	ANOX-20	0.58	0.55	0.58	0.92	0.11 - 0.12

* the value is, more accurately, the specific *degradation* rate constant, since it is not elucidated that the elimination is due to biological processes.

** best case scenario: it is assumed that the start concentration of ASA was 2 mg/l and time period between first sample and the addition of pharmaceuticals was 0.2 h.

Obviously, the kinetics in the aerobic, anoxic and anaerobic tests differed. In all cases, the aerobic tests (as well as at 10 and 20^{o}C) the rates were higher than in the anoxic tests and much higher than in the anaerobic tests. Both anaerobic tests resulted in very low biodegration kinetics, with a k_{biol} in ANAER-1 and ANAER-2 for ASA of 0.1 and 0.25 L/gTS/d respectively.
Between pharmaceuticals, the kinetics differed as well as can be observed in table 5-2. With regard to the AER-20-2 test, ASA was degraded most fast (25 L/gTS/d), followed by IBU (0.87-1.1 L/gTS/d), MTP (0.57-0.69 L/gTS/d) and BZF (0.038-0.043 L/gTS/d).

For aerobic condition, literature values are reported for some of the selected pharmaceutical (section 3.3). In this research the specific degradation rate were lower than as observed in Joss (2006). This can be caused by the difference in concentration. In the results of this test, high concentration of pharmaceuticals were used, representing pharmaceutical concentration in source separated sanitation. In the article of Joss (2006) concentration as in conventional sewage systems were used. Also in de Mes (2007) in where the biodegradation kinetics of estrogens in concentrated waste streams is investigated, lower kinetic constants were reported compared to those observed when using low pharmaceutical concentration (as in sewage). The high concentration and the mixture of pharmaceuticals perhaps inhibit the activity of bacteria to a certain extent.

Moreover, the tests were performed without the addition of an external carbon source in order to research the biodegradation of the pharmaceuticals. When adding an external carbon source the degradation rate might be higher, because co-metabolism of pharmaceuticals can than easily take place. This could also caused the lower biodegradation rate in comparison of those reported by Joss (2006).

Differences in kinetics at different temperature were observed next to differences in redox conditions. At a temperature of 20^{o}C the biodegradation rates were mostly higher as observed in section 5.3-5.5. For the MTP and IBU, the temperature coefficient κ of the Arrhenius equation (eq. 4.8) is calculated to quantify this difference. In case of the other pharmaceuticals, the exponential fitting was improper or a lower temperature resulted in no exponential decrease within 2 days (in case of BZF) so that no temperature coefficient could be determined. The κ is expected to be in the range of 0.03-0.09 for pharmaceuticals (Ternes 2006).

Table 5-3: The influence of temperature on biodegradation rate.
κ is calculated (eq. 4.8) based on k_{biol} range of AER-20-1, AER-20-2 and the AER-10 results.

Pharmaceutical	Test results	κ (-)
MTP	AER-20-1 / AER-10	0.17-0.16
	AER-20-2 / AER-10	0.14-0.11
IBU	AER-20-1 / AER-10	0.03-0.05
	AER-20-2 / AER-10	No sig. difference

The temperature coefficient κ is in case of MTP higher than the expected range, although in the same order of magnitude. For ibuprofen, the difference in temperature measured between AER-10 and AER-20-2 was not significant. Comparing AER-20-1 and AER-10 gives κ- values ranging from 0.03-0.05. This difference in AER-20-1 and AER-20-2 could be due to improved sample preservation in AER-20-2.

It should be stressed that only the degradation of the original pharmaceutical was analyzed. Whether a pharmaceutical completely mineralized and thus if the subsequent produced metabolites were degraded is at this moment unclear. Regarding the section on metabolites in chapter 3, for 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 biodegradable. The possible 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.

5.7 Sorption

This section elaborates the sorption behaviour of the selected pharmaceuticals in the tests. In all samples, the concentration of pharmaceutical compounds present in water and solid phase was analyzed. The contribution of the concentration in the solid phase to the total concentration is important for determination of the removal of pharmaceuticals via sludge.

Note that the obtained measured concentration are used in the section. The gap in mass balances between control and test, as uncertain, are at this moment not taken into account.

Sorption of pharmaceuticals to the activated sludge from WWTP Bennekom was analyzed with the data from the anoxic tests. The anoxic tests were used for this purpose because compared to the aerobic tests which were performed with sludge from the same origin, the least degradation of pharmaceuticals was observed and thus the highest sorption results could be calculated. The sorption to anaerobic sludge was analyzed with the use of the results of ANAER-2 since pharmaceutical concentrations in the solid phase in the ANAER-1 were not available for majority of the samples. All other sorption results can be found in the appendix.

To be sure equilibrium of pharmaceuticals between the solid and liquid phase could be assumed, only those samples were taken into consideration which showed no decrease in total pharmaceutical concentration compared to the starting value. Moreover, concentrations at the start(t=0) were left out of the calculation for this purpose as well.

The sorption behaviour of pharmaceuticals to activated and anaerobic sludges are shown in figure 5-29.The figure shows clearly that the pharmaceutical fraction in the liquid phase prevailed. Only a small part of the total pharmaceutical concentration was present in the solid phase.
Differences between pharmaceuticals and between the different types of sludge were there.

Sorption turned out to be most relevant for FNF, CBZ and MTP. The percentages of these pharmaceuticals sorbed to solid were 84-87%, 9 -21% and 8.2-17% respectively for the anoxic tests. In the anaerobic test (ANAER-2) the percentages pharmaceutical in the solid phase were much more varying. This is because the low concentrations in the beginning of the experiment (especially the first hours) and the higher liquid concentrations after 15 days.
It is difficult to asses which values are correct if the difference in liquid concentration is due to measuring errors. In figure 5-28 the averages of these percentages are given. The percentages are varying between 74-79%, 3-50% and 5-63% respectively for FNF, CBZ and MTP. To add, in ANAER-1 at t=77 days the percentage pharmaceutical absorbed to solid is known. This is 23% for CBZ and 36% for MTP, slightly higher than for activated sludge.

Regarding ASA, IBU, DCF, BZF and CFA, less than 10% of the total concentration was absorbed in both anoxic and anaerobic tests as can be observed in figure 5-28.

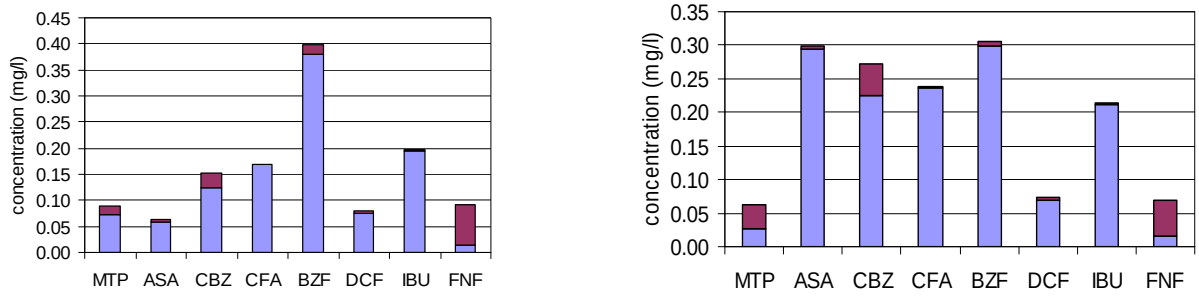


Figure 5-28: Average concentration of pharmaceuticals in both liquid (blue) and solid (red) phase during the time interval in which the pharmaceutical concentrations were constant. Results from the ANOX-10 test (left) and ANAER-2 test (right).

The low sorption affinity of ASA is as expected since the compound is hydrophilic and acidic. Apparently, hydrophobic character of IBU, DCF, CFA and BZF is also too small to obtain high pharmaceutical concentration in the solid phase.

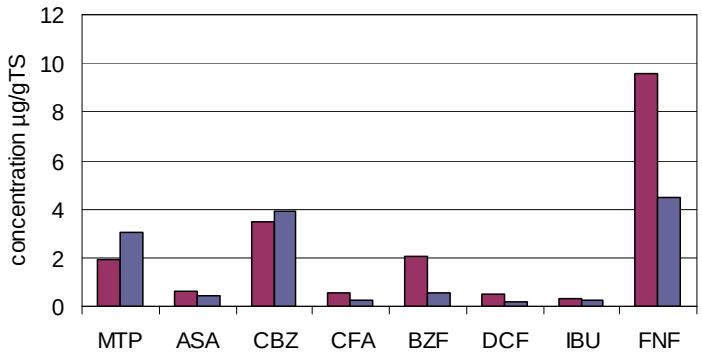


Figure 5-29: Pharmaceutical concentration per g of TS in ANOX-10 test (red) and in ANAER-2 (blue) test.

In figure 5-29 the concentration absorbed per g TS is presented. These figures are independent of measuring errors of the liquid concentrations in the anaerobic tests. Moreover, because the concentration is expressed per g of TS, comparison between anaerobic sludge and activated sludge is possible. The sorption to sludge is not differing much between the sludge's according to figure 5-29. Sorption in the ANOX-10 test was for FNF, BZF, CFA, DCF, IBU and ASA higher and for MTP and CBZ lower compared to sorption in the ANAER-2.

To compare the results also with literature, the concentration in the solid is divided by the concentration in the liquid, since it influences the sorption equilibrium as well. The sorption partition coefficient (K_d) is obtained in this way. In table 5-4, the calculated K_d values from this research are compared with K_d values from literature as presented in section 3.2.2. K_d values of activated sludge were obtained from the average sorption results of ANOX-20 and ANOX-10 test. Since the percentages of pharmaceuticals sorbed to anaerobic sludge are varying a lot over time, for this test a range for K_d is given covering the different results.

Table 5-4: Comparison of the assessed solid distribution coefficients (K_d) with literature values for activated sludge and anaerobic sludge. Observed K_d for activated sludge were determined based on the concentrations of pharmaceuticals in the ANOX-10 and ANOX-20 tests. K_d for the anaerobic sludge were determined using results from ANAER-2. Literature values are from Ternes (2004). n.a. = not available;

	MTP	ASA	CBZ	CFA	BZF	DCF	IBU	FNF
K_d (L/kg TS)								
Activated sludge (this test)	24	10	29	3.1	7.1	5.9	1.7	6.5^{E}+02
K_d (L/kg TS)								
Anaerobic sludge (this test)	5-110	1.5	4-18	0.5-1.0	0.5-1.9	1.4-4.7	1.3	2.8^{E}+02
Kd (l/kg)								
Activated sludge (literature)	n.a.	n.a.	1.2	4.8	n.a.	16	7.1	n.a.

