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Sustainable Water Management in the City of the Future

Integrated Project
Global Change and Ecosystems

Deliverable 4.1.3

Biodegradability and fate of pharmaceutical impact compounds in different treatment processes

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Authors:
Katarzyna Kujawa-Roeleveld, Els Schuman WU, Environmental Technology, Wageningen, The Netherlands

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SWITCH Deliverable Briefing Note Template

SWITCH Document Deliverable 4.1.3 entitled **Biodegradability of selected pharmaceuticals:**

Audience This document is targeted mainly at engineers, scientists and technologists. To a lesser extent it addresses policy makers, the pharmaceutical industry (awareness) and the general public.

Purpose

Biological treatment of wastewater is a core technology in most sanitation systems. In this work the biodegradability of eight selected human pharmaceutical compounds, each having very different characteristics, has been assessed. The results, complemented with literature research, contributes to increased understanding whether more advanced treatment of source separated wastewater streams containing elevated concentrations of pharmaceutical compounds, is needed in order to avoid risks for humans and the environment.

Background

Human pharmaceuticals are consumed in high quantities world wide. The consumption is in the range of tons per year per one pharmaceutical compound, depending on the size of a country. It is expected that these amounts will keep on increasing because of improving health care systems and longer life expectations of people.

The diversity of pharmaceuticals is large. E.g., in the Netherlands, there are 12,000 human pharmaceuticals approved (authorised). From an environmental point of view, there are 850 active compounds in human pharmaceuticals, that are important (Derksen 2004). The pharmaceuticals that are administered (a medical term, indicating 'consumed') by humans after the required action in the body, get excreted with urine and faeces as a parent (original) compound and usually as a number of metabolites. In conventional wastewater systems, the toilet wastewater (consisting of urine and faeces flushed with clean water; often called black water) is mixed with other wastewater streams forming sewage that enters the municipal sewer. Research shows that in sewage treatment plants (STPs), many pharmaceuticals compounds do not get removed to a sufficient degree. The reason is that the configurations (designs) of the current STPs that are not efficient enough to remove these micro pollutants. Consequently, they enter surface water systems where they may pose effects on aquatic life and ultimately may enter the human water cycle through the intake of surface water for the production of tap water. There is evidence that harmful effects are there.

Our knowledge on the fate of pharmaceuticals in wastewater treatment systems (biological, physical-chemical) is still limited. Especially systems dealing with concentrated streams, such as urine or black water, have not been not subject of many investigations. In the research described in this report, the behaviour of eight selected pharmaceuticals was investigated in biological systems for treatment of concentrated wastewater.

Potential Impact

This work contributes to other contributes to increased understanding on the fate of pharmaceuticals in different environmental compartments. Studies were done on a number of representative pharmaceuticals, each having different chemical-physical property. The knowledge obtained in the research can be generalized to other, 'similar' pharmaceutical compounds. The knowledge on the potential of biological treatment for environmentally relevant compounds could facilitate further research into the optimization of biological STPs to achieve an improved efficiency towards the removal of pharmaceuticals. The research also shows that for persistent and semi-persistent compounds it is inevitable to develop more efficient treatment methods. These are most likely advanced chemical-physical treatment methods.

Issues

- Although the issue of the pharmaceutical in environment attracts a lot of attention, especially, of scientific world, there is no policy, no standards defining which compounds should be removed and to which level.
- The analytical methods to determine pharmaceuticals in a complex matrix like wastewater are still difficult, time consuming and costly; for many pharmaceutical compounds not even developed/validated yet
- The fate of excreted (active) metabolites/conjugates in treatment systems is very unclear.
- The number of environmentally relevant pharmaceuticals excreted to the environment is large; examining all of them is impossible. Therefore the simplifications are required and it is recommendable to work with a restricted number of so called representative compounds;
- Separation and concentration of wastewater streams has an advantage of having pharmaceuticals in a very small, perhaps better controllable volume; on the other hand the matrix becomes more complex.

Recommendations

- More research should be devoted to the biodegradability of human pharmaceuticals from source separated concentrated wastewater streams; the selection of representative pharmaceuticals should be enlarged;
- Both anaerobic and aerobic biodegradation are of importance in the expected overall treatment schemes;
- This research worked with laboratory batch tests. It is recommended to also investigate the fate of these pharmaceuticals in continuous biological treatment systems during a long term period in order to study the adaptation of the biological sludge.
- The information on potential biodegradability of human pharmaceuticals should be bundled into a model enabling the prediction of the behaviour of a given compound in a biological treatment systems.

Table of content

Preface

Human pharmaceuticals are consumed in high quantities worldwide; the consumption is in the range of tons per year per pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of a improving health care system and longer life expectations of people.

Our current sanitation systems are characterised by a high degree of dilution. Dilution is one of the reasons why pharmaceutical compounds are not sufficiently removed. When discharged to surface water they may form a threat to aquatic life and in the worse case may re-enter the water cycle. Source control, i.e. sanitation approaches based on separation at source, are based on separation and separation of wastewater streams of different origin (black water, grey water). Specific treatments, targeting different flows, may enable elimination of pharmaceuticals and minimisation of the emission of human pharmaceuticals to the environment.

In this document a final selection of representative pharmaceutical compounds to be tested in various biological- (2^{nd} year of the project) and later on physical-chemical (3^{rd} year of the project) wastewater treatment systems was done. The selected compounds: acetylsalicylic acid (aspirin), diclofenac, ibuprofen, carbamazepine, metoprolol, clofibric acid, bezafibrate and fenofibrate represent 4 therapeutic groups. A brief overview of a found behaviour of selected compounds in physical systems (STP, batch experiments) was given.

During the second year of the project a number of biodegradation tests was performed under various process conditions. The applied concentrations of the selected pharmaceuticals were relatively high (low mg/L range) in order to simulate situation where concentrated wastewater sub-streams solely containing pharmaceutical (urine, black water) are biological (pre/post)-treated. Different process conditions (redo-ox, temperature, sludge origin) were applied in order to translate the obtained results to various process configurations.

Summary

The biodegradability of eight selected pharmaceutically active compounds (PhAC) was assessed under various environmental conditions (varying with respect to red-ox conditions, temperature and the character of the seed biological sludge). The selected PhACs were characterized by different physical-chemical-biological properties in order to be able to extend the results of this research to the broader group of environmentally relevant micro-pollutants. The selected compounds were: acetylsalicylic acid (ASA), bezafibrate (BZF), carbamazepine (CBZ), clofibric acid (CFA), diclofenac (DCF), fenofibrate (FNF) and metoprolol (MTP).

Many PhAC can be biodegraded under aerobic conditions. The extent of biodegradation depends in many cases on the exposure time of a biomass to a given compound. Aerobic biodegradation is faster than anoxic degradation. Elevating operational temperatures speed up the biodegradation processes, as expected. Under anaerobic conditions and relatively long retention times (HRT=30 d) some PhAC can be degraded (ASA, IBU, FNF) but at much lower rate than under aerobic or anoxic conditions. The anaerobic digestion process, is however not expected, to contribute significantly to elimination of majority of PhACs. Optimisation of process conditions for a (semi)persistent group of PhAC (CBZ, CLF, DCF) will only result in their partial (if any) biodegradation. For new sanitation concepts for source separated wastewater, where anaerobic digestion is applied as an efficient pre-treatment for a bulk of organic matter, and aerobic as a main treatment, addition of a physical or chemical polishing unit to eliminate persistent compounds (when demanded) will be unavoidable.

1 Introduction

Human pharmaceuticals are consumed in high quantities world wide. The consumption is in the range of tons per year per one pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of a improving health care system and longer life expectations of people.
The diversity of the human pharmaceuticals is large. In the Netherlands, for instance, there are 12000 human pharmaceuticals approved (authorised). There are 850 active compounds in human pharmaceuticals, important fact from environmental point of view (Derksen 2004).
Pharmaceuticals administered (it is a medical term, in other words consumed) by humans after required action in the body get excreted with urine and feaces as a parent (original) compound and usually as a number of metabolites. The toilet wastewater (consisting of urine and faeces flushed with clean water; often called black water) is mixed with other wastewater streams forming finally a sewage that enter the municipal sewer. In a sewage treatment plant (STP) effluents many pharmaceuticals compounds do not get removed to a sufficient degree. This is because of the configurations of the current STPs that are not efficient enough to remove these micropolllutants. Consequently they are present in the effluents of STPs, enter the surface water where they may pose effects onto aquatic life. There are already evidences that they do so.
Knowledge on the fate of pharmaceutical in wastewater treatment system (biological, physical-chemical) is still insufficient. Especially systems dealing with concentrated streams, such as urine or black water, were not subject of many investigations. In this part of the project the behaviour of eight selected pharmaceuticals will be investigated in biological systems for treatment of concentrated wastewater.

1.1 Presence of human (and veterinary) pharmaceuticals in environment

The presence of human and veterinary pharmaceuticals in various environmental compartments (aquatic and terrestrial) were described to some extent in deliverable 4.1.2 (Kujawa-Roeleveld 2007)

1.2 Consumption human pharmaceuticals (in NL)

The consumption and abundance of pharmaceutical compounds differ per country. The global consumption of pharmaceuticals used by humans is predicted as 100,000 tons per year. This number corresponds to a worldwide average pro capita consumption of 15 $g.cap^{-1}.a^{-1}$ (Ternes 2006) (Kummerer 2004).
The consumption of all therapeutic groups of pharmaceuticals in the Netherlands in years 2002 till 2006 expressed in number of users is given in Table 1.1.

Table 1.1: Users per ATC group of pharmaceuticals (* 1000) in the Netherlands (CVZ 2007)

ATC group	2002	2003	2004	2005	2006
A Alimentary tract and metabolism	2.910	3.003	2.769	2.969	3.348
B Blood and blood forming organs	1.651	1.663	1.668	1.674	1.853
C Cardiovascular system	2.676	2.759	2.910	2.982	3.470
D Dermatologicals	3.419	3.463	3.190	3.164	3.423
G Genito urinary system and sex hormones	2.767	2.696	1.412	1.406	1.530
H Systematic hormonal preparations	828	854	890	927	1.017
J Antiinfectives for systematic use	3.840	3.826	3.775	3.945	4.233
L Antineoplastic and immunomodulating agents	145	157	169	179	215
M Musculo-skeletal system	3.403	3.423	3.322	3.136	3.236
N Nervous system	3.590	3.603	3.351	3.313	3.477
P Antiparasitic agents, insecticides, repellents	144	148	160	162	174
R Respiratory system	3.149	3.064	3.033	3.099	3.410
S Sensory organs	1.786	1.802	1.759	1.754	2.104
V Various	34	37	40	43	55

Group A, C, D, J, M, N and R are characterised by the highest number of users, above 3,2 mln people per ATC group. A significant increase of users for all therapeutic groups is to observe especially from year 2005 to 2006. An increase up to 20% for some groups was reported by CVZ (CVZ 2007). In Table 1.2 number of DDDs sold between 2002 and 2006 are listed per ATC main group. The prevailing groups are then A, B, C, D, N and R. The difference between number of users for group B (relatively small) and DDDs sold (high) is that preparates from this group are used chronically (between 17 to 575 DDD per user per year depending on the sub-group used). In contrary drugs from group J are used by a large number of people but amount of DDD sold is relatively small as anti-infectives are used for a short time per patient (between 1 (vaccine) to 169 DDD/p/y (anti-mycobaterials).

Table 1.2 : Amount of DDDs (* 1000) used in The Netherlands in years 2001-2006 (CVZ 2006)

ATC group	2002	2003	2004	2005	2006
A Alimentary tract and metabolism	839.970	897.320	828.640	924.480	992.680
B Blood and blood forming organs	546.890	589.220	612.920	652.010	690.970
C Cardiovascular system	1.713.300	1.870.900	2.047.900	2.190.500	2.435.000
D Dermatologicals	495.010	522.170	472.040	486.820	504.050
G Genito urinary system and sex hormones	790.320	798.740	277.350	283.660	307.660
H Systematic hormonal preparations	113.920	120.200	125.290	129.510	149.510
J Antiinfectives for systematic use	63.606	64.400	64.951	69.285	73.436
L Antineoplastic and immunomodulating agents	34.981	40.856	47.140	52.408	62.288
M Musculo-skeletal system	241.730	256.170	250.080	238.100	237.680
N Nervous system	670.680	699.650	686.690	691.830	666.840
P Antiparasitic agents, insecticides, repellents	4.249	4.502	5.207	5.052	5.277
R Respiratory system	592.680	582.240	565.290	568.710	576.030
S Sensory organs	208.450	220.610	220.300	222.780	255.180
V Various	2.979	3.706	4.647	5.752	7.317

Again a significant increase can be observed between year 2005 and 2006 regarding the consumption of the medicines from almost all groups (except of N and P). Again increase up to 20% for certain groups was reported.

1.3 Source separation based sanitation concept

A number of different wastewater streams are produced in households as a consequence of various human activities (Figure 1.1). In the existing combined sanitation system, all the streams originating from the households are collected with the same piping system and end up to the conventional WWTPs. Wastewater streams can be separated based on their composition and concentrations (STOWA 2005). Black water originating from the toilets is one of the most concentrated streams and consists of faeces, urine and flush water (Otterpohl, Albold et al. 1999; Kujawa-Roeleveld and Zeeman 2006). Grey water is the combination of the sub-streams originating from shower, bath, laundry and kitchen and is relatively diluted (Kujawa-Roeleveld and Zeeman 2006). Black water contains high organic contents as well as the major fraction of the nutrients in domestic wastewater. Besides, most of the pathogens and mico pollutants (pharmaceuticals, hormones etc.) are also present in this stream which has a small volume. Separating urine or black water stream from the others enables to concentrate the risks in a very small volume. This gives an opportunity to have a better control, enabling the recovery of nutrients and energy and limit the negative environmental effects (Kujawa-Roeleveld and Zeeman 2006).

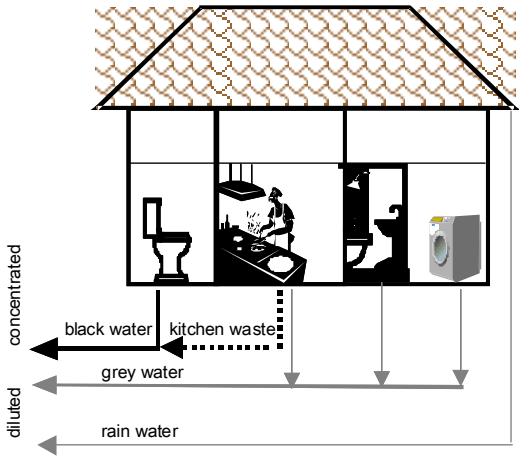


Figure 1.1: Wastewater streams produced in households.

1.4 Objectives

The objective of this sub-study was to asses biodegradability of the 8 selected pharmaceutical compounds under various process conditions. The results of the study will enable to predict the fate of pharmaceutical compounds in biological systems treating wastewater containing increased concentrations of pharmaceuticals (urine, black water).

2 Selected pharmaceuticals

2.1 Introduction

In this section the motivation for the selection of eight pharmaceuticals: acetylsalicylic acid (aspirin), diclofenac, ibuprofen, carbamazepine, metoprolol, clofibric acid, bezafibrate and fenofibrate for this research is elaborated.

The following selection criteria were used:

- 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.

The last criteria was fulfilled for all selected pharmaceuticals.
An attempt was taken to include as much as possible of different criteria per selected compound to obtain a good representation of pharmaceuticals released to the environment. A strong variety of selected compounds may enable to translate to translate results of this study to other compounds having similar properties.

The relevancy of the selected pharmaceuticals could be justified by a research of Dutch Institute for Public Health and Environment (RIVM) in which all the selected pharmaceuticals were detected in drinking water sources (Versteegh 2007), Figure 2.1.