There is the high K_d value for FNF. Sorption of FNF is one or two magnitudes higher than for the other pharmaceuticals. It is explained with the high K_{ow} value of FNF.
For MTP, the K_d range from the results of the ANAER-2 is quite high reflecting the differences in analyzed liquid concentrations over time. Since especially the low concentrations are uncertain, the high K_d values could be a overestimated values.
Moreover, since a gap between the mass balances of the control en test batches is noticed for the anoxic tests and ANAER-2, the K_d value could be higher or lower depending on the cause of this gap (error in solid or liquid phase).

For activated sludge from municipal WWTPs, K_d values are reported in literature. Values for CFA obtained in this study are very similar to those reported by Ternes (1998). The K_d value of IBU and DCF are a bit lower, the value for CBZ was on contrary higher in this research.
Differences between the K_d values can be explained by different sludge characteristics as this is very important for sorption behaviour. Also the pH in the batches could have affected this. The pH determines the deprotonated fraction of an acidic compound (f.e. IBU) and therefore fraction of a pharmaceutical which is negatively charged. Moreover, in case of DCF the log K_{ow} which indicates the hydrophobic character, is influenced by pH (Ternes 2006).
The pH in the batch tests were all between 7-8.5. Only at the aerobic tests after 30 days, the pH dropped to 5.6-6.4. But a clear effect of the pH drop is not observed. For the non-biodegraded pharmaceutical CFA, the sorption decreased in AER-20-2 from to 0.24 ng/gTS to 0.14 ng/gTS which was not expected since the decrease in pH was supposed to enhance sorption. For CBZ, a increase was observed from 0.7 µg/gTS to 1.4 µg/gTS.

The results showed that FNF, the most hydrophobic pharmaceutical with a K_{ow} of 5.2 was absorbed the most.
The results further point out that the electrostatic interactions between pharmaceuticals and sludge are relevant processes too. MTP and CBZ which are both the only non-acidic compounds, showed compared to CFA, BZF and IBU a higher sorption although the log K_{ow} values are similar or lower.
These findings are in consistency with Suarez (2007). Suarez (2007) reported the highest removal efficiencies for lipophilic pharmaceuticals and personal care products and a highest removal of DCF at lowest tested pH during flotation processes.
Moreover, the calculated distribution coefficient, K_d, of activated sludge makes clear that for all selected pharmaceuticals, except for FNF, sorption is not a relevant removal mechanism in a conventional municipal WWTP as their value is lower than 500 L/kg TS.
FNF has a calculated K_d higher than 500 L/kg TS and therefore for this pharmaceutical sorption could be an important removal process in a WWTP. However, since it is decomposed very fast, the sludge concentration drops in the ANOX-10 test from about 10 to 0.5 µg/gTS after 48 hours. For this pharmaceutical sorption is therefore also only a minor

elimination process. For other pharmaceuticals, which are as hydrophobic as FNF and persistent, sorption can be important in removing the pharmaceutical from the wastewater.

5.8 Implications for biological systems

The batch tests demonstrated biodegradability of selected pharmaceuticals. The implications of the obtained results for source separated urine application on the field and biological treatment system of black water is further elaborated.

5.8.1 Application of urine on soil

In the STOWA project in Anderen the possibilities of applying urine on the field as fertilizer has been tested.
Applying untreated urine on the field as a fertilizer will result in the input of pharmaceuticals onto the soil as well.
The fate of these compounds in soil and their transport to ground water depends on many factors, amongst others: sorption/desorption, biodegradation, abiotic conversions and the (rain) water flow in the soil.
To assess the biodegradation kinetics in soil several factors are important: presence and abundance of micro-organisms, bioavailability of absorbed pharmaceuticals, temperature, pH, uptake by plant roots and the redox conditions.
The density of micro-organisms will be lower than in biological water treatment systems. In case of a sandy soil, it will be highest in the top soil because there oxygen and organic matter will be present most abundant. Over the whole soil column different conditions might be present. At a lower soil depth, the conditions become anoxic and /or anaerobic.
Assuming bioavailability and the presence of micro-organisms, the well biodegradable pharmaceuticals (ASA, FNF, IBU) are most likely to be, at least partly, biodegraded in aerobic, anoxic and anaerobic zones. For MTP and BZF biodegradation seems possible as well in the aerobic and anoxic zones of soil, although this will be at a relative slow rate. The same counts for DCF, although it can only be biodegraded aerobically. When the vertical flux of (rain) water is low, the retention time of pharmaceuticals can be very high (up to several years). This is thus much higher than in the laboratory tests of this study (with a duration of 30 days). This will enhance the removal of pharmaceuticals. Moreover, urine will be applied on the soil only once or twice a year.

No leakage to groundwater takes place when the biological degradation rates at different soil depths covers the flux of pharmaceuticals in the liquid phase through the soil. Processes as (de)sorption and bioavailability of pharmaceuticals are crucial here.

For the pharmaceuticals which were not removed at all (CFA and CBZ) in the batch tests, no biodegradation in soil is expected. In this case, the pharmaceuticals will be absorbed to soil particles or they will be transported with the water flow to groundwater. An increase in concentration of pharmaceuticals absorbed over years is foreseen when urine is applied yearly. The sorption capacity of the soil can be very high, but leakage to groundwater might be possible. A possibility to eliminate the pharmaceuticals might be the development of specific bacteria over the years in these soils which are able to convert specific pharmaceuticals but no literature about this is known for the selected pharmaceuticals.

Although the presence of pharmaceuticals in urine can be very low, depending on the consumption of pharmaceuticals, not all pharmaceuticals consumed nowadays can be biodegraded. If any risk of contamination of soil and/or groundwater should be eliminated than pre-treatment of urine is necessary.

5.8.2 Black water treatment system

To asses the fate of pharmaceuticals in black water treatment system, two selected pharmaceuticals were used to calculate their fate in the system as presented in figure 5-30.
The treatment consists of an anaerobic part (UASB-ST), like in the demonstration project in Sneek (Zeeman 2006), and an aerobic treatment consisting of a MBR. The MBR is chosen because of the higher SRT which can be obtained in this system in comparison to conventional activated sludge system. In this way it is assumed that bacteria get more time to adapt to the elevated concentrations of pharmaceuticals originating from black water. Moreover, the absorbed fraction of the pharmaceuticals can be biodegraded over a longer time period.
The assumed design parameters are given in figure 5-30. An influent flow of 7 L/d was assumed per person (vacuum toilet 1L/flush, 6 visits, 1 L waste produced), resembling the flow in demonstration plant Sneek. The other parameters of UASB-ST resemble to those in demonstration plant Sneek as well. Parameters of the aerobic MBR were taken from a high loaded MBR system in the Netherlands treating municipal wastewater (Benthem 2006).

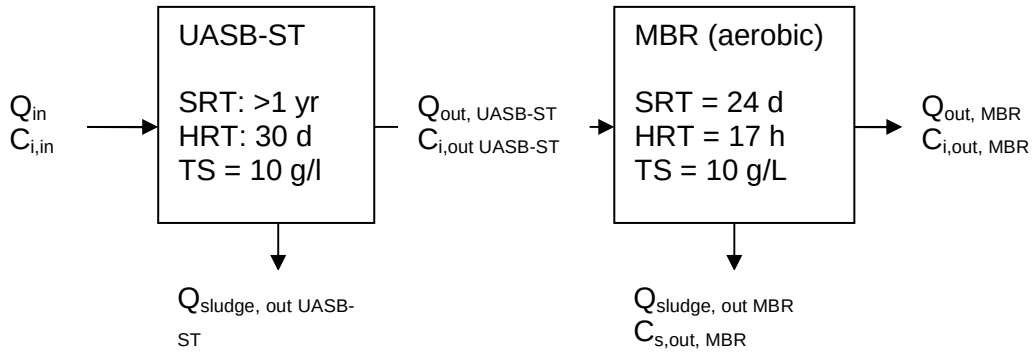


Figure 5-30: Blackwater treatment system with UASB-ST and an aerobic membrane bioreactor (MBR). Parameters are taken from Kujawa (2005) and Benthem (2006) respectively.

The calculations are performed with the moderately biodegradable metoprolol (MTP) and the non-biodegradable pharmaceutical carbamazepine (CBZ).

Table 5-5: Mass balance of MTP and CBZ during treatment of black water (black water flow is equal to that of 1 person). Numbers between the brackets show the percentage of pharmaceutical left with respect to the influent concentration.

	CBZ	MTP
UASB-ST		
Influent concentration (C_{in}) (µg/l)	1070	2900
Effluent concentration UASB-ST ($C_{i,out\ UASB-ST}$) (µg/l)	1070 *(100%)*	2900 *(100%)*
Aerobic MBR		
Influent concentration (=$C_{i,out\ UASB-ST}$) (µg/l)	1070	2900
Effluent concentration MBR ($C_{i,\ out\ MBR}$) (µg/l)	1060 *(99%)*	530 *(20%)*
Total Mass Balance		
Flow in (µg/d)	7,500	20,000
Flow out (water phase) (µg/d)	7,450	3,700
Flow out (solids phase) (µg/d)	50	30

Influent concentration is calculated with eq. 4.9. The influent concentrations are calculated without taking into account the user fraction of CBZ and MTP. Therefore the concentrations in table 5-5 belong to those expected in a worst case scenario.

For each treatment tank (UASB-ST and MBR) a mass balance (eq. 4.10) is given and the influent and effluent concentration of MTP and CBZ.
Because the SRT in UASB-ST is very high, only part of the sludge is removed maximally once a year, and because the absorption to sludge is limited, the removal of pharmaceuticals with solids in the UASB-ST is neglected.

In table 5-5 it is shown that in the mass balances of the overall system CBZ passes the wastewater treatment system without a significant decrease in concentration and it leaves the plant at a concentration of about 1 mg/l. The fate of MTP is different. The concentration stays unchanged after treatment of the water in the UASB-ST, but in the aerobic system it decreases with 80% to 0.53 mg/l.
The amount of CBZ and MTP leaving the aerobic MBR via the solid phase, is in both cases limited compared to the amount in the liquid phase.

The reduction of 80% in case of MTP shows some that pharmaceuticals can be biodegraded to a large extent. However, still a relative high concentration is present in the effluent. A longer HRT in the aerobic system will be beneficial with respect to the MTP effluent concentration, but this will of course enlarge the volume of the treatment tank.
Calculations for CBZ show that this pharmaceutical, and likely more pharmaceuticals, will pass the biological water treatment system without any change. To remove these compounds from urine or black water other systems are required like physical-chemical treatment options such as treatment with eg. activated carbon or nanofiltration (Maurer et al., 2006).

Conclusions

Fate of pharmaceuticals was researched in biological treatment systems under various environmental conditions. The pharmaceuticals, which were selected in order to represent a large group of pharmaceuticals, have different biodegradability potentials.
A summary of the biotransformation of pharmaceuticals and the influence of different environmental conditions on this biodegradation is presented in table 6-1.

Of all pharmaceuticals, acetylsalicylic acid (ASA) and fenofibrate (FNF), are eliminated most fast. ASA and FNF can be eliminated well at aerobic, anoxic and anaerobic conditions (>99% within 2 days of the aerobic and anoxic tests and within 30 days of anaerobic test). Biological processes play a role in this, but not solely since abiotic processes were detected as well: concentration in the batches without sludge decreased in some cases as well.
Ibuprofen (IBU) can be biodegraded as well under the three different redox conditions. Metoprolol (MTP) can be biodegraded under aerobic and anoxic conditions but at a slower rate than ASA, FNF and IBU (>80% within 2 days at aerobic conditions). Under anaerobic conditions, biodegradation of MTP was not observed.
Bezafibrate (BZF) can be slowly biodegraded under aerobic and anoxic conditions (about 40% removed after 2 days in the aerobic test at 20^{o}C). Diclofenac (DCF) has the potential to be biodegradable under aerobic conditions at a relatively high retention time (up to 90% removal in 30 days). At anoxic and anaerobic conditions, DCF is not degraded.
Clofibric acid (CFA) and carbamazepine (CBZ) are not biodegraded at all during any tested condition.

Table 6-1: Comparison of biodegradation rate of the selected pharmaceuticals at different environmental conditions. Biodegradability: +++ = very well, ++ = well, + = moderately, +/- = degradable (but including abiotic processes), - = not biodegradable within the test period.

	Aerobic-20^{o}C	Aerobic-10^{o}C	Anoxic-20^{o}C	Anoxic-10^{o}C	Anaerobic-30^{o}C
ASA	+++	+++	++	++	+/-
FNF	+++	++	++	++	+/-
IBU	++	++	+	-	+/-
MTP	++	+	+	-	-
BZF	+	+	+	-	-
DCF	+	+	-	-	-
CBZ	-	-	-	-	-
CFA	-	-	-	-	-

The biodegradation of pharmaceuticals follows pseudo first order kinetics and therefore biological degradation rate constants could be determined for ASA, FNF, IBU, MTP and BZF. In these batch tests, the biological degradation rate constants were lower than those reported in literature (Joss 2006).

Aerobic conditions resulted in general, in highest biodegradation rates, varying from 0.038 L/gTS/d for BZF up to 44 L/gTS/d for ASA. Biodegradation rates were lower during anoxic conditions, followed by subsequently anaerobic conditions. Temperature decrease from 20^{o}C to 10^{o}C in aerobic and anoxic environments influences the biodegradation rates. Differences varied from no significant differences to distinct differences.

The fraction absorbed to sludge is for selected pharmaceuticals of minor importance. Sorption turns out to be highest for non-acidic pharmaceuticals and pharmaceuticals with a very high hydrophobic character. For most pharmaceuticals concentration in the solid is <10%. For the non-acidic pharmaceuticals, MTP and CBZ, sorption is higher. The very hydrophobic but fast eliminated FNF is absorbed most.

Overall, the pharmaceuticals can be divided into three groups according to their biodegradability. With regard to the selected pharmaceuticals, the first group includes ASA, FNF and IBU. This group of pharmaceuticals can be biodegraded under various environmental conditions and therefore these compounds can be removed in biological treatment systems under different redox conditions. The second group consists of MTP, BZF and DCF. These pharmaceuticals can be biodegraded but under certain conditions. Biological treatment systems can be chosen such that the biodegradation of these pharmaceuticals is enhanced as much as possible by selecting favourable temperature, retention time and redox potentials. Aerobic systems will be most efficient in the removal of these pharmaceuticals. The third group consists of pharmaceuticals which cannot be biodegraded. In this research, these were CBZ and CFA. The group of non-biodegradable pharmaceuticals is likely to pass the biological treatment systems in almost unchanged concentration and additional treatment steps will be necessary to remove these compounds.

Recommendations for further research

Based on the results and conclusions of this thesis the following recommendations for further research are made:

- ✓ The disappearance of FNF, ASA and IBU in anaerobic systems should be further researched to clarify if it results from biological processes and/or abiotic processes.
- ✓ Biodegradation rate of pharmaceuticals should be researched with a pilot continuous system. The activated sludge originating from municipal WWTP Bennekom used for the aerobic and anoxic tests was not adapted to the high pharmaceutical concentrations as present in source separated wastewater. An pilot continuous system allows adaptation of bacteria and research about this could give information about the possible enhanced biodegradation rates of pharmaceuticals using adapted biomass.
- ✓ Analyzing pharmaceuticals at low concentrations is complicated. In the results gaps in mass balances very found indicating possible loss of pharmaceuticals during sampling or analysis method. More research in the analytical part is recommended to acquire more insights in the analysis of pharmaceuticals, in both liquid and solid phase samples.
- ✓ In this research the fate of original compounds was analyzed and not the fate of the produced metabolites. As e.g. the metabolite of clofibrate, CFA, shows, metabolites can be very persistent. Only for a small group of pharmaceuticals, metabolites and their biodegradability have been determined in other researches. The main recommendation therefore is to research the biodegradability of produced metabolites, thus the complete mineralization of pharmaceuticals.
- ✓ Some pharmaceuticals turn out to be persistent to biotransformation, like CBZ. When only biological systems are applied to treat black water, a relative high concentration of the persistent pharmaceuticals will be released in the environment. Size of the group non-biodegradable pharmaceuticals and its relevancy should be researched in order to assess to necessity of applying physical-chemical treatment systems (e.g. nanofiltration or activated carbon). Moreover, these physical-chemical treatment options should be researched in their performance to degrade or remove these pharmaceuticals in a sustainable way.
- ✓ Besides researching at the end of the user chain, the whole chain of production, prescription, consumption and wasting of pharmaceuticals should be researched in order to reduce as much as possible the release of pharmaceuticals to the environment.

Acknowledgements

The state research institute RIVM ARO is greatly acknowledged for the analysis of pharmaceuticals and for its inputs in the discussions of the results.

The EU-SWITCH program and STOWA are acknowledged for funding the project.

Acknowledgements also to Katarzyna Kujawa for supervision, Darja Kragic, Ime Akanyeti and all others who helped in this thesis.

List of abbreviations

AER-20-1	first aerobic batch test at 20^{o}C
AER-20-2	second aerobic batch test at 20^{o}C
AER-10	aerobic batch test at 10^{o}C
ANAER-1	first anaerobic batch test at 30^{o}C
ANAER-2	second anaerobic batch test at 30^{o}C
ANOX-20	anoxic batch test at 20^{o}C
ANOX-10	anoxic batch test at 10^{o}C
ASA	acetylsalicylic acid
BZF	bezafibrate
CBZ	carbamazepine
CFA	clofibrate
DCF	diclofenac
FNF	fenofibrate
IBU	ibuprofen
MTP	metoprolol
TS	total solids
WWTP	waste water treatment plant

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Appendix

APPENDIX I: DATA OF PHARMACEUTICAL CONCENTRATIONS IN ALL TESTS

AER-20-1 concentrations in mg/L

Water	Time (hr)	Metoprolol		Acetylsalicylic acid		Carbamazepine		Clofibric acid		Bezafibrate		Diclofenac		Ibuprofen		Fenofibrate	
Backgroundvalue																	
B1	0.0	0.464		2.548		0.885		0.389		1.383		0.242		0.610		0.050	
B1	0.5	0.411		0.518		0.890		0.403		1.429		0.249		0.535		0.042	
B1	1.0	0.361		0.074		0.857		0.393		1.417		0.245		0.484		0.036	
B1	2.5	0.277		0.000		0.890		0.395		1.405		0.247		0.317		0.012	
B1	24.0	0.007		0.000		0.822		0.369		1.198		0.244		0.000		0.000	
	48.0	0.001		0.034		0.784		0.323		0.850		0.232		0.000		0.000	
B2	0.0	0.414		0.861		0.809		0.365		1.330		0.234		0.507		0.035	
B2	0.5	0.416		0.426		0.832		0.375		1.349		0.232		0.471		0.035	
B2	1.0	0.356		0.070		0.806		0.360		1.299		0.233		0.422		0.031	
B2	2.5	0.253		0.000		0.809		0.357		1.317		0.248		0.342		0.014	
B2	24.0	0.007		0.000		0.789		0.330		1.111		0.218		0.021		0.000	
B2	48.0	0.001		0.019		0.719		0.318		0.890		0.209		0.000		0.000	
B2																	
Controls																	
W1	0.0	0.684		4.272		0.855		0.282		1.347		0.210		0.532		0.588	
W2	0.0	0.694		4.244		0.846		0.285		1.323		0.200		0.514		0.335	
W1	48.0	0.634		3.069		0.776		0.268		1.286		0.192		0.427		0.014	
W2	48.0	0.671		3.152		0.831		0.269		1.285		0.189		0.427		0.028	
W1																	
W2																	

AVERAGES

Water (mg/L)	Time (hr)		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV
B1&B2	0.0	0.439	0.035	1.705	1.193	0.847	0.054	0.377	0.017	1.356	0.038	0.238	0.005	0.559	0.073	0.042	0.011
	0.5	0.414	0.003	0.472	0.065	0.861	0.041	0.389	0.019	1.389	0.057	0.241	0.012	0.503	0.045	0.038	0.004
	1.0	0.359	0.003	0.072	0.003	0.831	0.036	0.377	0.024	1.358	0.084	0.239	0.009	0.453	0.044	0.033	0.004
	2.5	0.265	0.017	0.000	0.000	0.849	0.057	0.376	0.027	1.361	0.062	0.247	0.000	0.329	0.018	0.013	0.001
	24.0	0.007	0.000	0.000	0.000	0.805	0.023	0.350	0.027	1.154	0.062	0.231	0.018	0.011	0.015	0.000	0.000
	48.0	0.001	0.000	0.026	0.011	0.751	0.046	0.320	0.004	0.870	0.028	0.220	0.016	0.000	0.000	0.000	0.000
Controls																	
W1&W2	0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	48.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