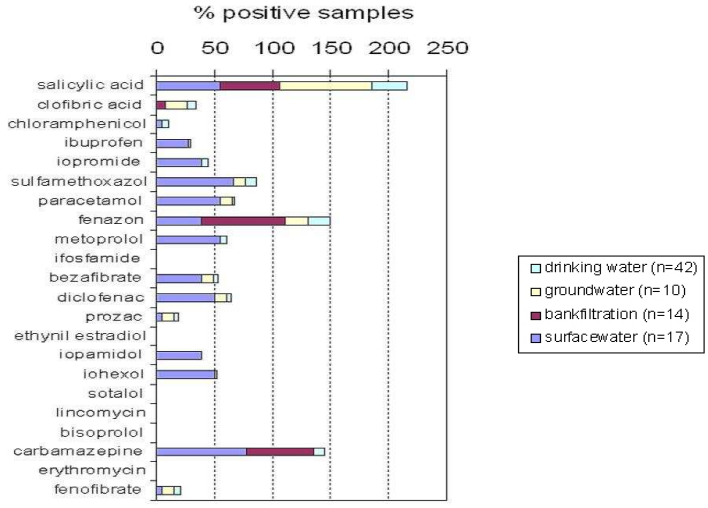


Figure 2.1. Percentage of the positive samples in the measurement campaign (2005/2006) for the presence of human pharmaceuticals in drinking water and drinking water resources in the Netherlands (Versteegh *et al*. 2007)

A short characteristics of the selected compounds is given below.

2.1.1 Acetylsalicilic acid (ASA)

Aspirine or acetylsalicylic acid (ASA) is a drug often used as an analgesic (to relieve minor aches and pains), antipyretic (to reduce fever), and as an anti-inflammatory. It also has an antiplatelet ("anti-clotting") effect and is used in long-term, low doses to prevent heart attacks and blood clot formation in people at high risk for developing blood clots.
Aspirin is consumed in high quantities in the NL . It can be prescribed but it can also be sold over-the-counter. This makes the estimation of the (real) consumption rate more difficult. When only the prescribed use of aspirin is taken into account, the amount of DDDs sold in 2006 in the Netherlands was 362.564 as analgesic (DDD = 3000 mg) and 344 mln as antiplatelet (dose between 30 and 100 mg/p/d (CVZ, 2007), (WHO 2006) resulting in environmental emissions as high as 18.3 tone/year.
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 (its main metabolite) was measured in the research of (Fent 2006).
Besides the high consumption rate, aspirin was also selected because of its hydrophilic character and good biodegradability according to literature.

2.1.2 Diclofenac (DCLF)

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and an analgesic reducing pain in conditions such as in arthritis or acute injury. It can also be used to reduce menstrual pain, dysmenorrhea. The name is derived from its chemical name: 2-(2,6-dichloranilino) phenylacetic acid.
Diclofenac is also consumed in high quantities in the NL . Analogously to aspirin It can be prescribed but it can also be sold over-the-counter. The number of prescribed DDDs for diclofenac (including combination preparates) amounted in 2006 at 68 323 300 (CVZ 2007). Assuming that all prescribed DDDs were consumed the emission to the environment would amount to approximately 6.8 tones/year (DDD = 100 mg, WHO, 2006).
Diclofenac is known as persistent to biodegradation and relatively harmful to aquatic organisms. Reported removal rates of diclofenac in WWTPs are between 0-69% (Table 3.3). Observed concentration in WWTP effluents are in the range of 0.17 – 2.5 ug/l (Fent 2006), (Lindqvist, Tuhkanen et al. 2005).
Further, diclofenac has shown to cause some harmful effects even at low concentrations. For diclofenac a lowest observed effect concentration (LOEC) of only 1 ug/l for fish was determined (Triebskorn, Casper et al. 2004).
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 was detected, have died because of problems related to renal failure (Oaks, Gilbert et al. 2004).

2.1.3 Ibuprofen (IBU)

Ibuprofen belongs also to the NSAID group. It reduces pain, inflammation and the fever. Ibuprofen has a high consumption and is often measured in the environment. In the Netherlands ibuprofen can be prescribed and sold over-the-counter like aspirin and diclofenac.
Ibuprofen is consumed in high quantities in the NL . The number of prescribed DDDs for ibuprofen (including combination preparates) in 2006 amounted 23 232 100 (CVZ 2007). Assuming that all prescribed DDDs were consumed the emission to the environment would amount to approximately 28 tones/year (DDD = 1200 mg, WHO, 2006).

According to the results of several researches the influent of ibuprofen to WWTPs ranges from 3 – 39 ug/l (Fent 2006). Ibuprofen can be degraded in WWTPs up to more than 90%, however because of the continuous and significant input of this pharmaceutical, still the presence of the compound is measured in surface waters and drinking water. Mean concentrations measured in WWTP effluents and surface waters are in the ranges of up to 10 µg/l and about 4 ng/l respectively (Jones 2005).
Ibuprofen has a high hydrophobic character. Adsorption to sludge of this pharmaceutical will be relative high compared to other selected pharmaceuticals.

2.1.4 Carbamazepine (CBZ)

Carbamazepine is an anti-epileptic drug, 'prominently' present in the aquatic environment. This compound was e.g. measured 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 2006), (Jones-Lepp 2001), (Fent 2006) and (Weigel 2003). Highest mean concentration measured in a river is 1.2 µg/l (Weigel 2003). Carbamazepine turned out to be persistent towards biological degradation. Measured median concentration for STPs effluents range from 0.70 to 2.1 µg/l (Petrovic et al., 2005).
Carbamazepine has been one of the substances which is detected most often in drinking water sources in the Netherlands (Versteegh 2007).

2.1.5 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 the most (50% of the used beta-blockers concern metoprolol) (CVZ 2007).
Metoprolol is a selective beta1 receptor blocker used in treatment of several diseases of the cardiovascular system, especially hypertension.
In the Netherlands in 2006 there were 826.100 users of metoprolol consuming 144.373.800 DDDs. The maximum emission of this drug to the environment was then 21.6 t taking into account that DDD is 150 mg (WHO 2006).
Metoprolol can have effects on the heart on invertebrates in the environment. In *D. Magna* for example metoprolol caused at low concentration acceleration of the heart beat (Fent 2006).

2.1.6 Clofibric acid (CFA)

Clofibric acid (also named: clofibrin or chlorofibrinic acid) is the active metabolite of clofibrate, etofibrate and etofyllin clofibrate (Reemtsma 2006) - lipid lowering agent.
It is poorly degraded in WWTPs. The measured removal percentages range from 0 – 51 % (Fent 2006).
The pollutant was detected in inland surface waters, in Guanabara Bay of Brazil (Stumpf, Ternes et al. 1999), ground water and in tap water (Heberer, 1998).

2.1.7 Bezafibrate (BZF)

Bezafibrate is a fibrate drug used for the treatment of hyperlipidaemia. It helps to lower (low-density lipoprotein (LDL called also 'bad cholesterol') cholesterol and triglyceride in the blood, and increase HDL (High-density lipoproteins – good cholesterol).
The observed removal percentages of bezafibrate at the WWTP vary a lot. In different researches elimination rates between 15-100% were reported (Fent 2006). Its biodegradation potential is therefore unclear.
Bezafibrate was especially chosen because of its high low K_{ow} value. Absorption to sludge will be important removal mechanism compared with the more hydrophilic pharmaceuticals.

2.1.8 Fenofibrate (FNF)

Fenofibrate is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. Like other fibrates, it reduces both low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as increasing high-density liporotein (HDL) levels and reducing tryglycerides level.

Fenofibrate is a drugs which is not used in the Netherlands nowadays but it is still used internationally (KNMP 2006). Fenofibrate was measured in drinking water samples in the Netherlands (Versteegh 2007).

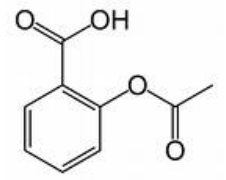
Fenofibate was selected for this study, analogously to bezafibrate, for its hydrophobic character (exceptionally high K_{ow} value, Table 2.1).

The consumption figures in the Netherlands and the properties of the selected pharmaceuticals are summarized in tables 2.1 and 2.2.

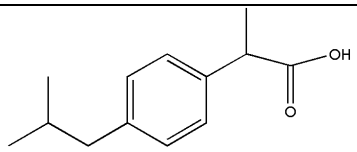
Table 2.1: Prescribed amounts of the selected pharmaceuticals in the Netherlands in 2006 (source: (CVZ,2007)

Thera-peutic class	Compound	Amount of users in NL (2006)	Fraction of users (%) in NL	DDD (mg/p/d)	Amount of DDDs sold in NL	Amount sold (ton/yr)
		23.631 (3000) 576.920 (30-100)		3000 or 30-100	362564(3000) 166334800 (50)	9.4
B & N	Aspirin		4.1			
M	Ibuprofen	853.980	7.2	1200	23,627,700	27.9
M & S	Diclofenac	1 755 610[1)]	9.3	100	52,189,600	5.2
C	Metoprolol	826 100	5.4	150	144 373 800	21.6
N	Carbamazepine	57 779	0.4	1000	8 762 300	23
C	Bezafibrate	3,222	0.02	600	532,670	0.88
C	Clofibrate	275	0.002	2000	31,590	0.17
C	Fenofibrate	0	0	200	0	0

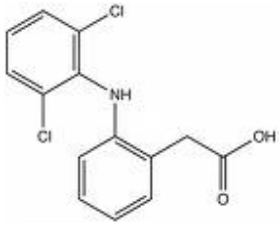
B - Blood and blood forming organs, C - Cardiovascular system, M - Musculo-skeletal system, N - Nervous system and S - Sensory organs; [1)] including combination preparates



aspirine



ibuprofen



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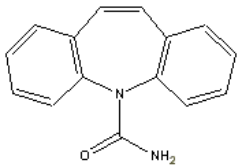
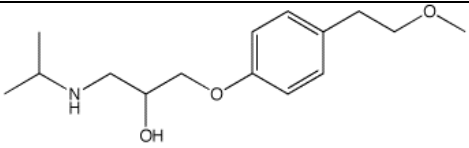


carbamazepine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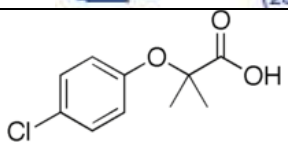
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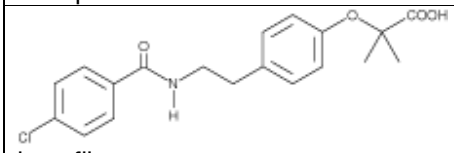
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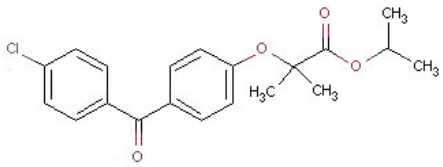

fenofibrate

2.2 Characteristics of selected pharmaceuticals

The physical-chemical properties of the selected pharmaceuticals are given in Table 2.2.

Table 2.1: Physical-chemical properties of the selected pharmaceuticals

Pharmaceutical	Therapeutic group	Log Kow[2]	Hydrophilic / hydrophobic	pKa value at T = 20 ^{0}C[2]	k_{biol} for CAS (L/ gSS/d)[1]
Aspirin	anti-inflammatory	1.426	hydrophilic	3.5	n.a.
Ibuprofen	anti-inflammatory	3.481	Moderately hydrophobic	4.5-5.2	21–35
Diclofenac	anti-inflammatory	0.7-4.5 depending on pH	varying	4.15	<0.1
Metoprolol	β – blocker	1.9	hydrophilic	9.7	n.a.
Carbamazepine	anti-epileptic	2.69	Moderately hydrophobic	<1, 13.9	n.a.
Clofibric acid	lipid regulating	2.57	Moderately hydrophobic	3.0	0.3–0.8
Bezafibrate	lipid regulating	4.25	hydrophobic	3.6	2.1–3.0
Fenofibrate	lipid regulating	5.19[3]	hydrophobic	n.a.	n.a.

[1]Joss et al., 2006, [2]Ternes et al., 2006 ; [3] van Beelen, 2007 CAS = conventional activated sludge

3 Fate of selected pharmaceuticals during wastewater treatment (literature study)

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 (e.g. photolytic degradation).

3.1.1 Biodegradation

Biodegradation is a very important process in the transformation of organic pollutants including such as pharmaceuticals. It can result in an energy gain for bacteria growth but it can also occur co-metabolically during degradation of other organic compounds. Biodegradation can happen partially or completely. The latter, complete biodegradation to carbon dioxide (CO_2) and water (H_20), is called mineralization. Partial biotransformation includes the conjugation of the pharmaceutical and the degradation of a compound to a metabolite (also occurring in human body). A conjugate is a complex formation of the pharmaceutical with glucuronic acid or sulphate. Conjugation of the pharmaceutical takes plays usually before excretion in the human body. The result of this conjugation is that the compound is becoming more polar and can be excreted more easily. A conjugated compound can be easily transformed back to the free original compound in the sewage system or in a WWTP.
Metabolites of pharmaceuticals can be produced the human metabolism and in WWTPs. Like the original pharmaceuticals, metabolites can be persistent and they might demonstrate effects in the environment. Identification of these metabolites in wastewater is therefore important.
Some studies have identified degradation products and possible degradation pathways of selected pharmaceuticals.

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 3.1).
These metabolites are also known to be formed during the human metabolism of ibuprofen.

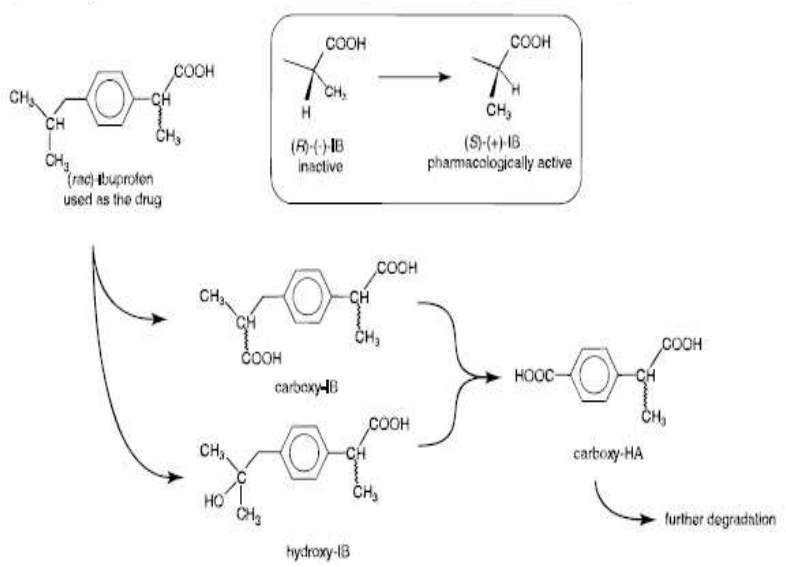


Figure 3.1: Part of the degradation path of ibuprofen. Source: (Buser, Poiger et al. 1999).

The metabolites of ibuprofen are/were found to be removed efficiently. Only small amounts are/were detected in WWTPs effluents. In lakes, only ibuprofen is detected and none of its metabolites (Buser, Poiger et al. 1999), (Reemtsma 2006).
Zwiener (2002) identified in an aerobic batch reactor fed with sewage sludge of WWTP, hydroxy-ibuprofen as the major metabolite of ibuprofen. However, the concentration of this metabolite was rather low. In this aerobic batch test lasting for two days a maximum of 7% of the degraded ibuprofen could be assigned to the formation of OH-ibuprofen.
In an anoxic batch test almost no OH-ibuprofen was detected. On the other hand, carboxy-ibuprofen was detected in highest concentration of all metabolites (up to 1.8% of the degraded ibuprofen concentration) under anoxic conditions. Concentrations of CA-HA were at quantification limits both under oxic (aerobic) and anoxic conditions (up to 0.3% of degraded ibuprofen).
All together, the concentration of metabolites did not exceed 10% of the initial ibuprofen concentration and not more than 10% of the degraded ibuprofen can be explained by the formation of these metabolites (Zwiener 2002).

Acetylsalicylic acid

Acetylsalicylic acid can be transformed to salicylic acid (Zwiener 2002). Other metabolites of acetylsalicylic acid are salicyluric acid and gentisic acid (Hansen, Jensen et al. 1998). Biodegradation of salicylic acid takes place easily because it is produced by nature itself as well. Removal rates upto 99% of salicylic acid were observed in WWTPs (Fent 2006).

Diclofenac

Diclofenac is quite persistent and little is known about its degradation pathways. Degradation products of diclofenac have been identified by Kosjek et al. (2007) (Kosjek, Heath et al. 2007) in effluent of a pilot WWTP (with aerobic and anaerobic compartments). The products are given in Figure 3.2.

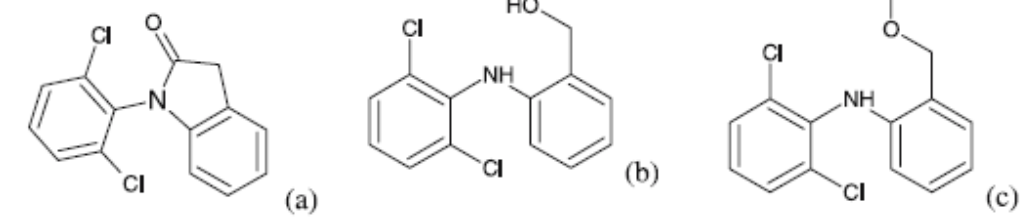


Figure 3.2: Degradation products of diclofenac. 2,6-dichlorophenyl-1,3-dihydro-2H-indol-2-one (a), 2-((2,6-dichlorophenyl)amino)benzyl alcohol (b) and 2-((2,6-dichlorophenyl)amino)benzyl alcohol methyl ether. Source: (Kosjek, Heath et al. 2007).

Carbamazepine (CBZ)

Carbamazepine is degraded in 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 3.3. The main metabolic pathway for CBZ 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 (Canada) the highest mean concentrations were for carbamazepine and for CBZ-DiOH. Other metabolites have been detected as well but in lower concentrations. In biosolids (sludge) mainly the parent compound carbamazepine was present. The overall removal of CBZ was about 29% (including UV-treatment step). Comparison of the metabolite concentrations of influent and effluent showed that the metabolites were not degraded. The effluent concentration was the highest for CBZ-DiOH followed by carbamazepine and other metabolites.

The toxicity of the metabolite CBZ-DiOH is not known. For CBZ-EP it was observed that it has (still) anti-epileptic properties as its parent compound (Miao 2005).

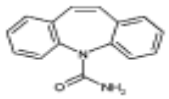
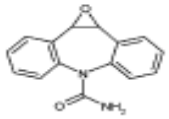
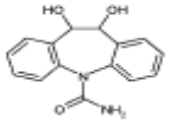
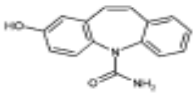
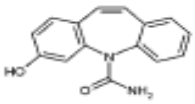
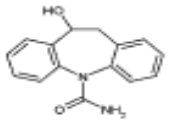
Structure	Analyte	Abbreviation [CASRN[a]]
	Carbamazepine	CBZ [298-46-4]
	10,11-dihydro-10,11-epoxycarbamazepine	CBZ-EP [36507-30-9]
	10,11-dihydro-10,11-dihydroxycarbamazepine	CBZ-DiOH [35079-97-1]
	2-hydroxycarbamazepine	CBZ-2OH [68011-66-5]
	3-hydroxycarbamazepine	CBZ-3OH [68011-67-6]
	10,11-dihydro-10-hydroxycarbamazepine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

Figure 3.3: Five main metabolites of carbamazepine. Source: (Miao 2005).

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 3.4. Metabolites of metoprolol are, according to the figure: metoprolol α-hydroxymetoprolol, O-demethylmetoprolol, metoprolol acid and deaminated metoprolol.

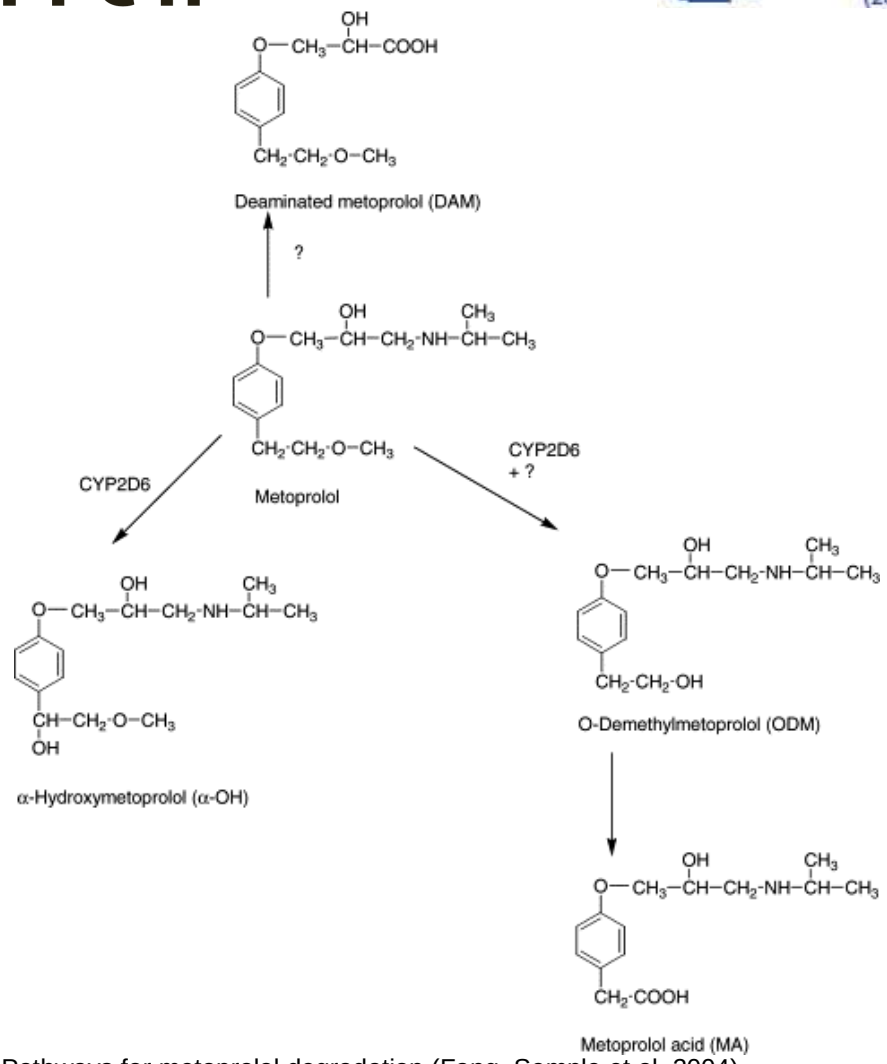


Figure 3.4: Pathways for metoprolol degradation (Fang, Semple et al. 2004).

Clofibric acid

Clofibric acid is the active metabolite of three fibrates: clofibrate, etofibrate and etofyllin clofibrate (Reemtsma 2006). Biodegradation products of clofibric acid and their behavior in environment were not found in literature.

Bezafibrate

The degradation of bezafibrate has not been much a subject of research. 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).

Fenofibrate

Fenofibrate is in the human body easily hydrolyzed with esterases to fenofibric acid. This is the pharmaceutically active compound. The microbial metabolic pathway of fenofibric acid is not known.

3.1.2 Sorption

Sorption to sludge can be an important removal mechanism especially when a pharmaceutical is slowly biodegradable 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.

Absorption

Absorption is related to hydrophobic interactions of aliphatic and aromatic groups of a compound with the lipid fractions of the sludge (solids) (Ternes and von Gunten 2005). The hydrophobic character of a compound can be indicated with the K_{ow} value. The 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. Based on the log K_{ow} values three groups of compounds can be distinguished regarding their sorption potential (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 log K_{ow} of the eight selected pharmaceuticals are listed in table 2.1. Bezafibrate and fenofibrate are the most hydrophobic pharmaceuticals, with a log K_{ow} >4,0. From all the selected pharmaceuticals, removal due to absorption will thus be the most important for these two compounds.

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.
The pKa value indicates whether a pharmaceutical is acidic or basic. The lower this value, the more acidic a compound is. Most of the selected pharmaceuticals are acidic and therefore at neutral pH, negatively charged. This decreases their adsorption affinity to sludge. Only metoprolol and carbamazepine are not acidic (Table 2.1), while, their log K_{ow} value is quite low (1.9 and 2.7 respectively).

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. The solid-liquid partition coefficient is calculated with the following formula under equilibrium conditions.

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

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 or production suspended solids in primary or secondary treatment (kg $L^{-1}_{wastewater}$);
S_i	the soluble concentration of a compound i *(mg/L)*;;

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

$$\frac{C_{(i,sorbed)}}{C_{(sorbed)} + C_{(i,solu ble)}} = \frac{K_{d,i} * SS}{1 + K_{d,i} * SS}$$

Sorption in municipal WWTP can be neglected when K_d value < 500 L/kgSS (<10% sorption if sludge production between 200 - 400 gSS/m^3 (Ternes, 2005 #106).

For three of the 8 selected pharmaceuticals, the K_d value was found in literature (Table 3.1). As it can be seen from the 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.

Table 3.1: Partitioning coefficients of 4 pharmaceuticals for primary and secondary sludge (Ternes and von Gunten 2005).

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

Considering above, only a minor part of the pharmaceuticals will be sorbed to the biological sludge during wastewater treatment. Taking into account the hydrophobic characters, sorption is likely to be, most significant for fenofibrate and bezafibrate.

3.1.3 Vaporisation

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

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

where:
K_{aw} = partitioning coefficient (-)
C_{air} = concentration of pollutant in air (mg/L)
C_{water} = soluble concentration of pollutant (mg/L)
H = Henry's law constant (atm m^3/mol)
R = gas constant (atm.m^3/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 3.2 shows that the Henry's law constant and the K_{aw} of pharmaceuticals are very low. As a result, vaporization is not regarded to as a significant mechanism for removal of the pharmaceuticals.

Table 3.2: Henry's law constants and partitioning coefficients for selected pharmaceuticals. (Source: US National Library of Medicine), T = 25 ^{o}C.

Pharmaceutical	MTP	DCLF	IBU	CBZ	ASA	CFA
Henry's Law constant (atm.m^3/mol)	1.40E-13	4.73E-12	1.50E-07	1.08E-10	1.30E-09	2.19E-08
K_{aw} (-)	5.73E-12	1.93E-10	6.13E-06	4.42E-09	5.32E-08	8.96E-07

3.1.4 Abiotic transformations

Abiotic transformation may occur via the processes of hydrolysis and photolysis. Andreozzi (1998) has determined half-lives of carbamazepine, clofibric acid and diclofenac in photolysis process. In a test with glas-disk reactors in a thermostatic bath at a temperature of 25 ^{o}C direct photolysis was analyzed in various seasons and at several latitudes (20 ^{o}N – 50 ^{o}N). During winter and 50^{o}N 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 $t_{1/2}$ for DCFL 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, Muller et al. 1998). A first order kinetic was determined in a laboratory experiment with a half-live of less than 1 hr in autumn at a latitude of 47^{o}N. Metabolites were not studied in that case, thus this elimination of diclofenac could also result from the production of OH-diclofenac or it could be more advanced.

3.1.5 Fate of selected pharmaceuticals in biological systems treating wastewater

Conventional Treatment
Removal in conventional municipal WWTPs was assessed for different treatment plants. Removal rates for the selected pharmaceuticals are listed in table 3.4. Removal includes transformation, mineralization and sorption to sludge. The different fate processes are not much researched separately.

Table 3.3: Removal of pharmaceuticals in municipal WWTP. (na = not available)

Pharmaceutical	Classification by Joss et al.(2006)	Removal in municipal WWTP or pilot WWTP (%)	References
Acetylsalicylic acid	Partial	-	
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)
Diclofenac	No removal	0- 69	(Kosjek, Heath et al. 2007) (Tauxe-Wuersch 2005), (Strenn 2004)
Metoprolol	n.a.	<10, 83	(Paxeus 2004), (Ternes 1998)
Carbamazepine	No removal	0-29	(Strenn 2004), (Miao 2005) (Ternes 1998),
Clofibric acid	Partial	0-51	(Ternes 1998), (Tauxe-Wuersch 2005), (Zwiener 2002)
Bezafibrate	Partial	0-97 , 83	(Strenn 2004), (Ternes 1998),
Fenofibric acid	Partial	64	(Ternes 1998)

In Table 2.1, biodegradation rate constants are listed for the eight pharmaceuticals for conventional activated sludge treatment of the wastewater. Based on these degradation constants the pharmaceuticals can be placed in different groups, according to the classification scheme set up by Joss *et al.* (2006).

The observed removal rates differ a lot for a certain pharmaceutical and also between pharmaceuticals. This can be due to different properties of the different WWTPs (like SRT and HRT) and of variation in climate.
Ibuprofen is some researches removed to a large extent. Some removal rates are however very low. The low removal rate of ibuprofen of 10% in the table, refers to winter conditions. In the same research (Tauxe *et al*., 2005) a high removal rate of 79% was obtained in summer for a WWTP with a HRT of 16 hr. Kosjek (2007) observed a removal of 91% for a pilot WWTP with a HRT of 48 hr.
Acetylsalicylic acid expected to biodegrade well, because its metabolite salicylic acid is a naturally occurring compound. Few data is available for this at the moment. The European Chemical Agency has performed a biodegradation test for acetylsalicylic acid. In this test, 98% was removed aerobically within a timeframe of 28 days and in 2 month >75% was degraded anaerobically.
Diclofenac is a compound for which a high variation in removal rates has been identified. Sludge age is likely to play an part in this (Reemtsma 2006). But no clear correlation between removal and operational factors could be concluded.
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. No significant removal for its metabolites was detected. In Italy, removal efficiencies of different pharmaceuticals in six different STPs were investigated. No significant removal of carbamazepine was detected in all STPs.

For carbamazepine, ibuprofen and diclofenac, the influence of temperature and sludge age on the removal efficiencies in different treatment systems was investigated (figure 3.5).