AER-20-2 concentrations in mg/L

	Time (hr)	Time (d)	Metoprolol	Acetylsalicylic acid	Carbamazepine	Clofibric acid	Bezafibrate	Diclofenac	Ibuprofen	Fenofibrate
Water			copy cells							
Backgroundvalue										
A1	0		0.001	0.000	0.002	0.000	0.004	0.013	0.000	0.012
A2	0		0.002	0.000	0.001	0.000	0.000	0.018	0.000	0.007
B1	0	0	0.300	0.394	0.289	0.294	1.893	0.165	0.245	0.021
B1	1	0.04	0.249	0.000	0.346	0.290	1.902	0.176	0.127	0.016
B1	2.5	0.10	0.280	0.000	0.509	0.432	1.360	0.194	0.111	0.014
B1	24	1	0.004	0.000	0.411	0.280	0.956	0.129	0.000	0.003
B1	48	2	0.001	0.000	0.502	0.342	0.965	0.158	0.000	0.006
	20	20	0.000	0.000	0.372	0.723	0.003	0.029	0.000	0.000
	30	30	0.000	0.000	0.884	0.408	0.000	0.007	0.000	0.000
B2	0	0	0.217	0.329	0.457	0.237	1.089	0.141	0.237	0.030
B2	1	0.04	0.168	0.000	0.175	0.159	1.198	0.125	0.137	0.014
B2	2.5	0.10	0.080	0.000	0.252	0.179	0.680	0.119	0.114	0.011
B2	24	1	0.002	0.000	0.391	0.320	1.054	0.165	0.000	0.004
B2	48	2	0.000	0.000	0.264	0.269	0.751	0.139	0.000	0.000
B2		20	0.000	0.000	0.931	0.881	0.000	0.046	0.000	0.000
B2		30	0.000	0.000	2.045	0.918	0.008	0.031	0.000	0.000
Controls										
W1	0	0.00	0.406	0.052	0.732	0.506	1.985	0.244	0.614	0.476
W2	0	0.00	0.450	0.041	0.757	0.539	1.977	0.265	0.646	0.075
W1	48	2.00	0.436	0.062	0.680	0.562	2.018	0.273	0.576	0.018
W2	48	2.00	0.427	0.075	0.747	0.464	1.916	0.113	0.489	0.015
W1		30.00	0.281	0.881	0.612	0.592	1.358	0.117	0.407	0.000
W2		30.00	0.471	0.905	2.232	0.906	3.720	0.112	0.595	0.003
Sludge										
Backgroundvalue										
1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		0.000								
B1	0.000	0.000	0.002	0.001	0.005	0.003	0.012	0.005	0.003	0.182
B1	1.000	0.042	0.002	0.000	0.005	0.002	0.008	0.002	0.001	0.069
B1	2.500	20.000	0.001	0.000	0.003	0.001	0.002	0.001	0.000	0.004
B1	24.000	30.000	0.000	0.000	0.004	0.002	0.004	0.002	0.001	0.000
B1	48.000	0.800	0.000	0.000	0.004	0.001	0.002	0.001	0.000	0.000
		20.000	0.000	0.000	0.011	0.005	0.000	0.002	0.000	0.000
		30.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000
B2	0.000	0.000	0.002	0.002	0.006	0.003	0.014	0.005	0.005	0.183
B2	1.000	0.042	0.003	0.000	0.005	0.002	0.010	0.003	0.002	0.114
B2	2.500	0.104	0.002	0.000	0.004	0.001	0.005	0.001	0.001	0.015
B2	24.000	1.000	0.000	0.000	0.005	0.002	0.005	0.005	0.000	0.004
B2	48.000	2.000	0.000	0.000	0.003	0.001	0.002	0.001	0.000	0.000
		20.000	0.000	0.000	0.005	0.001	0.000	0.000	0.000	0.000
		30.000	0.000	0.000	0.004	0.000	0.000	0.000	0.000	0.000

Concentrations total (water and sludge)

	Time (h).	Time (d)								
Backgroundvalue										
1		0.00	0.001	0.000	0.002	0.000	0.004	0.013	0.000	0.013
2		0.00	0.002	0.000	0.001	0.000	0.000	0.018	0.000	0.007
B1	0	0.0000	0.3019	0.395058	0.29434	0.297	1.90498	0.17026	0.24833	0.202608
B1	1	0.0417	0.2509	0.000050	0.35084	0.291	1.90972	0.17833	0.12866	0.084856
B1	2.5	0.1042	0.2813	0.000019	0.51136	0.432	1.36219	0.19528	0.11093	0.017733
B1	24	1.0000	0.0042	0.000359	0.41531	0.281	0.96009	0.13130	0.00056	0.003241
B1	48	2.0000	0.0006	0.000013	0.50592	0.343	0.96725	0.15972	0.00001	0.005787
		20.0000	0.0005	0.000029	0.38345	0.728	0.00307	0.03078	0.00001	0.000609
		30.0000	0.0003	0.000005	0.88845	0.409	0.00001	0.00658	0.00001	0.000011
B2	0	0.0000	0.2188	0.331235	0.46267	0.240	1.10302	0.14689	0.24273	0.212773
B2	1	0.0417	0.1701	0.000029	0.18063	0.161	1.20754	0.12813	0.13847	0.127884
B2	2.5	0.1042	0.0816	0.000034	0.25547	0.181	0.68516	0.12015	0.11500	0.026206
B2	24	1.0000	0.0024	0.000025	0.39530	0.322	1.05972	0.17051	0.00004	0.007992
B2	48	2.0000	0.0002	0.000014	0.26685	0.270	0.75301	0.14010	0.00001	0.000363
		20.0000	0.0003	0.000046	0.93647	0.881	0.00001	0.046	0.00001	0.000
		30.0000	0.0005	0.000418	excluded	0.918	0.00807	0.031	0.00001	0.000

AVERAGES			Metoprolol	STDEV	Acetylsalicylic acid	STDEV	Carbamazepine	STDEV	Clofibric acid	STDEV	Bezafibrate	STDEV	Diclofenac	STDEV	Ibuprofen	STDEV	Fenofibrate	STDEV
Water (mg/L)	Time (hr)																	
B1&B2	0	0	0.25810593	0.05880792	0.361411278	0.04581296	0.372971149	0.118897695	0.265361475	0.040442287	1.49096952	0.56875838	0.15316611	0.01662341	0.241236	0.005307	0.02537684	0.006602
	1	0.04	0.20814574	0.05738903	0.000005	0	0.26074603	0.120700598	0.224404963	0.092428006	1.54988436	0.49776467	0.15034439	0.03615669	0.1321541	0.0065862	0.01486999	0.001602
	2.5	0.10	0.17993924	0.14165308	0.000005	0	0.380153299	0.181909622	0.305501283	0.178193868	1.02009954	0.48049749	0.1565114	0.05343255	0.1125582	0.0025313	0.01232103	0.001886
	24	1	0.00307337	0.00112983	0.000005	0	0.400850144	0.01451799	0.299952237	0.028834695	1.00529405	0.06923074	0.14722991	0.02553354	0.000005	0	0.00324405	0.000568
	48	2	0.00038094	0.00029335	0.000005	0	0.383001689	0.168796364	0.305555709	0.051821573	0.85812513	0.15133047	0.14854918	0.01391336	0.000005	0	0.0028535	0.004028
		20	0.00033931	1.1438E-05	0.000005	0	0.651815822	0.395166939	0.8017801	0.11133358	0.0015393	0.00216982	0.03727093	0.01189211	0.000005	0	0.00010397	0.00014
		30	0.00034434	0.00010455	0.000211634	0.000292224	0.884	0.00	0.662955542	0.360355417	0.00403971	0.00570594	0.01879555	0.01731279	0.000005	0	0.000005	0
Sludge (mg/L)																		
	0	0	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.18	0.00
	1	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.09	0.03
	2.5	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
	24	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	48	2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		20	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Controls																		
W1&W2	0	0.00	0.428	0.031	0.047	0.008	0.745	0.018	0.523	0.023	1.981	0.006	0.254	0.015	0.630	0.022	0.275	0.283
	48	2.00	0.431	0.006	0.068	0.009	0.713	0.047	0.513	0.069	1.967	0.072	0.193	0.113	0.533	0.061	0.016	0.002
		30.00	0.376	0.134	0.893	0.017	0.612	0.000	0.592	0.000	1.358	0.000	0.115	0.003	0.501	0.133	0.001	0.002
Total concentration				STDEV optellen?!														
B1&B2	0	0	0.26	0.06	0.36	0.05	0.38	0.12	0.27	0.04	1.50	0.57	0.16	0.02	0.25	0.01	0.21	0.01
	1	0.04	0.21	0.06	0.00	0.00	0.27	0.12	0.23	0.09	1.56	0.50	0.15	0.04	0.13	0.01	0.11	0.03
	2.5	0.10	0.18	0.14	0.00	0.00	0.38	0.18	0.31	0.18	1.02	0.48	0.16	0.05	0.11	0.00	0.02	0.01
	24	1	0.00	0.00	0.00	0.00	0.41	0.01	0.30	0.03	1.01	0.07	0.15	0.03	0.00	0.00	0.01	0.00
	48	2	0.00	0.00	0.00	0.00	0.39	0.17	0.31	0.05	0.86	0.15	0.15	0.01	0.00	0.00	0.00	0.00
		20	0.00	0.00	0.00	0.00	0.66	0.40	0.80	0.11	0.00	0.00	0.04	0.01	0.00	0.00	0.00	0.00
		30	0.00	0.00	0.00	0.00	0.89	0.00	0.66	0.36	0.00	0.01	0.02	0.02	0.00	0.00	0.00	0.00

AER-10 concentrations in mg/L

	Time (hr)	Time (d)	Metoprolol	Acetylsalicylic acid	Carbamazepine	Clofibric acid	Bezafibrate	Diclofenac	Ibuprofen	Fenofibrate
Water										
Backgroundvalue										
A1	0	0	0	0	0	0	0.000	4.694	0.000	0.004
A2	0	0	0.002	0.000	0.000	0.000	0.000	11.000	0.000	0.002
B3	0	0	0.257	0.807	0.305	0.786	1.265	0.234	0.607	0.025
B3	0.6	0.03	0.348	0.926	0.905	0.773	2.338	0.219	0.414	0.022
B3	1.1	0.05	0.241	0.219	0.785	0.849	2.269	0.247	0.408	0.017
B3	2.5	0.1	0.248	0.000	0.355	0.898	1.377	0.234	0.227	0.010
B3	24	1	0.116	0.000	0.341	0.808	1.139	0.214	0.002	0.002
B3	48	2	0.046	0.000	0.373	0.939	1.124	0.238	0.001	0.000
B3		30	0.002	0.001	0.742	0.701	0.001	0.013	0.000	0.000
B4	0	0	0.259	0.838	0.810	0.874	2.255	0.228	0.567	0.029
B4	0.6	0.03	0.253	1.008	0.373	0.889	1.204	0.212	0.403	0.019
B4	1.1	0.05	0.250	0.678	0.311	0.753	1.215	0.204	0.370	0.026
B4	2.5	0.1	0.272	0.004	0.363	0.885	1.341	0.232	0.216	0.016
B4	24	1	0.117	0.000	0.343	0.862	1.133	0.226	0.002	0.003
B4	48	2	0.050	0.000	1.004	0.961	1.988	0.199	0.000	0.001
B4		30	0.001	0.000	0.712	0.601	0.000	0.016	0.000	0.000
Controls										
W3	0	0	0.437	0.261	0.891	0.878	2.075	0.274	0.744	0.470
W4	0	0	0.433	0.090	0.908	0.754	2.087	0.253	0.696	0.037
W3	48	2	0.339	0.279	0.343	0.740	1.392	0.284	0.546	0.013
W4	48	2	0.436	0.267	0.871	0.788	2.255	0.239	0.627	0.011
W3		30	0.306	0.003	0.624	0.630	1.504	0.199	0.286	0.000
W4		30	0.348	0.005	1.541	0.716	3.149	0.283	0.431	0.000
Sludge										
Backgroundvalue										
A3	0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A4	0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B3	0	0.00	0.002	0.003	0.004	0.002	0.003	0.000	0.001	0.009
B3	0.6	0.03	0.002	0.001	0.003	0.001	0.003	0.000	0.000	0.011
B3	1.1	0.05	0.002	0.001	0.003	0.002	0.004	0.001	0.001	0.014
B3	2.5	0.10	0.002	0.000	0.003	0.002	0.005	0.000	0.000	0.001
B3	24	1	0.001	0.000	0.004	0.002	0.004	0.000	0.000	0.000
B3	48	2	0.001	0.000	0.004	0.002	0.004	0.001	0.000	0.001
B3		30	0.000	0.000	0.010	0.001	0.000	0.000	0.000	0.000
B4	0	0.00	0.002	0.003	0.004	0.002	0.004	0.002	0.002	0.068
B4	0.6	0.03	0.002	0.002	0.002	0.002	0.003	0.000	0.000	0.004
B4	1.1	0.05	0.003	0.003	0.003	0.003	0.006	0.000	0.001	0.002
B4	2.5	0.10	0.003	0.000	0.004	0.002	0.003	0.000	0.000	0.003
B4	24	1	0.001	0.000	0.004	0.002	0.003	0.000	0.000	0.000
B4	48	2	0.001	0.000	0.003	0.002	0.003	0.000	0.000	0.000
B4		30	0.000	0.000	0.005	0.001	0.000	0.000	0.000	0.000
Concentrations total (water and sludge)										
Backgroundvalue										
1			0.000	0.000	0.000	0.000	0.000	4.694	0.000	0.004
2			0.002	0.000	0.000	0.000	0.000	11.000	0.000	0.002
B3	0	0.00	0.259	0.810	0.305	0.788	1.265	0.235	0.607	0.033
B3	0.6	0.03	0.349	0.927	0.905	0.774	2.338	0.219	0.414	0.033
B3	1.1	0.05	0.243	0.220	0.785	0.851	2.269	0.248	0.408	0.031
B3	2.5	0.10	0.251	0.000	0.355	0.900	1.377	0.234	0.227	0.011
B3	24	1	0.118	0.000	0.341	0.810	1.139	0.215	0.002	0.002
B3	48	2	0.046	0.000	0.373	0.941	1.124	0.240	0.001	0.001
B3		30	0.002	0.001	0.742	0.702	0.001	0.013	0.000	0.000
							0.000			
B4	0	0	0.261	0.840	0.810	0.875	2.255	0.230	0.569	0.096
B4	0.5	0.03	0.254	1.010	0.373	0.891	1.204	0.212	0.403	0.023
B4	1	0.05	0.253	0.682	0.311	0.756	1.215	0.205	0.371	0.029
B4	3	0.1	0.275	0.004	0.363	0.888	1.341	0.232	0.216	0.019
B4	24	1	0.119	0.000	0.343	0.864	1.133	0.226	0.002	0.003
B4	48	2	0.051	0.000	1.004	0.963	excluded	0.200	0.000	0.002
B4		30	0.001	0.000	0.712	0.602	0.000	0.016	0.000	0.000

AVERAGES

				STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV
Water (mg/L)																
B3&B4	0	0	0.258	0.001	0.823	0.021	0.557	0.357	0.830	0.062	1.760	0.700	0.231	0.005	0.587	0.028	0.027	0.003
	0.6	0.03	0.300	0.067	0.967	0.058	0.639	0.376	0.831	0.082	1.771	0.802	0.215	0.005	0.408	0.008	0.020	0.003
	1.1	0.05	0.245	0.006	0.449	0.325	0.548	0.335	0.801	0.068	1.742	0.745	0.226	0.030	0.389	0.027	0.022	0.006
	2.5	0.1	0.260	0.017	0.002	0.003	0.359	0.006	0.891	0.009	1.359	0.025	0.233	0.001	0.221	0.008	0.013	0.004
	24	1	0.117	0.001	0.000	0.000	0.342	0.001	0.835	0.038	1.136	0.005	0.220	0.008	0.002	0.000	0.002	0.001
	48	2	0.048	0.003	0.000	0.000	0.689	0.446	0.950	0.016	1.124	0.000	0.219	0.028	0.000	0.001	0.001	0.001
		30	0.001	0.000	0.000	0.001	0.727	0.021	0.651	0.071	0.000	0.001	0.014	0.002	0.000	0.000	0.000	0.000
Sludge (mg/L)																		
	0	0	0.002	0.000	0.003	0.000	0.004	0.000	0.002	0.000	0.004	0.000	0.001	0.001	0.001	0.001	0.038	0.042
	0.6	0.03	0.002	0.000	0.002	0.000	0.002	0.000	0.001	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.008	0.005
	1.1	0.05	0.003	0.000	0.002	0.002	0.003	0.000	0.003	0.001	0.005	0.001	0.000	0.000	0.001	0.000	0.008	0.008
	2.5	0.1	0.003	0.000	0.000	0.000	0.003	0.000	0.002	0.000	0.004	0.001	0.000	0.000	0.000	0.000	0.002	0.001
	24	1	0.001	0.000	0.000	0.000	0.004	0.000	0.002	0.000	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
	48	2	0.001	0.000	0.000	0.000	0.004	0.000	0.002	0.000	0.004	0.001	0.001	0.001	0.000	0.000	0.000	0.001
		30	0.000	0.000	0.000	0.000	0.008	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Controls																		
W3&W4	0	0	0.435	0.003	0.176	0.121	0.899	0.012	0.816	0.088	2.081	0.008	0.264	0.015	0.720	0.034	0.253	0.306
	48	2	0.388	0.068	0.273	0.008	0.607	0.374	0.764	0.034	1.824	0.610	0.262	0.031	0.586	0.058	0.012	0.002
		30	0.327	0.030	0.004	0.001	1.083	0.649	0.673	0.061	2.326	1.163	0.241	0.060	0.358	0.102	0.000	0.000
Total concentration				STDEV's optellen?!														
B3&B4	0	0	0.26	0.00	0.83	0.02	0.56	0.36	0.83	0.06	1.23	0.70	0.23	0.01	0.59	0.03	0.06	0.045
	0.6	0.03	0.30	0.07	0.97	0.06	0.64	0.38	0.83	0.08	1.78	0.80	0.22	0.01	0.41	0.01	0.03	0.008
	1.1	0.05	0.25	0.01	0.45	0.33	0.55	0.34	0.80	0.07	1.80	0.75	0.23	0.03	0.39	0.03	0.03	0.014
	2.5	0.1	0.26	0.02	0.00	0.00	0.36	0.01	0.89	0.01	1.25	0.03	0.23	0.00	0.22	0.01	0.01	0.006
	24	1	0.12	0.00	0.00	0.00	0.34	0.00	0.84	0.04	1.14	0.00	0.22	0.01	0.00	0.00	0.00	0.001
	48	2	0.05	0.00	0.00	0.00	0.69	0.45	0.95	0.02	1.12	0.00	0.22	0.03	0.00	0.00	0.00	0.001
		30	0.00	0.00	0.00	0.00	0.73	0.02	0.65	0.07	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.000