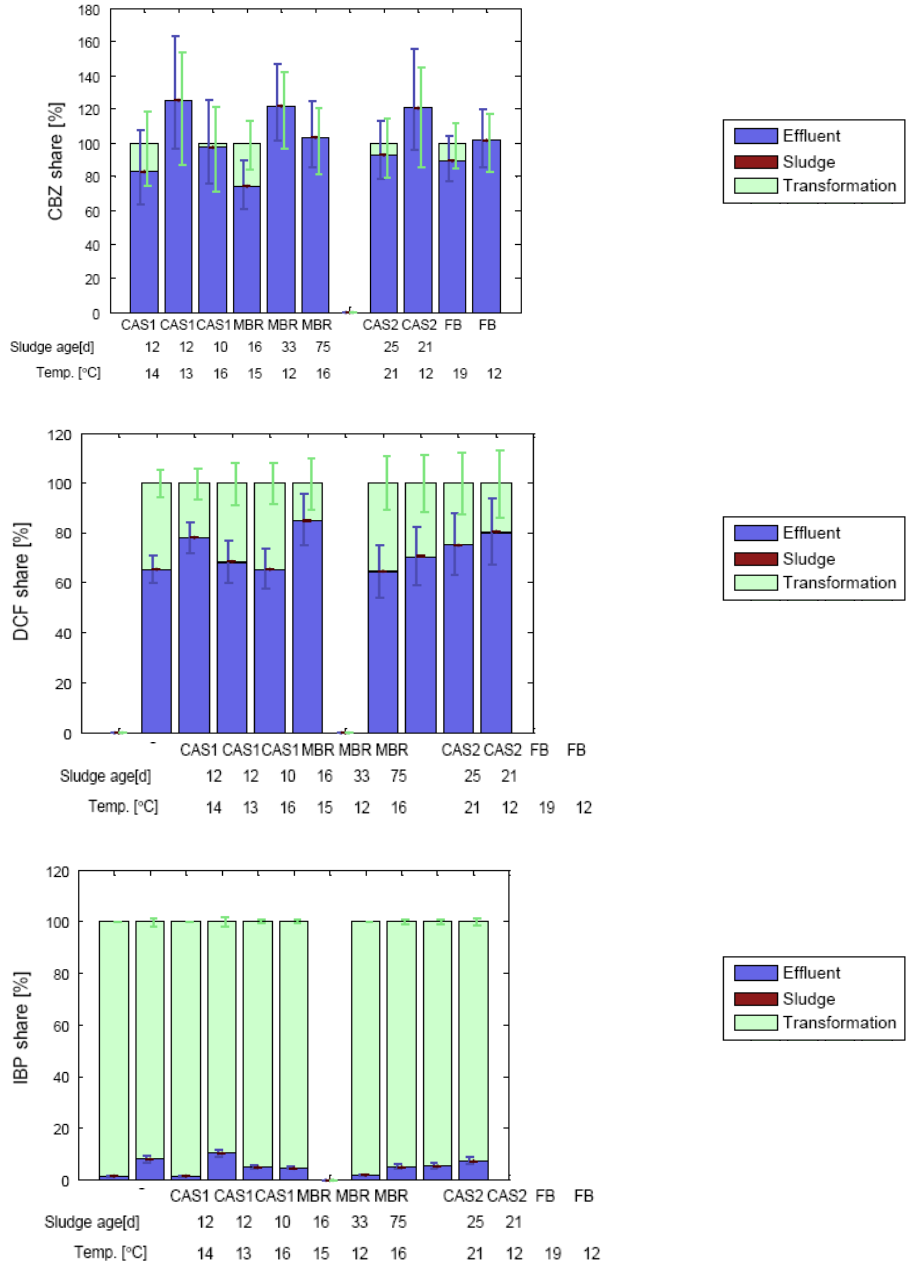


Figure 3.5. Removal of pharmaceutical compounds in full scale conventional activated sludge, membrane bioreactor and fixed bed reactor systems (Joss 2005); CBZ; carbamazepine, DCF; diclofenac, IBP; ibuprofen.

Clofibric acid is a metabolite of 3 pharmaceuticals: clofibrate, etofibrate and etofyllin clofibrate (Reemtsma 2006). It is poorly to moderately degraded in WWTPs. Removal percentages range from 0-51%.

Bezafibrate is partially removed in STPs. Ternes (1998) found a removal of 83%. In Strenn (2004) the removal varied between 0 and 97%.
Removal rates of fenofibrate are not available. However, fenofibrate can be transformed to fenofibric acid. Removal of fenofibric acid is partially (Joss *et al.* 2005).

The observed removal of pharmaceuticals is the result of several processes, mineralization is only one process.
Besides kinetics of the degradation of the pharmaceutical itself, also knowledge about the degradation of the metabolites of the pharmaceutical is important because they can be persistent and/or toxic as well. Quintana (2005) researched the mineralization of the pharmaceuticals: bezafibrate, diclofenac and ibuprofen in batch tests. The batch test consisted of synthetic wastewater and pharmaceuticals, operating with activated sludge. Within a timeframe of 28 days, bezafibrate is 100% transformed and 30% mineralized. Ibuprofen is for 96% mineralized and diclofenac is not mineralized at all.

Anaerobic digestion

Few information is available on the fate of pharmaceuticals in anaerobic systems. Two anaerobic pilot scale reactors, operated at mesophilic (37^0C) and thermophilic (55 oC) conditions were used to determine and assess the removal efficiencies of pharmaceuticals at different SRT (Carballa, Omil et al. 2007). The suspended solids concentrations was between 30-95 g/L. In this study of Carballa et al, (2007), some of the representative compounds were removed to some extent. For ibuprofen a medium removal (+/- 40%) for both reactors is measured. The removal efficiency of diclofenac was varying a lot between the different conditions. Removal was very low to quite high. SRT of 10 d gave the highest removal efficiency for ibuprofen and diclofenac. For all the other compounds, the SRT had no significant influence. For carbamazepine, no to very low removal was observed. (table 3.4) Temperature had in general no effect on the removal between mesophilic and thermophilic pilot reactors (Carballa, Omil et al. 2007).

Table 3.4: Removal of pharmaceuticals in anaerobic digestion of sludge. SA: 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%

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 3.5: 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.
In aquatic systems, ibuprofen is expected to be eliminated relative fast. Carbamazepine and clofibric acid will be quite persistent.
In Table 3.6 most of the reported removal rates in biological systems under various conditions of the selected pharmaceuticals are listed.

Table 3.6: Removal rates of pharmaceuticals alphabetical order.

Article	Compound	Removal rate*	Conditions and remarks
European Chemicals Bureau	Acetylsalicylic acid	98% aerobic >75 % anaerobic	Aerobic: over 28 days; Anaerobic: over 2 months readily biodegradable test (DOC measurement)
Joss et al., 2006	Acetylsalicylic acid	Between 20-90% (partial)	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Ternes et al., 1998	Acetylsalicylic acid	81%	Elimination in a municipal WWTP (also influence of rainfall is measured here, but not presented in this table)
Quintana et al., 2005	Bezafibrate	30% mineralization 100% transformation	Batch test with sludge of municipal WWTP; Concentration pharmaceutical 5 mg/l and added milk Time frame: 28 d Concentration pharmaceutical 20 mg/l & no additional C- source: no degradation
Ternes et al., 1998	Bezafibrate	83%	Elimination in a municipal WWTP flow rate: 60000m3/d
Joss et al., 2006	Bezafibrate	Between 20-90% (partial)	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Strenn et al., 2004	Bezafibrate	>90% lab 0%-97%	Lab scale experiments: activated sludge from WWTP, synthetic wastewater SRT: 4, 17 and 29d (conventional activated sludge system. HRT 2 d Full scale: several WWTP. SRT: 2 d - >40d Result: clear dependency on SRT
Ternes et al., 1998	Carbamazepine	7%	Elimination in a municipal WWTP flow rate: 60000m3/d
Joss et al., 2005	Carbamazepine	<10%	Municipal WWTP: CAS1: SRT 10-12 d and CAS2: 22 d Pilot MBR: SRT: 16,33,60 d HRT: 7.3 16.8, 13 respectively
Strenn et al., 2004	Carbamazepine	0% lab 0%	Lab scale experiments: activated sludge from WWTP, synthetic wastewater SRT: 4, 17 and 29d (conventional activated sludge system. HRT 2 d Full scale: several WWTP. SRT: 2 d - >40d Result: no dependency on SRT
Miao et al., 2005	Carbamazepine	29%	Removal in municipal WWTP HRT: 12-18 hr Aerobic treatment Metabolites not removed
Joss et al., 2006	Carbamazepine	<20% (no removal)	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Joss et al., 2006	Clofibric acid	Between 20-90% (partial)	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Ternes et al., 1998	Clofibric acid	51%	Elimination in a municipal WWTP flow rate: 60000m3/d
Zwiener et al., 2002	Clofibric acid	5% pilot WWTP and oxic BFR 30% anoxic BFR	Pilot WWTP (denitrification, activated sludge, sedimentation unit) 55hr Biofilm reactor oxic 48 hr

			Biofilm reactor anoxic 48hr
Tauxe et al., 2005	Clofibric acid	0 %	3 Municipal WWTPs with activated sludge HRT biological treatment: 9.3-15.9 hr and 7-9.7 hr and winter/summer samples
Strenn et al., 2004	Diclofenac	0% lab 0%, 39% 61%	Lab scale experiments: activated sludge from WWTP, synthetic wastewater SRT: 4, 17 and 29d (conventional activated sludge system. HRT 2 d Full scale: several WWTP. SRT: 2 d - >40d Result: no dependency on SRT
Quintana et al., 2005	Diclofenac	0 % mineralization	Batch test with sludge of municipal WWTP; Concentration pharmaceutical 5 mg/l and added milk Time frame: 28 d Concentration pharmaceutical 20 mg/l & no additional C- source: no degradation
Joss et al., 2006	Diclofenac	<20% (no removal)	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Tauxe et al., 2005	Diclofenac	0 %	3 Municipal WWTPs with activated sludge HRT biological treatment: 9.3-15.9 hr and 7-9.7 hr and winter/summer samples
Joss et al., 2005	Diclofenac	20-40%	Municipal WWTP: CAS1: SRT 10-12 d and CAS2: 22 d Pilot MBR: SRT: 16,33,60 d HRT: 7.3 16.8, 13 respectively
Kosjek et al., 2007	Diclofenac	49-59%	Pilot WWTP HRT: 48 hr; SRT: 15-25 d Aerobic treatment Operating time: 2 years
Zwiener et al., 2002	Diclofenac	<5% pilot and oxic BFR 35% anoxic	Pilot WWTP (denitritrification, activated sludge, sedimentation unit) 55hr Biofilm reactor oxic 48 hr Biofilm reactor anoxic 48hr
Ternes et al., 1998	Diclofenac	69%	Elimination in a municipal WWTP flow rate: 60000m3/d
Ternes et al., 1998	Ibuprofen	90%	Elimination in a municipal WWTP flow rate: 60000m3/d
Quintana et al., 2005	Ibuprofen	96% mineralization	Batch test with sludge of municipal WWTP; Concentration pharmaceutical 5 mg/l and added milk Time frame: 28 d Concentration pharmaceutical 20 mg/l & no additional C- source: no degradation
Kosjek et al., 2007	Ibuprofen	91%	Pilot WWTP HRT: 48 hr; SRT: 15-25 d Aerobic treatment Operating time: 2 years
Tauxe et al., 2005	Ibuprofen	10-79%	3 Municipal WWTPs with activated sludge HRT biological treatment and sedimentation tank: 9.3-15.9 hr and 7-9.7 hr and winter/summer samples 79%: summer, HRT 9.3-15.9 hr.
Strenn et al., 2004	Ibuprofen	>90% lab 12-86%	Lab scale experiments: activated sludge from WWTP, synthetic wastewater SRT: 4, 17 and 29d (conventional activated sludge system. HRT 2 d Full scale: several WWTP. SRT: 2 d - >40d
Joss et al., 2005	Ibuprofen	>90%	Municipal WWTP: CAS1: SRT 10-12 d and CAS2: 22 d Pilot MBR: SRT: 16,33,60 d HRT: 7.3 16.8, 13 respectively
Joss et al., 2006	Ibuprofen	>90%	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Zwiener et al., 2002	Ibuprofen	60% pilot 65% oxic BFR	Pilot WWTP (denitritrification, activated sludge, sedimentation unit): 55hr

		20% anoxic BFR	Biofilm reactor oxic 48 hr Biofilm reactor anoxic 48hr
Carballa et al., 2004	Ibuprofen	60-70%	Conventional activated sludge WWTP HRT: 24 hr
Joss et al., 2006	Fenofibric acid	Between 20-90% (partial)	Batch test, sludge from municipal WWTP CAS (including nitrification/denitrification) SRT: 11 d
Ternes et al., 1998	Fenofibric acid	64%	Elimination in a municipal WWTP flow rate: 60000m3/d
Ternes et al., 1998	Metroprolol	83%	Elimination in a municipal WWTP flow rate: 60000m3/d
Paxues, 2004	Metoprolol	<10%	Municipal WWTP

*only the parent compound, unless otherwise mentioned.

3.1.5 Removal of pharmaceuticals in source separated sanitation systems

In source separated sanitation, the pharmaceutical concentration is generally higher because the black water and urine streams in which the pharmaceuticals are present, are much more concentrated than conventional wastewater.

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) (Maurer 2006). Biological removal was tested in the study of (Escher 2006) but not elaborately. A removal efficiency of 0% for ibuprofen and propanol was observed in experiments with biomass (Escher 2006) .

Black water

Fate of pharmaceuticals during biological treatment of black water has not been researched much . Black water is usually (pre-)treated under anaerobic conditions. Therefore the fate of pharmaceuticals might be comparable with the ones observed in anaerobic digesters of municipal sludge. On the other hand the pharmaceutical concentration in black water is much higher than in sludge which can influence the removal rate. Further, the characteristics of the sludge in anaerobic digesters treating black water can differ much from the digested secondary and primary sludge.
The only information on the fate of organic micropollutants during anaerobic digestion of black water refer to estrogens (Mes 2007). Fate of the hormones estrogen (E1), 17α- ethynyloestradiol (EE2) was investigated during anaerobic treatment of blackwater. 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 and for E1 a half-live was determined at 42 d. Studied hormones are semi-hydrophobic. Based on above, low degradation rates of pharmaceuticals could be expected.

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

3.2.1 Aerobic conditions

The degradation of pharmaceuticals in batch tests was studied under aerobic conditions for many of the selected pharmaceuticals. Quintana (2005) (Quintana 2005) researched the mineralization of the pharmaceuticals: bezafibrate, diclofenac and ibuprofen in batch tests. The batch contained fresh activated sludge, a carbon source and pharmaceuticals. Within a timeframe of 28 days, bezafibrate

was transformed for 100% and 30% was mineralized. Ibuprofen was for 96% mineralized and diclofenac was not mineralized at all.

Joss (2005) (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°C (Joss 2005), n.a. not analysed

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

3.2.2 Anoxic conditions

Little is known on the fate of organic micro-pollutants 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).

4 Material and methods

In this part of the project the biodegradability tests for eight selected pharmaceutical compounds were performed under various process conditions. The following parameters were differentiated to get an insight into various processes during (concentrated) wastewater treatment. Three redox conditions were tested (aerobic, anoxic, anaerobic), three temperatures (10, 20 and 30°C) and different biological media (activated sludge from moderately loaded municipal WWTP, anaerobic sludge from digester treating concentrated black water).
A summary of five different experimental set-ups employing three red-ox potentials and temperatures is given in Table 4.1. The analysis of pharmaceuticals in wastewater and sludge samples is complex and it requires expertise. The samples have been analysed by the Dutch research institute RIVM Laboratory for Food and Residue Analysis.

Table 4.1: Summary of the different batch tests that were performed. The anaerobic and the aerobic test at 20ºC tests were repeated.

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

4.1 Predicted concentrations of pharmaceuticals in concentrated wastewaster streams

4.1.1 Urine

The calculated concentrations of pharmaceuticals in human urine (UC) were calculated from the Daily Defined Doses (DDD) and excretion rates 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). It was primarily assumed that everyone of the target group (e.g. residential district were the treatment is to be applied) uses the selected pharmaceuticals, that the undiluted urine is collected and its daily volume is 1.5 L/person.

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

Where:
V_{urine} = daily volume of urine produced (=1.5 L)
E_f = excretion fraction of the parent compound from the human body
The results of the calculations are shown in Table 4.2.