ANOX-20 concentrations in mg/L

	Time (h)	Time (d)	Metoprolol	Acetylsalicylic acid	Carbamazepine	Clofibric acid	Bezafibrate	Diclofenac	Ibuprofen	Fenofibrate
Water			copy cells							
Backgroundvalue										
A1	0		0.002	0.000	0.001	0.000	0.000	0.006	0.000	0.000
A2	0		0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
B1	0	0.0	0.116	0.294	0.194	0.299	0.537	0.127	0.306	0.018
B1	1.2	0.1	0.102	0.221	0.159	0.296	0.438	0.096	0.242	0.010
B1	3.3	0.1	0.079	0.040	0.132	0.202	0.382	0.103	0.239	0.000
B1	25	1	0.100	0.139	0.173	0.308	0.401	0.106	0.265	0.000
B1	48	2	0.056	0.014	0.094	0.114	0.156	0.080	0.143	0.000
		27	0.012	0.000	1.067	1.229	0.007	**0.275**	0.006	0.000
B2	0	0.0	0.087	0.154	0.137	0.216	0.360	0.104	0.253	0.016
B2	1.2	0.1	0.095	0.103	0.149	0.195	0.408	0.133	0.296	0.011
B2	3.3	0.1	0.095	0.090	0.150	0.234	0.433	0.124	0.229	0.006
B2	25	1	0.088	0.000	0.144	0.206	0.267	0.105	0.059	0.000
B2	48	2	0.056	0.000	0.096	0.124	0.112	0.051	0.007	0.000
		27	0.000	0.000	0.621	0.699	0.000	0.099	0.000	1.000
Controls										
W1	0	0.0	0.285	0.016	0.467	0.631	1.152	0.262	0.712	0.281
W2	0	0.0	0.311	0.035	0.517	0.628	1.269	0.252	0.705	0.263
W1	48	2.0	0.390	0.036	0.630	0.747	1.369	0.282	0.753	0.212
W2	48	2.0	0.425	0.006	0.625	0.855	1.376	0.309	0.771	0.168
W1		27.0	0.364	0.870	0.653	0.606	1.461	0.216	0.333	0.077
W2		27.0	0.392	0.001	1.066	0.670	1.605	**0.427**	0.380	0.339
Sludge										
Backgroundvalue										
A1	0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A2	0		0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
B1	0.0	0.0	0.008	0.004	0.018	0.003	0.016	0.002	0.001	0.021
B1	1.2	0.1	0.009	0.005	0.025	0.004	0.020	0.003	0.002	0.109
B1	3.3	1.0	0.010	0.003	0.024	0.003	0.017	0.002	0.002	0.049
B1	25.0	1	0.010	0.002	0.017	0.003	0.004	0.004	0.002	0.005
B1	48.0	0	0.009	0.000	0.017	0.003	0.007	0.002	0.001	0.000
B1		27	0.001	0.000	0.005	0.000	0.000	0.000	0.000	0.000
B2	0.0	0.0	0.010	0.007	0.013	0.004	0.003	0.001	0.001	0.005
B2	1.2	0.1	0.009	0.004	0.021	0.003	0.016	0.002	0.001	0.038
B2	3.3	1.0	0.010	0.003	0.023	0.003	0.017	0.002	0.001	0.035
B2	25.0	1	0.008	0.000	0.015	0.003	0.003	0.003	0.001	0.002
B2	48.0	0	0.008	0.000	0.016	0.002	0.004	0.002	0.000	0.000
B2		27	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000
Concentrations total (water and sludge)										
	Time (h)	Time (d)								
Backgroundvalue										
	1		0.002	0.000	0.001	0.000	0.000	0.006	0.000	0.000
	2		0.000	0.000	0.003	0.000	0.000	0.001	0.000	0.000
B1	0.0	0.0	0.124	0.298	0.212	0.302	0.553	0.129	0.307	0.039
B1	1.2	0.1	0.111	0.226	0.184	0.299	0.457	0.099	0.245	0.120
B1	3.3	0.1	0.089	0.043	0.157	0.205	0.398	0.105	0.241	0.049
B1	25.0	1	0.110	0.141	0.189	0.311	0.404	0.110	0.267	0.005
B1	48.0	2	0.065	0.015	0.111	0.116	0.163	0.081	0.144	0.000
		27	0.012	0.000	1.072	1.230	0.007	excluded	0.006	0.000
B2	0.0	0.0	0.097	0.161	0.149	0.220	0.363	0.105	0.254	0.021
B2	1.2	0.1	0.104	0.106	0.170	0.198	0.424	0.135	0.297	0.049
B2	3.3	0.1	0.105	0.093	0.173	0.237	0.450	0.126	0.231	0.041
B2	25.0	1	0.096	0.000	0.159	0.208	0.269	0.108	0.060	0.002
B2	48.0	2	0.063	0.000	0.111	0.126	0.116	0.053	0.007	0.000
		27	0.000	0.000	0.627	0.699	0.000	0.099	0.000	excluded
AVERAGES										
Water (mg/L)	Time (hr)									
B1&B2	0.0	0.0	0.101	0.019	0.224	0.097	0.165	0.045	0.258	0.058	0.448	0.134	0.115	0.017	0.279	0.038	0.017	0.013
	1.2	0.1	0.098	0.005	0.162	0.085	0.154	0.010	0.245	0.072	0.423	0.024	0.115	0.025	0.269	0.037	0.011	0.050
	3.3	0.1	0.087	0.011	0.065	0.035	0.141	0.012	0.218	0.023	0.408	0.037	0.114	0.014	0.234	0.007	0.003	0.005
	25.0	1	0.094	0.009	0.069	0.100	0.158	0.022	0.257	0.073	0.334	0.095	0.106	0.001	0.162	0.147	0.000	0.002
	48.0	2	0.056	0.001	0.007	0.010	0.095	0.000	0.119	0.007	0.134	0.033	0.065	0.020	0.075	0.097	0.000	0.000
		27	0.006	0.009	0.000	0.000	0.621	0.315	0.964	0.375	0.004	0.005	0.099	0.000	0.003	0.004	0.000	0.000
Sludge (mg/L)																		
	0.0	0.0	0.009	0.001	0.005	0.002	0.015	0.004	0.003	0.000	0.010	0.009	0.001	0.001	0.001	0.000	0.012880	0.012
	1.2	0.1	0.009	0.000	0.004	0.001	0.023	0.003	0.003	0.001	0.018	0.002	0.003	0.001	0.002	0.001	0.073678	0.050
	3.3	0.1	0.010	0.000	0.003	0.000	0.024	0.001	0.003	0.000	0.017	0.000	0.002	0.000	0.002	0.000	0.041694	0.010
	25.0	1	0.009	0.001	0.001	0.001	0.016	0.001	0.003	0.000	0.003	0.001	0.004	0.001	0.001	0.001	0.003686	0.002
	48.0	2	0.008	0.001	0.000	0.000	0.016	0.001	0.002	0.000	0.006	0.002	0.002	0.000	0.001	0.001	0.000297	0.000
		27	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000000	0.000
Controls																		
W1&W2	0	0	0.298	0.019	0.025	0.013	0.492	0.035	0.629	0.002	1.211	0.083	0.257	0.007	0.708	0.005	0.271970	0.013
	48	2	0.407	0.024	0.021	0.021	0.628	0.004	0.801	0.077	1.372	0.005	0.295	0.020	0.762	0.013	0.190068	0.031
		27	0.378	0.020	0.001	0.615	0.653	0.291	0.638	0.045	1.533	0.102	0.216	0.149	0.356	0.033	0.208019	0.185
Total concentration																		
B1&B2	0.0	0.0	0.110	0.019	0.230	0.097	0.181	0.045	0.261	0.058	0.458	0.134	0.117	0.017	0.280	0.038	0.030092	0.013
	1.2	0.1	0.107	0.005	0.166	0.085	0.177	0.010	0.249	0.072	0.440	0.024	0.117	0.025	0.271	0.037	0.084529	0.050
	3.3	0.1	0.097	0.011	0.068	0.035	0.165	0.012	0.221	0.023	0.424	0.037	0.116	0.014	0.236	0.007	0.044738	0.005
	25.0	1	0.103	0.009	0.070	0.100	0.174	0.022	0.260	0.073	0.337	0.095	0.109	0.001	0.163	0.147	0.003691	0.002
	48.0	2	0.064	0.001	0.008	0.010	0.111	0.000	0.121	0.007	0.140	0.033	0.067	0.020	0.076	0.097	0.000299	0.000
		27	0.006	0.009	0.000	0.000	0.627	0.315	0.965	0.375	0.004	0.005	0.099	0.000	0.003	0.004	0.000362	0.000

ANOX-10 concentrations in mg/L

	Time (hr)	Metoprolol	Acetylsalicylic acid	Carbamazepine	Clofibric acid	Bezafibrate	Diclofenac	Ibuprofen	Fenofibrate
Water									
Backgroundvalue									
B3	0	0.073	3.426	0.117	0.175	0.313	0.066	0.214	0.017
B3	1	0.075	0.045	0.118	0.139	0.356	0.078	0.188	0.013
B3	3	0.069	0.062	0.099	0.154	0.295	0.077	0.170	0.011
B3	24	0.059	0.040	0.109	0.120	0.299	0.043	0.144	0.000
B3	48	0.071	0.044	0.133	0.189	0.392	0.092	0.193	0.000
B4	0	0.108	0.113	0.165	0.250	0.528	0.107	0.253	0.015
B4	1	0.081	0.071	0.129	0.189	0.390	0.081	0.218	0.017
B4	3	0.083	0.054	0.132	0.207	0.484	0.075	0.205	0.008
B4	24	0.061	0.036	0.100	0.157	0.341	0.068	0.163	0.000
B4	48	0.089	0.111	0.166	0.198	0.484	0.087	0.029	0.000
Controls									
W3	0	0.476	0.028	0.851	0.902	1.760	0.369	0.845	0.084
W4	0	0.548	0.027	0.924	1.049	1.768	0.358	1.011	0.112
W3	48	0.458	0.027	0.777	0.830	1.650	0.318	0.862	0.060
W4	48	0.546	0.032	0.889	1.001	1.758	0.362	0.938	0.066
Sludge									
B3	0	0.014	0.006	0.024	0.000	0.007	0.004	0.003	0.065
B3	1	0.015	0.005	0.030	0.000	0.026	0.004	0.002	0.065
B3	3	0.015	0.005	0.031	0.000	0.028	0.004	0.003	0.067
B3	24	0.016	0.003	0.031	0.000	0.023	0.002	0.002	0.002
B3	48	0.016	0.002	0.033	0.000	0.018	0.005	0.003	0.003
B4	0	0.012	0.004	0.030	0.000	0.029	0.005	0.003	0.150
B4	1	0.013	0.004	0.029	0.000	0.025	0.004	0.003	0.086
B4	3	0.016	0.005	0.023	0.000	0.005	0.002	0.002	0.016
B4	24	0.016	0.003	0.023	0.000	0.005	0.004	0.003	0.006
B4	48	0.015	0.002	0.030	0.000	0.024	0.005	0.003	0.004
Concentrations total (water and sludge)									
B3	0	0.087	3.432	0.142	0.175	0.320	0.070	0.216	0.082
B3	1	0.090	0.050	0.148	0.139	0.382	0.081	0.190	0.078
B3	3	0.085	0.067	0.129	0.154	0.323	0.081	0.173	0.078
B3	24	0.075	0.043	0.140	0.120	0.323	0.045	0.147	0.002
B3	48	0.086	0.046	0.165	0.189	0.410	0.097	0.196	0.003
B4	0	0.120	0.117	0.195	0.250	0.556	0.112	0.256	0.164
B4	1	0.094	0.075	0.158	0.189	0.415	0.084	0.221	0.102
B4	3	0.099	0.059	0.155	0.207	0.488	0.077	0.206	0.023
B4	24	0.077	0.039	0.124	0.157	0.346	0.071	0.166	0.006
B4	48	0.104	0.113	0.196	0.198	0.508	0.092	0.032	0.004

AVERAGES		Metoprolol	STDEV	Acetylsalicylic acid	STDEV	Carbamazepine	STDEV	Clofibric acid	STDEV	Bezafibrate	STDEV	Diclofenac	STDEV	Ibuprofen	STDEV	Fenofibrate	STDEV
Water (mg/L)																	
B3&B4	0	0.090	0.025	1.769	2.343	0.141	0.034	0.213	0.053	0.421	0.152	0.086	0.029	0.233	0.028	0.016	0.002
	1	0.078	0.005	0.058	0.019	0.123	0.008	0.164	0.035	0.373	0.024	0.079	0.002	0.203	0.021	0.015	0.003
	3	0.076	0.010	0.058	0.005	0.115	0.024	0.181	0.038	0.389	0.134	0.076	0.001	0.187	0.024	0.009	0.002
	24	0.060	0.002	0.038	0.003	0.104	0.006	0.138	0.026	0.320	0.029	0.055	0.017	0.154	0.013	0.000	0.000
	48	0.080	0.013	0.078	0.047	0.149	0.023	0.193	0.006	0.438	0.065	0.090	0.004	0.111	0.116	0.000	0.000
Sludge (mg/L)																	
B3&B4	0	0.01	0.00	0.005	0.001	0.027	0.004	0.000	0.000	0.018	0.016	0.004	0.001	0.003	0.000	0.107	0.060
	1	0.01	0.00	0.005	0.001	0.030	0.001	0.000	0.000	0.026	0.001	0.004	0.000	0.003	0.000	0.076	0.014
	3	0.02	0.00	0.005	0.000	0.027	0.006	0.000	0.000	0.016	0.016	0.003	0.001	0.002	0.001	0.041	0.036
	24	0.02	0.00	0.003	0.000	0.027	0.006	0.000	0.000	0.014	0.013	0.003	0.001	0.003	0.000	0.004	0.003
	48	0.02	0.00	0.002	0.000	0.032	0.002	0.000	0.000	0.021	0.005	0.005	0.000	0.003	0.000	0.004	0.001
Controls																	
W3&W4	0	0.512	0.051	0.028	0.001	0.888	0.052	0.976	0.104	1.764	0.006	0.363	0.007	0.928	0.118	0.098	0.020
	48	0.503	0.064	0.027	0.000	0.850	0.104	0.940	0.155	1.709	0.084	0.338	0.028	0.937	0.106	0.086	0.037
Total concentration																	
B3&B4	0	0.103	0.026	1.774	2.344	0.168	0.037	0.213	0.053	0.438	0.167	0.091	0.030	0.236	0.028	0.123	0.061
	1	0.092	0.006	0.063	0.020	0.153	0.009	0.164	0.035	0.399	0.025	0.083	0.002	0.205	0.021	0.090	0.017
	3	0.092	0.010	0.063	0.005	0.142	0.030	0.181	0.038	0.406	0.150	0.079	0.002	0.190	0.025	0.051	0.038
	24	0.076	0.002	0.041	0.003	0.132	0.011	0.138	0.026	0.334	0.042	0.058	0.018	0.156	0.014	0.004	0.003
	48	0.095	0.014	0.079	0.047	0.181	0.025	0.193	0.006	0.459	0.070	0.095	0.004	0.114	0.116	0.004	0.001

ANAER-1 concentrations in mg/L

Water	Time (d)	Metoprolol		Acetylsalicylic acid		Carbamazepine		Clofibric acid		Bezafibrate		Diclofenac		Ibuprofen		Fenofibrate	
Backgroundvalue																	
A1	0	0.044		0.000		0.000		0.000		0.043		0.055		5.768		0.000	
B1	0	0.449		13.238		0.567		2.275		7.351		0.648		0.003		0.010	
B1	0.13	0.468		13.325		0.578		2.346		8.369		0.796		0.004		0.016	
B1	1	0.413		10.856		0.645		2.143		9.099		0.748		0.004		0.027	
B1	4	0.436		4.593		0.582		2.268		8.583		0.811		0.004		0.028	
B1	7	0.405		0.160		0.605		2.219		8.883		0.852		0.004		0.022	
B1	15	0.103		0.005		0.101		0.041		0.078		0.034		0.000		0.004	
B1	32	0.130		0.006		0.202		0.113		0.171		0.053		0.000		0.002	
B1	77	0.137		0.004		0.415		0.308		0.910		0.064		0.425		0.000	
B2	0	0.535		14.811		0.671		2.728		9.041		0.826		3.837		0.011	
B2	0.13	0.478		13.443		0.616		2.442		8.398		0.799		3.748		0.014	
B2	1	0.446		11.585		0.649		2.268		9.144		0.830		3.945		0.028	
B2	4	0.443		6.218		0.593		2.394		8.661		0.836		3.981		0.022	
B2	7	0.422		0.258		0.583		2.308		9.076		0.899		4.323		0.021	
B2	15	0.086		0.005		0.076		0.028		0.066		0.028		0.058		0.004	
B2	32	0.119		0.006		0.156		0.096		0.132		0.044		0.106		0.001	
B2	77	0.017		0.001		0.085		0.030		0.181		0.022		0.115		0.000	
Controls																	
W1	0	0.529		4.696		0.437		0.263		0.717		0.159		0.245		0.230	
W2	0	0.564		4.850		0.467		0.308		0.752		0.168		0.250		0.248	
W1	54	0.480		0.007		0.164		0.033		0.169		0.056		0.086		0.036	
W2	54	0.511		0.040		0.166		0.043		0.209		0.061		0.091		0.036	
W1	77	0.370		0.000		0.296		0.294		0.654		0.141		0.616		0.056	
W2	77	0.361		0.000		0.817		0.263		2.140		0.166		0.409		0.024	
Sludge																	
Backgroundvalue																	
	1	0.002		0.000		0.000		0.000		0.000		0.002		0.006		0.000	
	2	0.002		0.000		0.000		0.000		0.000		0.004		0.007		0.000	
B1	0																
B1	0.13																
B1	1																
B1	4																
B1	7																
B1	15	0.008		0.000		0.020		0.008		0.019		0.003		0.015		0.003	
B1	32	0.016		0.000		0.033		0.002		0.004		0.004		0.007		0.003	
B1	77	0.041		0.000		0.084		0.005		0.005		0.005		0.007		0.000	
														0.000		0.000	
B2	0																
B2	0.13																
B2	1																
B2	4																
B2	7																
B2	15	0.008		0.000		0.023		0.007		0.012		0.003		0.011		0.005	
B2	32	0.016		0.000		0.035		0.002		0.004		0.008		0.007		0.002	
b2	77	0.049		0.000		0.068		0.009		0.038		0.001		0.005		0.000	
Concentrations total (water and sludge)																	
	Time (d)																
Backgroundvalue																	
	1	0.044		0.000		0.000		0.000		0.043		0.055		5.768		0.000	
	2	0.002		0.000		0.000		0.000		0.000		0.002		0.006		0.000	
B1	0	0.449		13.238		0.567		2.275		7.351		0.648		0.003		0.010	
B1	0.13	0.468		13.325		0.578		2.346		8.369		0.796		0.004		0.016	
B1	1	0.413		10.856		0.645		2.143		9.099		0.748		0.004		0.027	
B1	4	0.436		4.593		0.582		2.268		8.583		0.811		0.004		0.028	
B1	7	0.405		0.160		0.605		2.219		8.883		0.852		0.004		0.022	
B1	15	0.111		0.005		0.121		0.049		0.098		0.036		0.015		0.007	
B1	32	0.146		0.006		0.236		0.114		0.175		0.057		0.007		0.005	
B1	77	0.179		0.005		0.499		0.313		0.915		0.069		0.432		0.000	
B2	0	0.535		14.811		0.671		2.728		9.041		0.826		3.837		0.011	
B2	0.13	0.478		13.443		0.616		2.442		8.398		0.799		3.748		0.014	
B2	1	0.446		11.585		0.649		2.268		9.144		0.830		3.945		0.028	
B2	4	0.443		6.218		0.593		2.394		8.661		0.836		3.981		0.022	
B2	7	0.422		0.258		0.583		2.308		9.076		0.899		4.323		0.021	
B2	15	0.095		0.005		0.099		0.035		0.078		0.031		0.069		0.009	
B2	32	0.134		0.006		0.192		0.097		0.136		0.052		0.113		0.003	
B2	77	0.066		0.001		0.153		0.039		0.219		0.023		0.120		0.000	
AVERAGES			STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV
Water (mg/L)	Time (d)																
B1&B2	0	0.492	0.061	14.025	1.112	0.619	0.073	2.502	0.320	8.196	1.194	0.737	0.125	1.920	2.711	0.011	0.001
	0.13	0.473	0.007	13.384	0.084	0.597	0.027	2.394	0.068	8.383	0.021	0.798	0.003	1.876	2.647	0.015	0.001
	1	0.429	0.024	11.220	0.516	0.647	0.003	2.206	0.088	9.122	0.032	0.789	0.058	1.974	2.787	0.027	0.001
	4	0.440	0.005	5.405	1.149	0.588	0.008	2.331	0.089	8.622	0.055	0.823	0.018	1.992	2.812	0.025	0.004
	7	0.413	0.012	0.209	0.069	0.594	0.015	2.264	0.063	8.980	0.136	0.876	0.033	2.163	3.054	0.021	0.001
	15	0.095	0.012	0.005	0.000	0.089	0.018	0.035	0.009	0.072	0.009	0.031	0.004	0.029	0.041	0.004	0.000
	32	0.124	0.008	0.006	0.000	0.179	0.033	0.104	0.012	0.152	0.028	0.048	0.006	0.052	0.075	0.002	0.001
	77	0.077	0.085	0.003	0.002	0.250	0.234	0.169	0.196	0.545	0.515	0.043	0.030	0.270	0.219	0.000	0.000
Sludge (mg/L)																	
B1&B2	0																
	0.13																
	1																
	4																
	7																
	15	0.008	0.000	0.000	0.000	0.021	0.002	0.007	0.001	0.016	0.005	0.003	0.001	0.013	0.003	0.004	0.001
	32	0.016	0.000	0.000	0.000	0.034	0.001	0.002	0.000	0.004	0.000	0.006	0.002	0.007	0.000	0.002	0.000
	77	0.045	0.005	0.000	0.000	0.076	0.011	0.007	0.003	0.022	0.023	0.003	0.002	0.006	0.002	0.000	0.000
Controls																	
W1&W2	0	0.547	0.025	4.773	0.109	0.452	0.021	0.286	0.032	0.735	0.025	0.164	0.006	0.248	0.004	0.239	0.013
	54	0.495	0.022	0.023	0.023	0.165	0.002	0.038	0.007	0.189	0.028	0.058	0.004	0.089	0.004	0.036	0.000
	77	0.366	0.006	0.000	0.000	0.556	0.368	0.279	0.022	0.654	1.050	0.153	0.017	0.513	0.147	0.040	0.022
Total concentration																	
B1&B2	0	0.492	0.061	14.025	1.112	0.619	0.073	2.502	0.320	8.196	1.194	0.737	0.125	1.920	2.711	0.011	0.001
	0.13	0.473	0.007	13.384	0.084	0.597	0.027	2.394	0.068	8.383	0.021	0.798	0.003	1.876	2.647	0.015	0.001
	1	0.429	0.024	11.220	0.516	0.647	0.003	2.206	0.088	9.122	0.032	0.789	0.058	1.974	2.787	0.027	0.001
	4	0.440	0.005	5.405	1.149	0.588	0.008	2.331	0.089	8.622	0.055	0.823	0.018	1.992	2.812	0.025	0.004
	7	0.413	0.012	0.209	0.069	0.594	0.015	2.264	0.063	8.980	0.136	0.876	0.033	2.163	3.054	0.021	0.001
	15	0.103	0.012	0.005	0.000	0.110	0.016	0.042	0.010	0.088	0.014	0.034	0.003	0.042	0.038	0.008	0.001
	32	0.140	0.008	0.006	0.000	0.214	0.031	0.106	0.012	0.156	0.027	0.054	0.004	0.060	0.075	0.004	0.001
	77	0.122	0.080	0.003	0.003	0.326	0.245	0.176	0.194	0.567	0.492	0.046	0.033	0.276	0.221	0.000	0.000