Table 4.2: Defined daily dosis (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)	Conc. used in biodegradation tests (motivate in the text) (mg/L)
Aspirin	3000	0.01	20.00	0.82	2
Diclofenac	100	0.05	3.33	0.30	0.3
Ibuprofen	1200	0.01	8.00	0.57	0.8
Carbamazepine	1000	0.02	13.33	0.05	0.9
Metoprolol	150	0.05	5.00	0.28	0.5
Clofibric acid	2000	0.06	80.00	0.001	0.8
Bezafibrate	600	0.5	200.00	0.04	2
Fenofibrate	200	0.14	18.67	0.00	2

Finally the used concentrations in the batch tests were $1/10^{th}$ of the calculated UC. This was due to the fact that in real-life situation urine is diluted and not all medicines are used chronically (e.g. diclofenac, ibuprofen) and not by all people of the target group. In the 5^{th} column of table 4.2, the calculated urine concentration is multiplied with the users fraction for the Netherland (CVZ, 2006). The selected concentrations for the tests are above these values. On the other hand 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.

Ternes (2006) proposed another formula to calculate a predicted urine concentration (PUC) for pharmaceuticals . This concerns undiluted urine as well.

$$\text{PUC} = \frac{A * E_f}{P * U * 365}$$

A = Amount of pharmaceutical sold per year (unit) (per country, district, unit?) .
P = number of inhabitants (country, city, residential district, number of resident, etc.)
U = produced urine per capita per day (=1 or 1.5L)

The equation takes already into account a fraction of user for a given medicine. Escher et al. (2006) computed this PUC for ibuprofen, carbamazepine and diclofenac for German situation and obtained respectively 2.7, 0.23 and 0.58 mg/L. These values are in the same range as used in the described here tests.
The fraction of excreted metabolism products (metabolites, conjugates) were not taken in this stage into account.

4.1.2 Black water

For black water the concentration will be a lower than in the urine because more flush water is used to transported combined waste. The dilution factor depends on the collection used (toilet). Vacuum toilets require 4.8-12 L of flush water a day. This means a dilution factor of 3-8 in relation to undiluted urine. 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 factor of 2-4. Considering these dilution factors and the uncertainty in UC, it seems plausible that degradation kinetics in the batch tests will also be valid for pharmaceuticals during 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 moderately loaded activated sludge treatment (nitrification, denitrification and biological phosphorus removal) and a secondary sedimentation tank (aeration circuit). Activated sludge samples for the aerobic and anoxic biodegradation batch tests were collected at the end of the circuit.
The sludge for the first anaerobic biodegradation test originated 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). Operational details of the treatments plants from which the sludge samples were taken are given in Table 4.3.

Table 4.3: Characteristics of the biological treatment systems, from which the sludge samples were taken. The black water used in the pilot reactor at Wetsus is the same as the one treated in Sneek.

Treatment plant	Bennekom[1]	Sneek	Wetsus
Reactor configuration	Moderately loaded aeration circuit	UASB septic tank	UASB tank
Type of waste water treated	Combined domestic sewage	Concentrated black water (vacuum toilets)	Concentrated black water (vacuum toilets)
Average flow rate (m^3/d)	3300	0.2	0.0059
HRT (d)	2	35	8-9
SRT (d)	40		220
ORL (kg COD/m^3/d)	0.396	0.4	0.07
Volume (of biological treatment tank) (m^3)	5700	7	0.05
Temperature (^oC)	ambient	35	25

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

4.4 Aerobic biodegradation experiment

4.4.1 Introduction

In the aerobic biodegradation experiment a mixture of pharmaceuticals was spiked to a volume of activated sludge mixture. The sludge was incubated and aerated at a constant temperature. During the experiment mixed liquor samples were taken; liquid and solid fraction were separated from each other to determine the concentration of the pharmaceuticals in both phases. From the declined amount of considered compounds the biodegraded fraction was determined as well as process kinetics.

4.4.2 Set-up of the experiment

Experiments were performed at two temperatures of 20°C (twice) and at 10°C.
Each test consisted of:

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

Table 4.4 gives an overview of the volumes of media added to the different batches. The experiment of 20°C has been performed twice under the same conditions because the sampling method was improved after the first aerobic tests (improved preservation of the samples and improved sampling of the solid phase). The 2nd aerobic test at 20°C and the aerobic test at 10°C were prolonged to 30 d 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	Methanol solution with a mixture of pharmaceuticals (ml)	Millipore Water (L)	Sludge (L)
Biodegradation test	0.5	0	1
Control	0.5	1	0

The activated sludge from WWTP Bennekom was aerated a few hours prior to start of the experiment to deplete the remaining carbon sources in the activated sludge and to bring the mixture to the 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 was ensured. During the experiment pH, T and O_2 measurements were regularly performed. Especially at 20°C water evaporates quickly. 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 foil to prevent photolytic degradation (if any).

4.4.3 Stock solution of PhAC

A stock solution of selected pharmaceuticals was prepared in 50 ml of methanol. A 0.5 ml of this concentrated stock solution was spiked to the batches for obtaining the desired concentration of each of 8 PhAC. The required and expected concentrations (based on calculated and exact weights of the substances respectively) of the eight pharmaceuticals in the batch experiments are given in Table 4.5.

Table 4.5: Required and expected concentrations of pharmaceuticals in all 3 aerobic batch tests. Expected concentrations are based on the added amount of pharmaceuticals to the solution.

Pharmaceutical	Required concentration in the batches (mg/L)	Expected concentration in the batches 20^0C (1^{st} test) (mg/L) AER-20-1	Expected concentration in the batches 20^0C (2^{nd} test) (mg/L) AER-20-2	Expected concentration in the batches 10^0C (mg/L) AER-10
acetyl salicylic acid	2	2.058	2.005	2.004
diclofenac	0.3	0.298	0.321	0.327
ibuprofen	0.8	0.808	0.819	0.818
carbamazepine	0.9	0.913	0.912	0.898
metoprolol	0.5	0.507	0.51	0.523
clofibric acid	0.8	0.814	0.794	0.805
bezafibrate	2	1.933	1.989	2.024
fenofibrate	2	2.040	1.960	2.005

4.4.4 Sampling intervals

The duration of the experiments for determining the biodegradation kinetics in AER-20-1 was set to 2 days. This duration refers to the maximum HRT in a conventional waste water treatment plant. Time intervals at which samples from that test were taken were: t_0 = 0 hr; t_1 = 0.5 hr, t_2 = 1 hr, $t_{3.}$ = 3 hr, t_4 = 20 hr, t_5 = 48 hr.

To assess a possible sludge adaptation or a utilization of (especially persistent) pharmaceutical compounds under stress conditions (no co-substrate supplied, long retention), the latter two experiments (AER-20-2 and AER-10) were prolonged to 30 days. During this period samples were taken in week 1, 2 and 4. The controls were sampled at 0 hr and t = 48 hr in all tests. Additionally controls from AER-20-1 and AER-10 were sampled at t=30 days.

4.5 Anoxic tests

4.5.1 Introduction

In the anoxic biodegradation experiment a mixture of pharmaceuticals was spiked to the activated sludge subjected to anoxic conditions. 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.

4.5.2 Set-up of the experiment

The anoxic experiments were performed at 20°C and 10°C (ANOX-20 and ANOX-10). Each experiment consisted of:

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

Table 4.3 gives an overview of the volumes of the substances and media added to the different bottles.

Table 4.6: Overview of the volumes of added media and solutions in the anoxic biodegradation experiment

Batch	Methanol solution with a mixture of pharmaceuticals (ml)	$NaNO_3$ solution (ml)	Millipore Water (L)	Sludge (L)
Biodegradation test	0.5	0.5 (final [N-NO_3]: 20 mg/L)	0	0.5
Control	0.5	0.5 final [N-NO_3]: 20 mg/L)	0.5	0

The activated sludge was taken municipal WWTP in Bennekom a day prior starting an experiment. The oxygen in a liquid and gas phase was depleted prior the 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 the beginning and the end of the experiment (t=0 and t=2 d for ANOX-10 and t=0, 2d and 30 d for ANOX-20). 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 20-40 mg/l N-NO_3. Nitrate concentration in the liquid was followed in time. When all nitrate was almost denitrified, an appropriate volume of $NaNO_3$ solution was added again to obtain a required NO_3 level in the remained volume.
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 re-dox potential, temperature and pH measurements were regularly performed.

4.5.3 Stock solution of pharmaceuticals

A mixture of pharmaceuticals was prepared in 50 ml of methanol like in the aerobic test. A 0.5 ml of this concentrated stock solution was spiked to the batches for obtaining the desired concentration of each compound. The required and expected (based on calculated and exact weights respectively) concentrations of the eight pharmaceuticals in the batch experiments are given in Table 4.7.

Table 4.7: Required and expected concentrations of pharmaceuticals in all 4 batch tests. Expected concentrations are based on the added amount of pharmaceuticals to the stock solution.

Pharmaceutical	Required concentration in the batches (mg/L)	Expected concentration in the batches 20^0C (mg/L) ANOX-20	Expected concentration in the batches 10^0C (mg/L) ANOX-10
acetyl salicylic acid	2	2.004	2.012
diclofenac	0.3	0.324	0.384
ibuprofen	0.8	0.826	0.838
carbamazepine	0.9	0.900	0.926
metoprolol	0.5	0,504	0.524
clofibric acid	0.8	0.796	0.808
bezafibrate	2	2.008	2.016
fenofibrate	2	1.984	1.992

4.5.4 Sampling intervals

The duration of the experiments for determining the anoxic biodegradation kinetics was primarily set at 2 days (ANOX-10). The time intervals at which samples were taken: t_0 = 0 h; t_1 = 1 h, t_2 = 3 h, t_3 = 20 h, t_4 = 48 h. To assess a possible sludge adaptation or a utilization of (especially persistent) pharmaceutical compounds under stress conditions (no co-substrate supplied, long test duration), the 20°C experiment was prolonged to 30 days. During this period samples the samples were taken in week 1, 2 and 4. The controls were sampled at t=0 hr, t=48 hr for ANOX-1- and additionally at t=30 days for ANOX-20.

4.6 Anaerobic tests

4.6.1 Introduction

In the anaerobic biodegradation experiment a mixture of pharmaceuticals was spiked to the anaerobic (mesophilic) sludge of two different origin see 3.3.2). The batches were incubated under anaerobic conditions at constant temperature of 30 °C and shaken continuously. Biodegradation of pharmaceuticals under anaerobic conditions is expected to be lower compared to aerobic and anoxic conditions. A relative high temperature of 30 °C was chosen for this test to observe the maximum biodegradation potential under these beneficial conditions. From the batches, liquid and solid samples were taken to analyze the concentration of the pharmaceuticals in both phases over time.

4.6.2 Set-up of the experiment

The anaerobic experiment, performed at 30 °C, consisted of:

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

This experiment has been repeated with this set-up for the same reasons as in the aerobic test at 20 °C: improved sampling of solid phase and sample preservation. In Table 4.8 an overview of the volumes of the substances and media used for the both experiments is given.

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	Methanol solution with a mixture of pharmaceuticals (ml)	(Millipore) Water (mL)	Sludge (mL)	Total volume (mL)
Biodegradation (1st test) ANAER-1	0.5	100	300	400
Control (1st test)	0.5	400	0	400
Biodegradation (2st test) ANAER-2	0.5	0	500	500
Control (2st test)	0.5	500	0	500

In the first anaerobic test (ANAER-1), the sludge was taken from the anaerobic pilot reactor treating concentrated black water (WETSUS, Leeuwarden, the Netherlands); the second test was performed with sludge from the anaerobic digester treating (the same) black water in Sneek (the Netherlands) – ANAER-2. 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 foil to prevent photolytic degradation (if any). After sampling, the gas phase of the bottles was flushed with nitrogen.

4.6.3 Stock solution of pharmaceuticals

A fresh concentrated solution of pharmaceuticals was prepared in 50 ml of methanol as in the other batch tests. A 0.5 ml of this stock solution was spiked to the batches to obtain the desired concentration of each pharmaceutical compound. The required and expected concentrations of the eight pharmaceuticals in the batch experiments are given in Table 4.9.

Table 4.9: Required and expected concentrations of pharmaceuticals in all batches. Expected concentrations are based on the added amount of pharmaceuticals to the stock solution of pharmaceuticals.

Pharmaceutical	Required concentration in the batches (mg/L)	Expected concentration in the batches (mg/L) (ANAER-1)	Expected concentration in the batches (mg/L) (ANAER-2)
Acetyl salicylic acid	2	2.000	2.040
diclofenac	0.3	0.300	0.448
ibuprofen	0.8	0.765	0.818
carbamazepine	0.9	1.000	0.976
metoprolol	0.5	0.523	0.500
clofibric acid	0.8	0.788	0.802
bezafibrate	2	2.008	1.968
fenofibrate	2	2.013	2.064

4.6.4 Sampling intervals

In both anaerobic tests a sampling period of about 30 days was applied 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 that test were t_0 = 0 h; $t_{1,}$ = 3 h; 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 = 30d) liquid samples were taken.
In addition to assess a possible sludge adaptation or a utilization of (especially persistent) pharmaceutical compounds under stress conditions (no substrate supplied), the first experiment was prolonged to 77 days. During that 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 d.

4.7 Analytical method

4.7.1 Sampling

A 30 mL mixed liquor samples were taken with a plastic syringe. For the anoxic tests also a long sampling needle was used. By flushing with nitrogen, 30 ml of liquid was taken out of the bottle in the anoxic test. To the 30 ml of sludge mixture sample 4-5 drops of chloroform were added with a Pasteur pipette. This step was not taken in the first aerobic test at 20°C (AER-20-1) and the first anaerobic test (ANAER-1).
Subsequently, the samples were centrifuged for 10 min at 4000 rpm / 2800 rpf using the centrifuge FirlabO SW12R (with rotor type FACENSW12001) and IEC thermo CL31R (with rotor type AC 100.10A).
After centrifuging, the solid (4 ml) and liquid phase (20ml) were separated. For the aerobic tests 20°C and the first anaerobic test, non-disposable centrifuge tubes were used. The solid phase had to be replaced therefore after centrifuging. Transferring solid was enabled using a little amount of demi-water. In AER-20-1 and ANAER-1 and 2, not all liquid from the solid sample could be removed. The sampling of the solid phase was later improved by removing all the remained liquid after centrifuging. In the anoxic tests and in the ANAER-2, centrifuging has been done with disposable centrifuge tubes (PP-Test tubes 50ml, CELLSTAR). Also for the aerobic experiments these tubes were used during the extended time period of the experiment (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 and stored directly.

4.7.2 Samples preservation

Chloroform was added to stop any microbial activity after sampling. In the samples without chloroform (the first aerobic and anaerobic tests), activity of bacteria was observed. By addition of chloroform this is attempted to be prevented. An additional test was performed by RIVM on the effect on chloroform in the samples (see appendix), showing the positive effect of using chloroform.
All samples were immediately stored in the freezer (-75 °C). The series of samples were sent for analysis to RIVM (ARO-CRL) during and after finishing the experiment.

4.7.3 Analysis of other parameters

VS and TS concentration are measured according to standard method. Nitrate concentration was analyzed with Dr. de Lange method using kits for measuring range of $N\text{-}NO_3$ concentration of 5 to 35 mg/l. The pH, O_2 and ORP are measured with appropriate electrodes.

4.7.4 Materials

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

4.7.5 Apparatus

Liquid chromatography (LC): Waters Chromatography Acquity UPLC separation module. Column: Acquity UPLC BEH C_{18} 1.7 µm (100 * 2.1 mm ID). Column temperature was 65°C. The LC mobile phase consisted of a mixture of 0.1 percent acetic acid (solution A) and acetonitrile (100%). The gradient used was linear, started at 10% B and progressed to 30% B in 3 minutes after which it was increased to 100% B in 6 minutes . After 9 min the mobile phase was kept for 2 min at 100% B, then the percentage B was decreased to 10 percent in 0.01 minute . The mobile phase flow was set at 0.4 ml min^{-1}. The injection volume was 20 µl.
Mass-spectrometer (MS) analysis was carried out on a Waters-Micromass Ultima Platinum. Depending on a compound the measurement was carried out in a 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°C and desolvation temperature: 325°C. 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°C and desolvation temperature: 325°C. 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. In Table 4.2. MRM transitions are given for the measured pharmaceuticals.