ANAER-2 concentrations in mg/L

	Time (d)	Metoprolol	Acetylsalicylic acid	Carbamazepine	Clofibric acid	Bezafibrate	Diclofenac	Ibuprofen	Fenofibrate
Water									
Backgroundvalue									
A1	0	0.006	0.010	0.002	0.004	0.006	0.003	0.145	0.006
A2	0	0.006	0.003	0.005	0.003	0.000	0.009	0.048	0.000
B3	0.0	0.027	0.232	0.045	0.081	0.090	0.059	0.222	0.019
B3	0.10	0.025	0.093	0.054	0.089	0.085	0.064	0.223	0.014
B3	1.0	0.026	0.401	0.053	0.088	0.115	0.065	0.230	0.016
B3	7.0	0.053	0.000	0.337	0.222	0.790	0.111	0.423	0.002
B3	15.0	0.277	0.000	0.747	0.577	0.628	0.104	0.004	0.003
B3	27.0	0.049	0.000	1.000	0.236	0.193	0.003	0.001	0.001
B4	0.0	0.026	0.477	0.201	0.107	0.458	0.072	0.250	0.023
B4	0.10	0.020	0.098	0.042	0.083	0.094	0.061	0.236	0.016
B4	1.0	0.021	0.186	0.042	0.075	0.081	0.058	0.159	0.017
B4	7.0	0.017	0.000	0.091	0.045	0.171	0.047	0.097	0.000
B4	15.0	0.235	0.000	0.432	0.441	0.361	0.073	0.002	0.002
B4	27.0	0.147	0.000	0.789	0.504	0.464	0.106	0.016	0.001
Controls									
W3	0	0.340	0.162	0.344	0.283	0.650	0.319	0.679	0.152
W4	0	0.312	0.119	0.958	0.245	2.071	0.255	0.677	0.168
W3	27	0.888	0.000	0.483	0.030	0.141	0.055	0.028	0.013
W4	27	0.880	0.000	0.409	0.026	0.159	0.046	0.027	0.015
Sludge									
Backgroundvalue									
1	0	0.006	0.000	0.000	0.000	0.000	0.000	0.002	0.000
2	0	0.006	0.000	0.000	0.000	0.000	0.000	0.001	0.000
B3	0.0	0.030	0.001	0.046	0.003	0.002	0.002	0.003	0.064
B3	0.10	0.035	0.001	0.048	0.003	0.009	0.003	0.003	0.066
B3	1.0	0.032	0.001	0.050	0.003	0.007	0.002	0.004	0.077
B3	7.0	0.032	0.000	0.051	0.003	0.004	0.010	0.007	0.004
B3	15.0	0.015	0.000	0.050	0.001	0.003	0.002	0.000	0.000
B3	27.0	0.008	0.000	0.027	0.002	0.003	0.000	0.000	0.000
B4	0.0	0.030	0.002	0.039	0.003	0.011	0.002	0.003	0.029
B4	0.10	0.042	0.001	0.050	0.003	0.012	0.003	0.003	0.050
B4	1.0	0.039	0.010	0.046	0.006	0.020	0.001	0.003	0.017
B4	7.0	0.033	0.000	0.051	0.003	0.005	0.014	0.005	0.005
B4	15.0	0.012	0.000	0.025	0.001	0.003	0.002	0.002	0.000
B4	27.0	0.007	0.000	0.023	0.001	0.001	0.001	0.000	0.000
Concentrations total (water and sludge)									
	Time (d)								
Backgroundvalue									
1		0.012	0.010	0.002	0.004	0.006	0.003	0.146	0.006
2		0.013	0.003	0.005	0.003	0.000	0.009	0.050	0.000
B3	0.0	0.057	0.233	0.091	0.084	0.092	0.061	0.225	0.083
B3	0.10	0.060	0.093	0.101	0.092	0.094	0.067	0.226	0.081
B3	1.0	0.059	0.402	0.104	0.091	0.121	0.067	0.234	0.092
B3	7.0	0.085	0.000	0.387	0.225	0.794	0.121	excluded	0.005
B3	15.0	0.292	0.000	0.797	0.579	0.631	0.106	0.004	0.003
B3	27.0	0.057	0.000	1.027	0.238	0.197	0.003	0.001	0.001
								0.000	0.000
B4	0.0	0.056	0.479	0.241	0.110	0.469	0.073	0.253	0.052
B4	0.10	0.062	0.099	0.092	0.087	0.106	0.063	0.239	0.066
B4	1.0	0.060	0.195	0.088	0.080	0.101	0.059	0.162	0.035
B4	7.0	0.051	0.000	0.142	0.048	0.176	0.061	0.102	0.005
B4	15.0	0.247	0.000	0.457	0.443	0.364	0.075	0.002	0.002
B4	27.0	0.154	0.000	0.812	0.505	0.465	0.107	0.016	0.001

AVERAGES

			STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV		STDEV
Water (mg/L)	Time (d)																
B3&B4	0.0	0.026	0.00099872	0.354769547	0.173076724	0.12321764	0.110530763	0.094281101	0.018253021	0.27397997	0.26059035	0.06529737	0.00914082	0.2360318	0.0200194	0.02096886	0.003208
	0.10	0.02231668	0.00312993	0.095457441	0.004076696	0.047792594	0.00809393	0.086067732	0.003733476	0.08962015	0.00638587	0.06255072	0.00273163	0.229101	0.0090659	0.01505855	0.0008
	1.0	0.02391932	0.00343685	0.293382193	0.152392373	0.047589427	0.00824447	0.081222886	0.009482339	0.09789297	0.02382425	0.06124531	0.00490563	0.1945933	0.0502924	0.01644652	0.001072
	7.0	0.03524686	0.02532029	0.000005	0	0.213823998	0.173683162	0.133122436	0.125147429	0.48073463	0.43780713	0.07890306	0.04519543	0.097	0	0.00086071	0.00111
	15.0	0.2558203	0.02969327	0.000005	0	0.589436206	0.222552523	0.509249106	0.096050745	0.49477394	0.18888451	0.08884403	0.02170568	0.0031582	0.0017699	0.00216505	0.000601
	27.0	0.09807697	0.0691393	0.000005	0	0.894655218	0.14968245	0.369919884	0.189697873	0.32880347	0.19155662	0.05452244	0.07290467	0.0085549	0.0102183	0.00088593	0.000149
Sludge (mg/L)																	
B3&B4	0.0	0.030	0.000	0.002	0.001	0.043	0.005	0.003	0.000	0.007	0.006	0.002	0.001	0.003	0.000	0.047	0.025
	0.10	0.039	0.005	0.001	0.000	0.049	0.002	0.003	0.000	0.010	0.002	0.003	0.000	0.003	0.000	0.058	0.011
	1.0	0.036	0.004	0.005	0.006	0.048	0.003	0.004	0.002	0.013	0.009	0.002	0.001	0.003	0.000	0.047	0.042
	7.0	0.033	0.001	0.000	0.000	0.051	0.000	0.003	0.000	0.004	0.001	0.012	0.003	0.006	0.001	0.004	0.001
	15.0	0.014	0.002	0.000	0.000	0.037	0.018	0.001	0.000	0.003	0.000	0.002	0.000	0.000	0.000	0.000	0.000
	27.0	0.007	0.001	0.000	0.000	0.025	0.003	0.002	0.001	0.002	0.002	0.001	0.000	0.000	0.000	0.000	0.000
Controls																	
W3&W4	0	0.326	0.01917393	0.140060394	0.030325986	0.650939201	0.434732782	0.263786676	0.02696684	1.36040372	1.00445845	0.28733936	0.04544403	0.6778807	0.0013052	0.16012687	0.01102
	27	0.884	0.00604247	0.000	0	0.446	0.051684257	0.028	0.002711669	0.150	0.01289119	0.051	0.00586194	0.027	0.0003992	0.014	0.001027
Total concentration																	
B3&B4	0.0	0.06	0.00	0.36	0.17	0.17	0.12	0.10	0.02	0.28	0.27	0.07	0.01	0.24	0.02	0.07	0.028
	0.10	0.06	0.01	0.10	0.00	0.10	0.01	0.09	0.00	0.10	0.01	0.07	0.00	0.23	0.01	0.07	0.012
	1.0	0.06	0.01	0.30	0.16	0.10	0.01	0.09	0.01	0.11	0.03	0.06	0.01	0.20	0.05	0.06	0.043
	7.0	0.07	0.03	0.00	0.00	0.26	0.17	0.14	0.13	0.48	0.44	0.09	0.05	0.10	0.00	0.01	0.002
	15.0	0.27	0.03	0.00	0.00	0.63	0.24	0.51	0.10	0.50	0.19	0.09	0.02	0.00	0.00	0.00	0.001
	27.0	0.11	0.07	0.00	0.00	0.92	0.15	0.37	0.19	0.33	0.19	0.06	0.07	0.01	0.01	0.00	0.000

APPENDIX II: RESULTS OF CHOLOROFORM TEST

Influence of chloroform addition on pharmaceutical concentration

Table II-1: Analyzed concentration of pharmaceuticals (mg/l) of samples preserved at different conditions.

	MTP	ASA	CBZ	CFA	BZF	DCF	IBU	FNF
5 ml sludge spiked with PhAC stock solution,n 1 night in fridge	0.72	0.00	3.82	5.85	64.17	7.30	9.00	0.84
5 ml sludge spiked with PhAC stock solution + 100 µl chloroform, 1 night in fridge	0.65	1.62	4.73	6.23	73.70	6.73	16.72	4.86
5 ml sludge spiked with PhAC stock solution, 1 night at -30 oC	0.61	0.79	5.15	4.76	65.08	5.37	11.63	2.94
5 ml sludge spiked with PhAC stock solution + 100 µl chloroform, 1 night at -30oC	0.59	1.12	5.27	5.30	70.28	6.08	14.62	3.35
5 ml sludge 1 night at -30oC, than spiked with PhAC stock solution	0.91	4.71	6.77	6.55	94.37	1.94	12.76	4.56
5 ml sludge 1 night at -30oC, than spiked with PhAC stock solution + 100 µl chloroform	0.98	7.64	6.59	6.57	96.39	2.82	15.31	18.87
expected start concentration	0.539	2.06	0.916	0.806	1.994	0.3055	0.81	2.056

APPENDIX III: NO3-N CONCENTRATION IN THE ANOXIC BATCH TESTS

Table III-1: NO3-N concentration (mg/l) in the ANOX-20 test

	t=0 d	t=0.2 d	t=1 d	t=2 d	t=17 d	t=30 d
B1 (duplicate 1)	40*	6.07	<5,0	36.8	<5.0	>35
B2 (duplicate 2)	40*	8.26	<5.0	<5.0	>35	>35
addition of NaNO3 stock			yes	yes, to B2		

* assumed concentration, based on the spiked amount of NaNO3 stock solution

Table III-2: NO3-N concentration (mg/l) in the ANOX-10 test:

	t=0 d	t=0.2 d	t=0.8 d	t=1.1 d	t=1.8 d
B3 (=duplicate 1)	40*	21.9	62.8	<5.0	41.2
B4 (=duplicate 2)	40*	21.4	13.8	<5.0	48.6
Addition of NaNO3 stock		yes		yes	

* assumed concentration based on the spiked amount of NaNO3 stock solution