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

4.7.6 Sample Clean-up

Sample clean-up of the liquids was straightforward. The samples were 10 times diluted in LC-eluents 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 sludge 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°C. The dried sample was reconstituted in one millilitre of eluens A followed by 10 minutes ultrasonification.

4.7.7 Calibration curves

To correct for losses due to sample storage and to correct for signal suppression due to matrix compounds the calibration curves were 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.

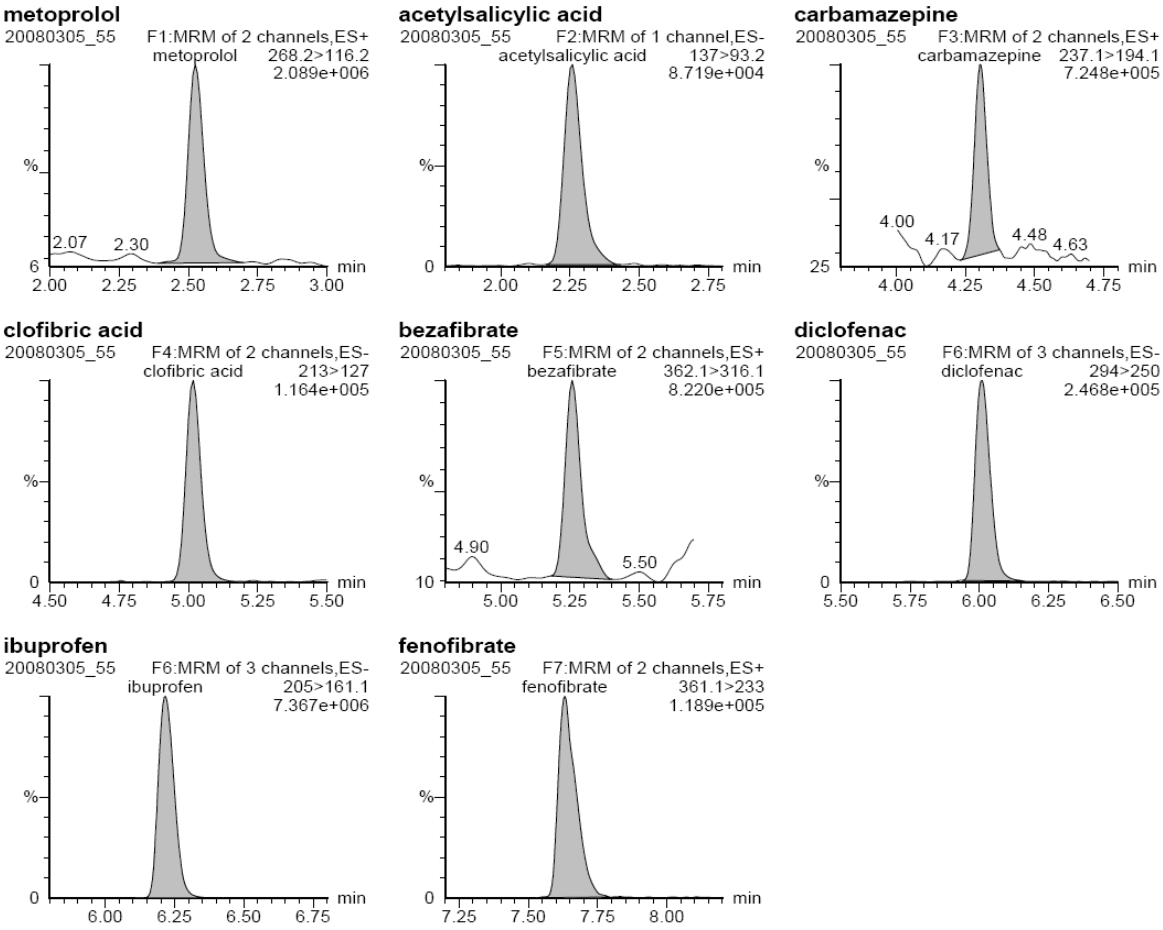


Figure 4.1: Reversed phase microbore LC-ESI MSMS profiles of an anaerobe sample spiked (5 ng/ml) with a mixture pharmaceuticals

4.7.8 Calculations used for biodegradation tests

The equations used to assess the biodegradation and sorption of selected pharmaceuticals are 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 the formule:

$$C_{t,i} = C_{l,i} + C_{s,i} = C_{l,i} + X_i TS$$

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*, $K_{d,i}$ was calculated with the formula:

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

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

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

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

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/hr or L/gTS/d)
k_i = biological degradation constant of pharmaceutical i (1/h or 1/d).
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:

$$C_i(t) = C_i(0) * e^{-k_{biol,i} * TS * t} \quad \text{and} \quad C_i(t) = C_i(0) \cdot e^{-k_{biol,i} * t}$$

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

$k_2 = k_1 * e\ \kappa * (T_2-T_1)$
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 (-).

5 Results of biodegradation batch experiments

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) and oxidation reduction potential (ORP, mV), volatile solids (VS, g/L)) and total solids (TS, g/L). The measurement procedures and equipment applied were described in chapter 4. The list of the controlled parameters and their values in all tests is given in Table 5.1. (all parameters were controlled in the beginning and the end of experiment, some also during the test).

Table 5.1: Operational conditions during all performed biodegradation tests; DO= dissolved oxygen, VS= volatile solids, TS= total solids, ORP= oxidation reduction potential. Duplicates are marked 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)			**ORP (mV)**			
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	13.2	68			

	II	12.0	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°C (ANAER-30-1)			**ORP (mV)**			
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°C (ANAER-30-2)			**ORP (mV)**			
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°C were finally performed at 18-19 ^{0}C. The lower temperature of a duplicate in the first aerobic test after 2 days was 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°C) were over the first 2 days around 10^{0}C, after this the temperature in the cooling system increased to 12^{0}C. Moreover, the cooling system has been broken for 1 week during this period so temperature was then not controlled, which means the bottles' contents 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). After 30 days, the pH was rather low (no buffer was added to the medium) for the aerobic test at 20 and 10 °C both. Biological activity of the sludge is likely retarded at such pH values After 30 days the VS and TS concentration decreased significantly as no substrate was added (endogenous respiration).

The anoxic tests were performed at slightly higher temperatures as originally planned: 12 (instead of 10) and 22-23 (instead of 20) °C. For the anoxic 10°C test, the same cooling system was used as for the aerobic 10°C test. In these tests the temperature was not controlled for about 1 week between the 2-30 days period of the experiment.
The pH during the experiment was close to neutral or higher. 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)..
The ORP indicated the presence of anoxic conditions in the first 2 days of both anoxic tests. After 15 days, probably some oxygen diffused into the system because the redox potential became higher. Still denitrification can take place at these higher ORP (Hong, 1998).
The nitrate concentration at the start of the test was approximately 40 mg/L N-NO_3. A concentrated $NaNO_3$ 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.
The anaerobic experiments were finally performed at 29 °C instead of planned 30°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, perhaps due to diffusion of some oxygen to the test bottles.

5.2 Background concentrations of pharmaceuticals in sludge mixtures

To assess the contribution of the background concentration of the sewage sludge mixture to the total measured concentration in the batches, as well as to acquaint information on the occurrence of selected compounds in the (effluent of) wastewater treatment systems, these sludge samples have been analysed for the presence of the selected compounds. The concentration of pharmaceuticals in the effluent of the activated sludge treatment tank of municipal WWTP Bennekom are shown in Figure 5.1.

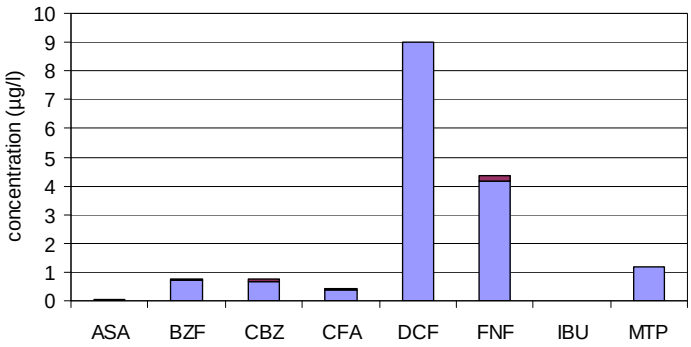


Figure 5.1: Background concentration of selected pharmaceuticals in activated sludge mixture of municipal WWTP Bennekom. Liquid and solid fraction are distinguished; the darker color indicates pharmaceutical compounds in solid fraction. Presented data is obtained from three activated sludge samples taken in January and February 2008.

All selected pharmaceuticals were detected in the activated sludge, except for ibuprofen. Diclofenac was present in relatively high concentration. Presence of fenofibrate was unexpected as 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.
The graphs shows 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.

In the anaerobic sludge obtained from pilot-scale UASB and from the demonstration scale UASB septic tank treating concentrated black water, the pharmaceutical concentrations were much higher (up to 150 µg/L). Ibuprofen, metoprolol, diclofenac and acetylsalicylic acid were present in the highest concentration. Figure 5.2 and 5.3 present the background concentrations for the pilot UASB and demonstration UASB septic tank respectively. These higher concentrations confirmed expectations as the mentioned reactors treat only concentrated (vacuum toilets) black water. Moreover, the expected (based on literature findings) removal efficiency of pharmaceuticals compounds in the anaerobic systems is also lower (proof ASA and IBU).

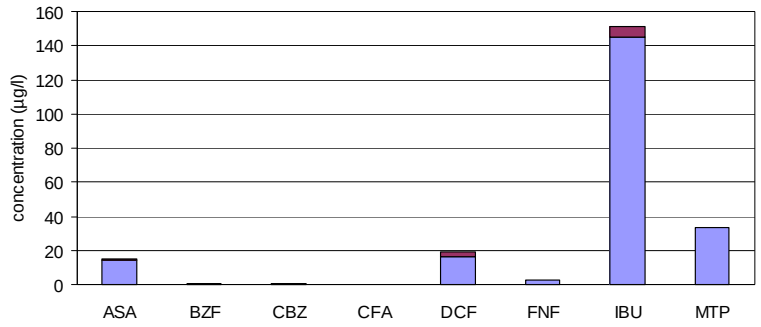


Figure 5.2. Background concentration of pharmaceuticals in anaerobic sludge sampled from pilot-scale UASB reactor fed with concentrated black water investigated in Leeuwarden, the Netherlands (de Graaff et al., 2008). Liquid and solid fraction are distinguished; the darker color indicates pharmaceutical compounds in solid fraction.

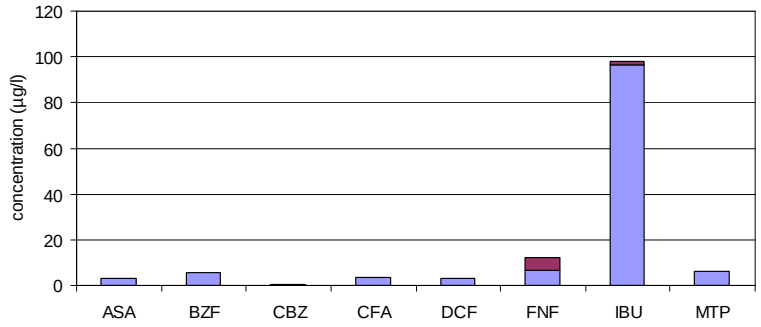


Figure 5.3: Background concentration of pharmaceuticals in anaerobic sludge obtained from demonstration-scale UASB septic tank treating concentrated black water (the same as the pilot-scale UASB, Leeuwarden) in Sneek, the Netherlands. Liquid and solid fraction are distinguished; the darker color indicates pharmaceutical compounds in solid fraction.

In graphs 5.1-5.3, the pharmaceutical concentration result from both, liquid and solid phase. All graphs show the prevailed pharmaceutical concentration in the liquid phase.

5.3 (Bio)degradation in aerobic batch tests

The aerobic batch tests were performed twice at 20°C and once at 10 °C. The mentioned temperatures were target temperatures; in real they varied between 16.3 to 19.8°C and 10.0 up to 12.8°C respectively. For simplicity however they will be referred in the text as 10 and 20°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). 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 hydraulic retention time (HRT) in a conventional municipal WWTP (HRT of 2 d). The sampling was continued up to 30 days (but less frequent) to determine whether some persistent pharmaceuticals would be eliminated when bacteria are subjected to stress

conditions (no other external carbon source added) and longer exposed to a given compound (adaptation).

The results of the aerobic tests are given in the figures 5.4-5.12. The graphs show the total pharmaceutical concentration consisting of the sum of the pharmaceutical concentration in water and solid phase in the batch tests with sludge (so sorption is taken into account). Also the concentrations of pharmaceuticals in the controls (without sludge) are plotted. The detection limit of the pharmaceutical concentration in the liquid phase was 0.005 µg/l and 0.005 ng/gTS 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 h (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.
Surprisingly the initial concentration of ASA expected to be 2.0 mg/L (at t=0) was never obtained in controls and the test bottles. As significantly lower concentration of ASA in test bottles could be attributed to fast sorption to sludge and its fast/direct degradation, this can not explain in the control bottles.

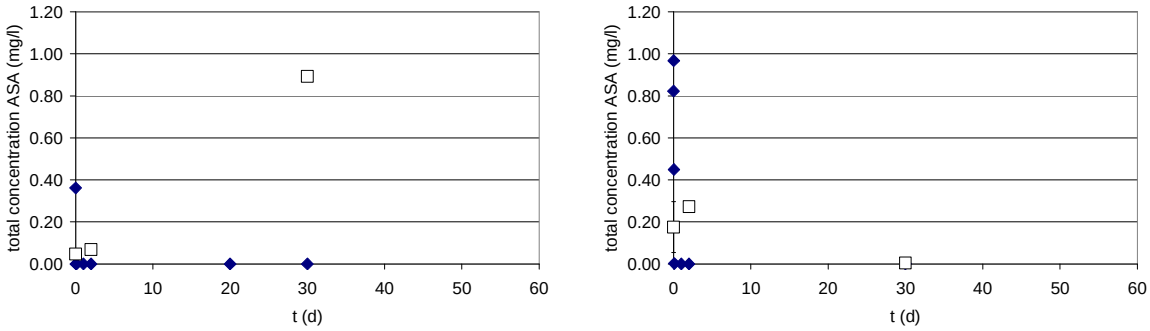


Figure 5.4: Total concentration of acetylsalicylic acid (ASA) in time in AER-20-2 (left) and AER-10 (right). (♦ with sludge, □ without sludge)

A fast/immediate decrease in concentration of fenofibrate (FNF) was observed (Figure 5.5). Both tests at 20°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°C a disappearance of FNF in the biodegradation test and in the control was measured as well. For this reason it is uncertain which part of the FNF reduction was due to biological activity and which part was caused by abiotic reactions. The initial concentration of FNF in controls and test bottles was expected to be 2 mg/L; this concentration could not be measured in any of these tests. Surprisingly initial concentrations in test bottles (with sludge) were higher than in controls.
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.

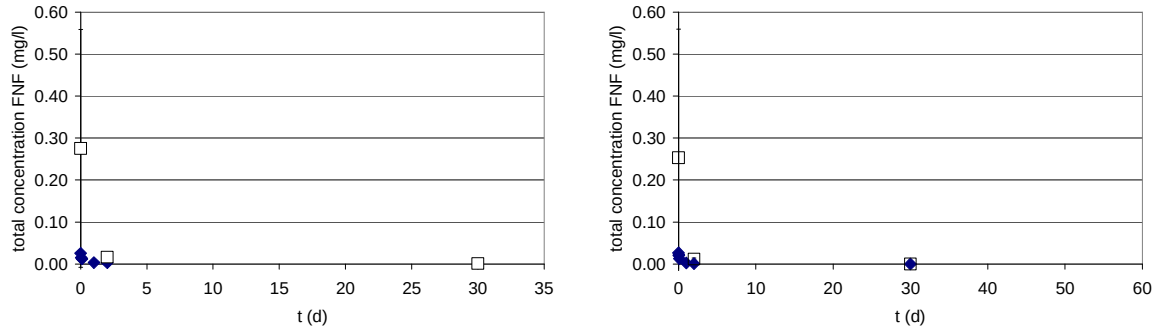


Figure 5.5: Total concentration of fenofibrate (FNF) in time in the aerobic batch test at 20o (AER-20-1, left) and at 10°C (AER-10, right). (♦ with sludge, □ without sludge)

Within 2 days ibuprofen (IBU) was effectively eliminated to concentrations under or close to the detection limit (Figure 5-6). 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°C compared to both tests performed at 20°C.
The biodegradation of IBU confirm literature findings. Removal of IBU higher than 90% 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/L) was not completely confirmed in controls, but the values were close, especially in AER-10; in AER-20-2 there was a significant lost of IBU in test bottles, probably due to sorption and poor extraction in the analytical method.

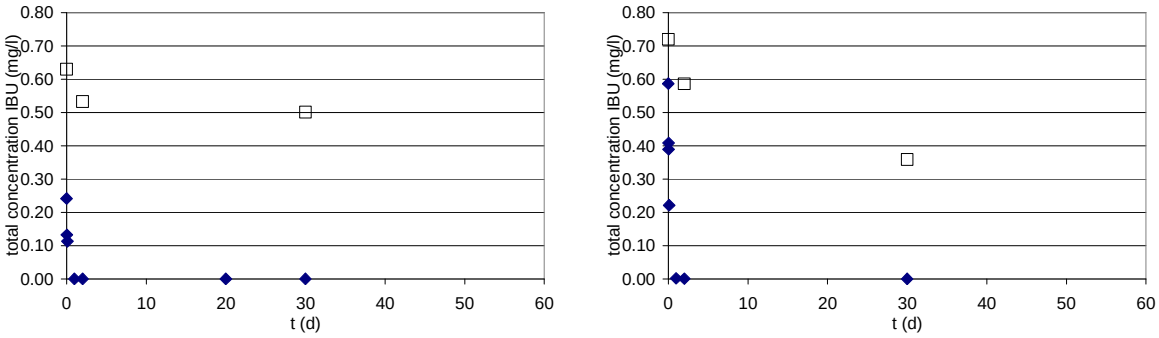


Figure 5.6: Total concentration of ibuprofen (IBU) in the aerobic test at 20°C (AER-20-2, left) and 10° (AER-10, right). (♦ with sludge, □ without sludge)

Metoprolol (MTP) was eliminated also exponentially (Figure 5.7). Compared to IBU the concentration decreased at a slower rate. In both tests at 20°C, the pharmaceutical was eliminated to concentrations under the detection limit within 2 days. In the AER-10 a 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 relatively strong sorption and insufficient recovery; a very rapid biodegradation is not expected in case of MTP.

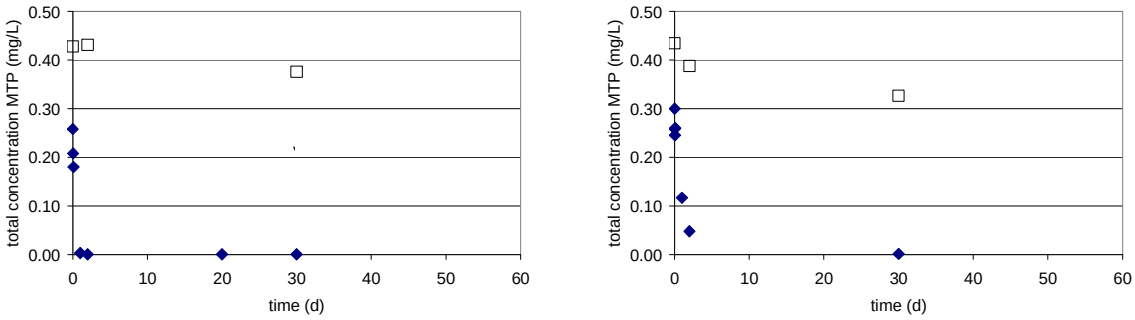


Figure 5.7: Concentration of metoprolol (MTP) in the aerobic test at 20oC (AER-20-2, left) and 10o (AER-10, right). (♦ with sludge, □ without sludge)

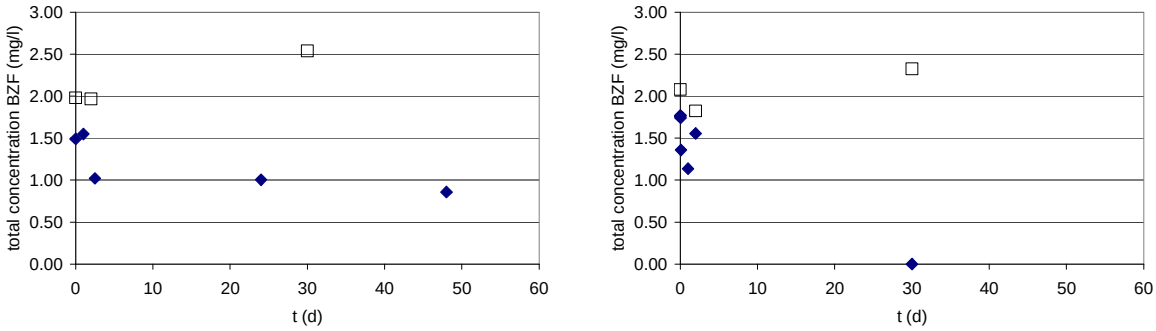


Figure 5.8: Concentration bezafibrate (BZF) in time in the aerobic test at 20 ^{o}C (AER-20-2, left) and at 10^{o}C (AER-10, right). (♦ with sludge, □ without sludge)

Bezafibrate (BZF) was removed less efficiently than previously described compounds (Figure 5.8). The AER-20-1 and AER-20-2 tests showed an inconsistent decrease in BZF concentration after 2 days, of 15% and 40% respectively. In the AER-10 test the decrease of BZF concentration was less significant. After 30 days the BZF in all aerobic tests was under the detection limit. This showed that BZF can be eventually also biodegraded at lower temperatures. The concentration in the controls stayed more or less constant although the standard deviation of the concentration in the controls of AER-10 test was quite high. After 30 days concentration of BZF decreased in AER-20-2 and increased in AER-10, which is not really consistent.

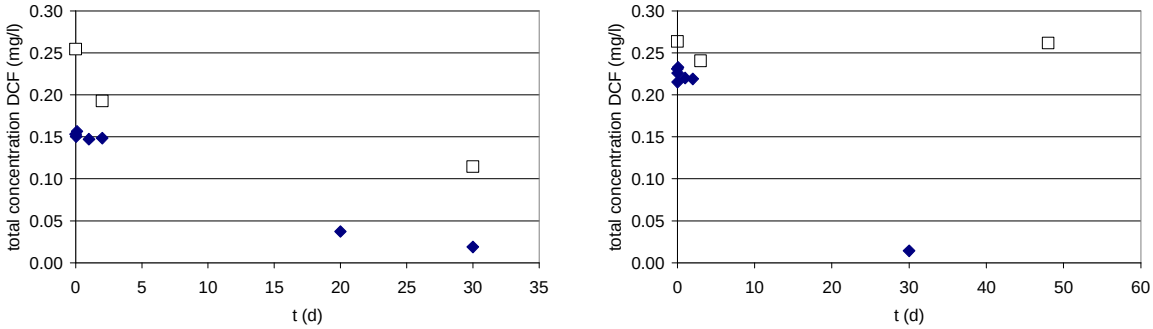


Figure 5.9: Concentration diclofenac (DCF) in time in the aerobic test at 20 ^{o}C (AER-20-2, left) and at 10^{o}C (AER-10, right). (♦ with sludge, □ without sludge)

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. 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. At this moment it seems that DCF can be potentially eliminated in biological systems.

The fate of DCF in controls was not consistent (in AER-20-2 decrease of DCF and in AER-10 it remained stable) indicating that other processes than biodegradation could have also played a role. The causes of the decrease in AER-20-2 are, besides the possibility of measuring errors, unknown.

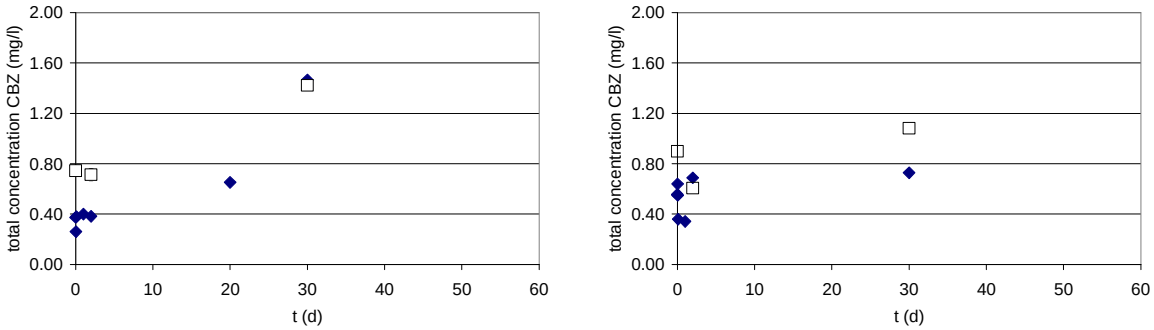


Figure 5.10. Concentration carbamazepine (CBZ) in time in the aerobic test at 20 °C (AER-20-2, left) and at 10°C (AER-10, right). (♦ with sludge, □ without sludge)

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 (stronger at higher temperature). This increase can be explained by a fast sorption of CBZ in the beginning of the experiment (the difference between a control and a test bottle was significant) and than its desorption due to aging (decay, changing of structure of activated sludge enabling a better extraction of a considered compound in the analytical method) of the activated sludge.

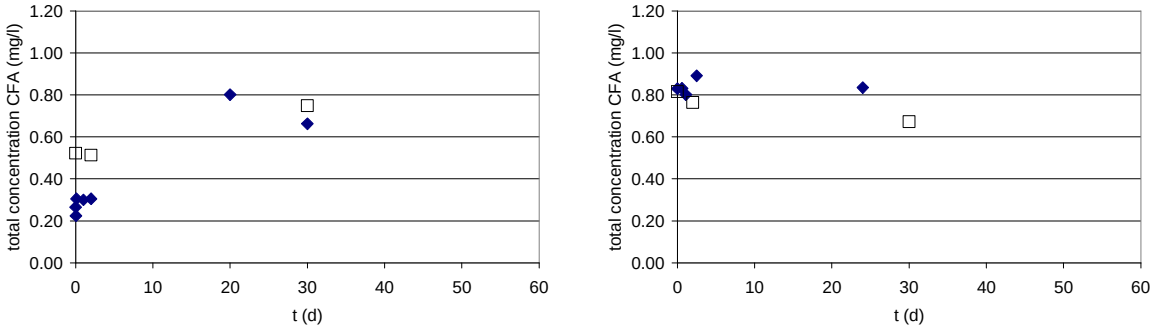


Figure 5.11. Concentration clofibric acid (CFA) in time in the Aerobic test at 20°C (AER-20-2, left) and at 10°C (AER-10, right). (♦ with sludge, □ without sludge)

The course of concentration of clofibric acid (CFA) during the 30 days lasting test is similar to CBZ (Figure 5-11). No decrease in concentration of CBZ was observed over the entire duration of the test. On the other hand the concentration in the AER-20-2 increased significantly, which could be caused

by a strong sorption of a compound in the beginning and than its desorption due to a long test duration and changes in activated sludge structure enabling a better extraction of the compound in the analytical method.

Altogether, the aerobic tests showed an exponential decrease in concentration of ASA, FNF, IBU and MTP. This observed biotransformation is conform literature findings. The pharmaceuticals BZF and DCF are not or only to a limited extent eliminated within 2 days of the test. After 30 days, their concentration was reduced to a large extent, showing the slow but possible biodegradation of these two compounds. Besides a slow degradation rate, also an adaptation phase could have been required before degradation of a specific compound could take place or stress conditions caused an ultimate reduction of certain compounds.
Literature also indicates the persistency of DCF to biodegradation. For BZF the elimination after 2 days was expected to be higher since amongst others (Ternes 1998) observed 83% removal of BZF in a municipal WWTP. The CBZ and CFA are the pharmaceuticals which did not show any biodegradability in all three tests. Apparently they are persistent to biodegradation. This observation is according to literature, which reported no removal for both compounds in aerobic wastewater treatment. For CFA however, also higher removal efficiencies, up to 51%, were found in literature.

5.4 (Bio)degradation in anoxic batch tests

In figures 5.12 to 5-19, the results of the anoxic biodegradation tests performed at target temperatures of 10 and 20 °C are plotted. The mentioned temperatures were target temperatures; in real they varied between 21.5 to even 23.0 °C and 11.8 up to 13.9°C respectively. For simplicity however they will be still referred in the text as 10 and 20°C
The batch test at 10 °C (ANOX-10) was performed over a time period of 2 days while the test at 20°C (ANOX-20) over 30 days.
ASA disappeared completely in both tests; within 48 hour it was under the detection limits. The decrease in concentration of ASA was faster at 20°C than at 10°C. 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 initial concentration of ASA of 2.0 mg/L. The initial concentration of ASA in test ANOX-20 was much lower, while in ANOX-10 close to expected.

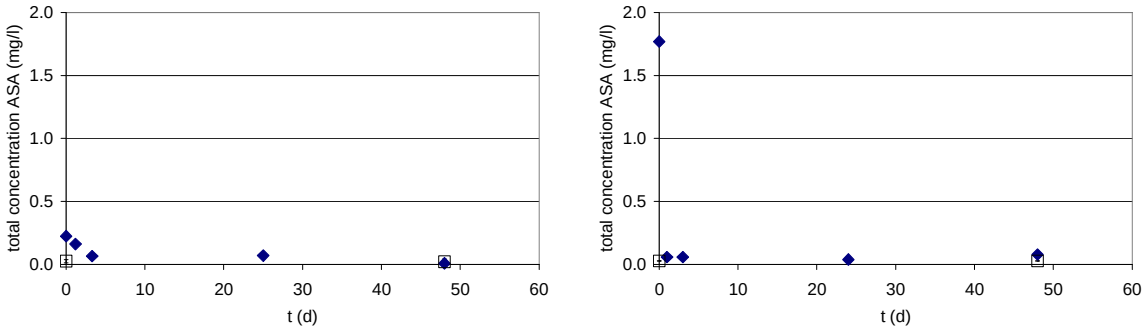


Figure 5.12: Total concentration of aspirin (ASA) in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

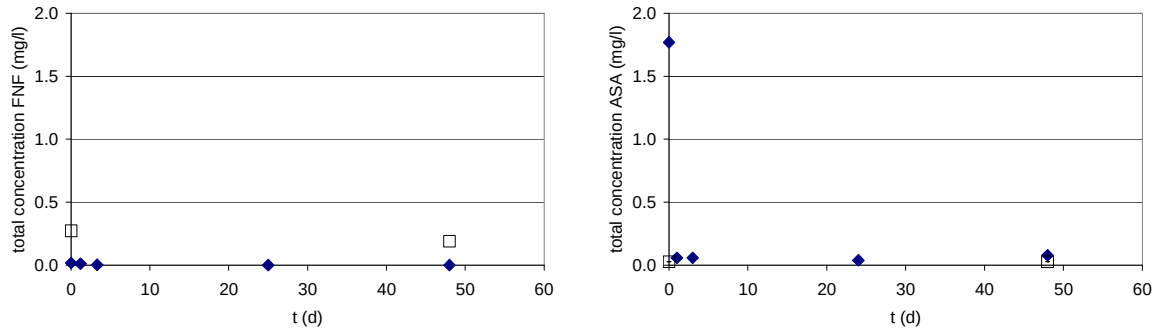


Figure 5.13. Total concentration of fenofibrate (FNF) in time in the anoxic test at 20 °C (ANOX-20, left) and 10°C (ANOX-10, right) batch tests (♦ with sludge, □ without sludge).

At anoxic conditions FNF was eliminated relatively fast like in the aerobic tests. Differences in transformation rate between different temperatures were not significant. Other tests showed a decrease in FNF concentration in controls, but not the anoxic ones, although the measured concentration was a way far from expected one (0.1- 0.2 mg/L against 2 mg/L respectively). A relative stability of the FNF concentration in the controls, show that a biological activity played a role in the disappearance/degradation of FNF.

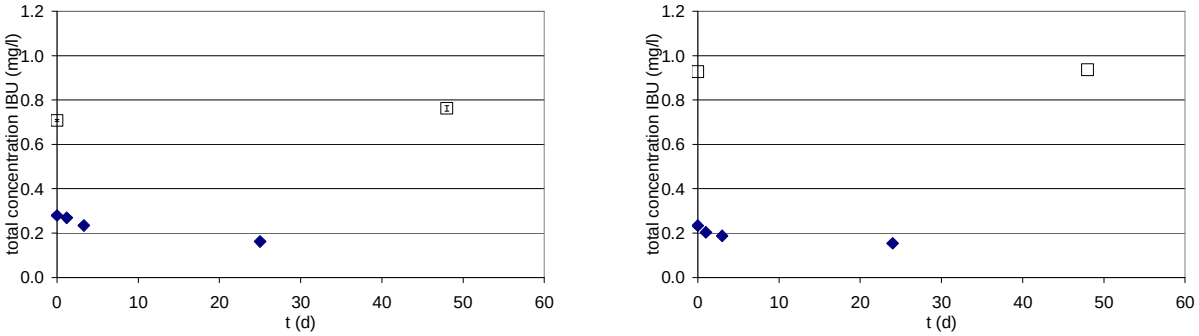


Figure 5.14. Total concentration of ibuprofen (IBU) in time in the anoxic test at 10 °C (ANOX-10, left) and 20°C (ANOX-20, right) batch tests (♦ with sludge, □ without sludge)

Ibuprofen (IBU) was removed in the anoxic tests but slower than at higher oxidation-reduction potentials. Furthermore, a large variation between the duplicates was observed (52% vs. 97% respectively). Both removal efficiencies were higher than reported 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°C was measured, as 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 disappearance of IBU at 20°C could not have been attributed to biodegradation only.
The overall removal rate of IBU under anoxic conditions might increase when taking a longer adaptation time for biomass. This was shown in the research of (Suarez Martinez 2008). 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 2008).

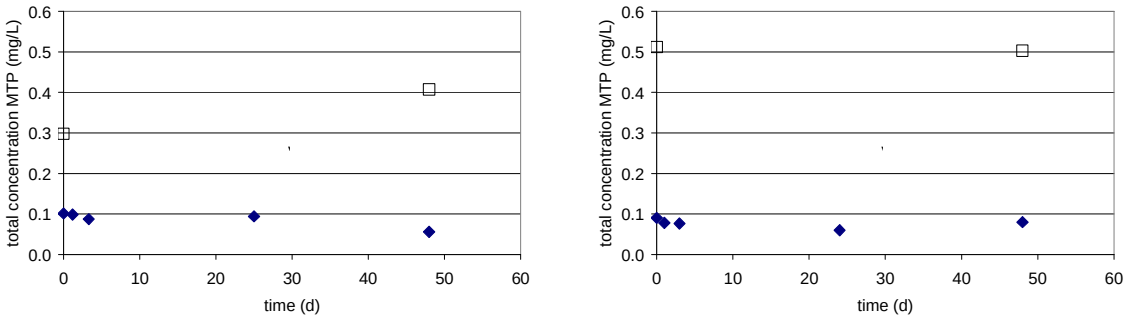


Figure 5.15. Total concentration of metoprolol (MTP) in time in the anoxic test at 10 °C (ANOX-10, left) and 20°C (ANOX-20, right) batch tests (♦ with sludge, □ without sludge)

In contrary to aerobic tests, metoprolol (MTP) was only eliminated to a small extent within 48 hours (Figure 5.15). At 20°C, MTP tended to decrease in concentration 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 ANOX-20.

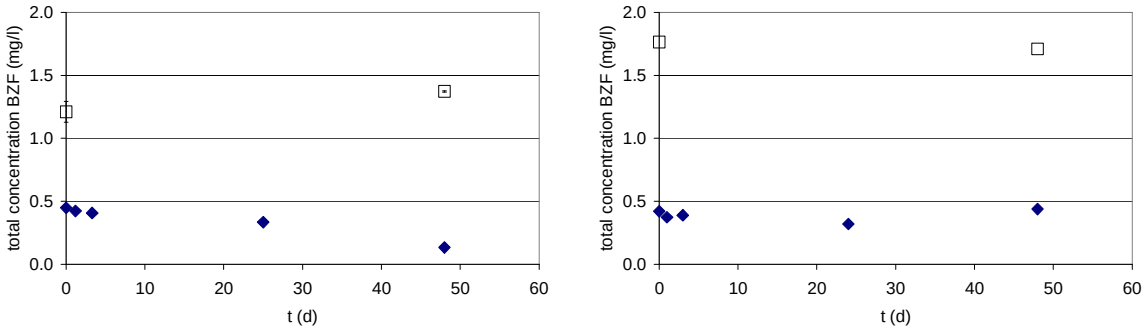


Figure 5.16. Total concentration of bezafibrate (BZF) in time in the anoxic batch test at 10 °C (ANOX-10, left) and 20°C (ANOX-20, right) batch tests (♦ with sludge, □ without sludge)

In the ANOX-20 a significant elimination of BZF was observed (about 70% reduction). On the other hand, BZF was not decreased in concentration in the ANOX-10 test; a clear difference thus between both anoxic tests (Figure 5.16). After 30 days, the BZF concentration was close and under the detection limit in the ANOX-20 (duplicates).
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 BZF concentrations in the controls were close to the expected (added) values – 2 mg/L. In the bottles with sludge this initial concentration was however significantly lower, indicating a probable fast sorption of BZF onto the sludge but also its insufficient recovery from sludge in analytical method.

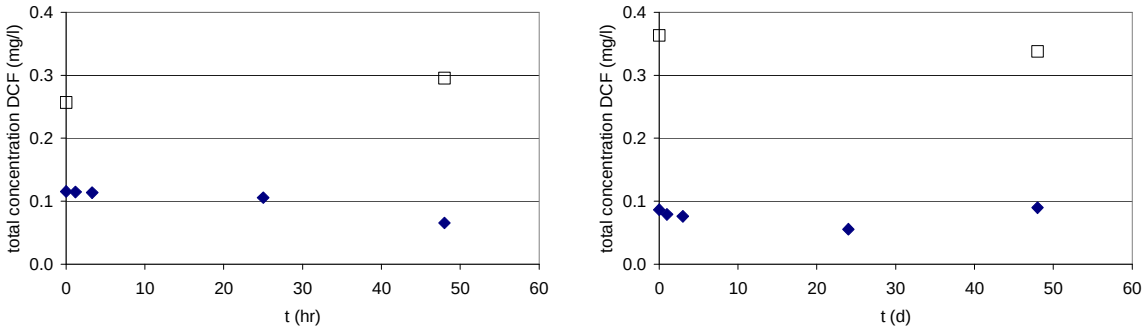


Figure 5.17: Total concentration of diclofenac (DCF) in time in the anoxic test at 10 °C (ANOX-10, left) and 20°C (ANOX-20, right) batch tests (♦ with sludge, □ without sludge)

At a temperature of 10°C, diclofenac (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 demanded concentration of 0.3 mg/L, while in the tests this concentration was much lower. The latter would indicate again a strong sorption of DCF and its insufficient recovery from the sludge in the analytical method.

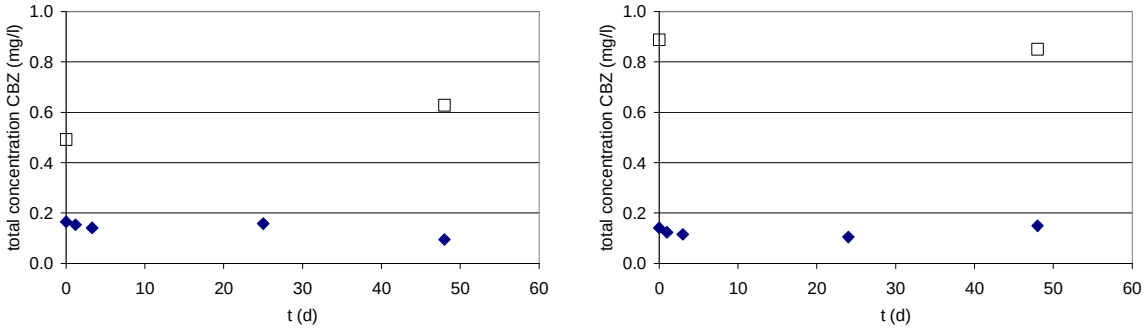


Figure 5.18. Total concentration of carbamazepine (CBZ) in time in the anoxic test at 10 °C (ANOX-10, left) and 20°C (ANOX-20, right) batch tests (♦ with sludge, □ without sludge)

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 (Figure 5.18). The difference between the initial concentrations and the tests were again significant, indicating (the most probably) a strong sorption of a compound in the beginning of the contact time. Long duration of the experiment resulted in the desorption of the compound, but certainly not its degradation.

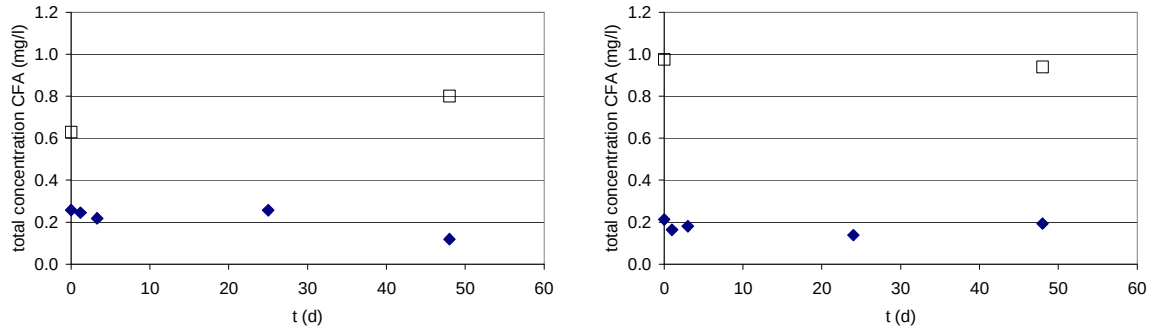


Figure 5.19. Total concentration of clofibric acid (CFA) in time in the anoxic test at 10 °C (ANOX-10, left) and 20°C (ANOX-20, right) batch tests (♦ with sludge, □ without sludge)

For clofibric acid (CFA) the same was observed as for CBZ: no removal of this pharmaceutical under anoxic conditions and a increase in measured concentration after 1 month (Figure 5.19). On the other hand the initial concentrations of CFA in controls were almost as expected, while in the beginning of the tests with sludge these concentrations were significantly lower. This would indicate again a strong sorption of CFA onto the sludge in the beginning of the biodegradation test and then its desorption at the end of the test due to change in the structure of sludge and stress conditions.
Summarizing, the pharmaceuticals, which showed to be (partly) degradable under aerobic conditions showed a lower degradation rate under anoxic conditions, with the exception of BZF in anoxic test at 20°C. The lower biotransformation rate is as expected since organic compounds are faster degraded under aerobic conditions than under anoxic ones. The reason for the higher rate for BZF at anoxic conditions is unclear. Next to this, ASA, IBU, MTP and BZF showed a different degradation rate between the tests at 20° and 10°C. For MTP and BZF this temperature difference resulted in a small removal at 20°C and no significant removal at 10°C within 48 hours.
After 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-aerophilic conditions); this increase of ORP could have influenced this degradation positively. The ORP is not likely to have affected the differences in both temperature tests; the ORP of the 10 and 20°C tests were similar. In addition sorption seems to be underestimated indicating that extraction of the considered compound from the sludge is not always optimal.
In general, in the anoxic tests the control concentrations stayed constant in the time interval in which pharmaceutical concentrations decreased. Elimination of pharmaceuticals in anoxic tests is therefore most likely a result of biotransformation/sorption processes.

5.5 (Bio)degradation in anaerobic batch tests

The anaerobic experiments were performed twice at a temperature of 30°C. The mentioned temperature was a target temperature; in real it varied between 28.5 to 29.5 °C. For simplicity however it will be still referred in the text as 30°C.
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 from the liquid phase. On the other hand initial concentrations in the controls and tests were much lower than expected, and they reached zero after duration of the experiment indication any other processed than biological degradation. Again, measured and expected initial concentrations of ASA and FNF in controls and tests differed significantly from each other.

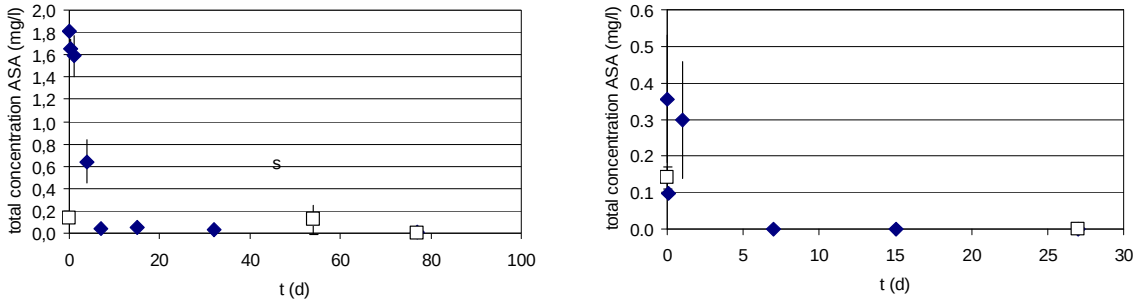


Figure 5.20: Aspirin (ASA) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

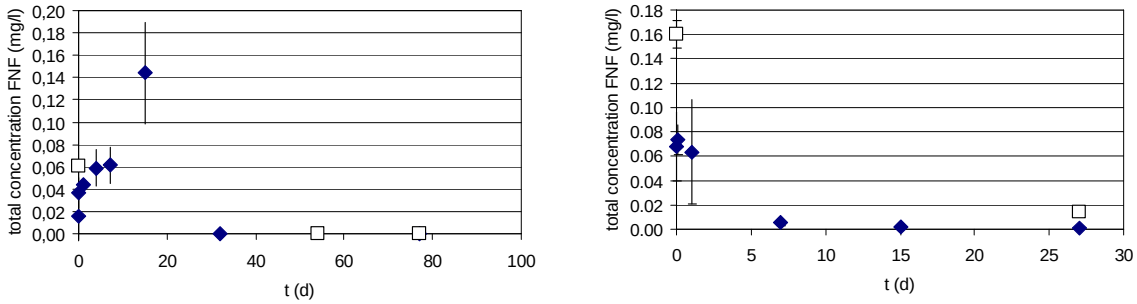


Figure 5.21: Fenofibrate (FNF) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

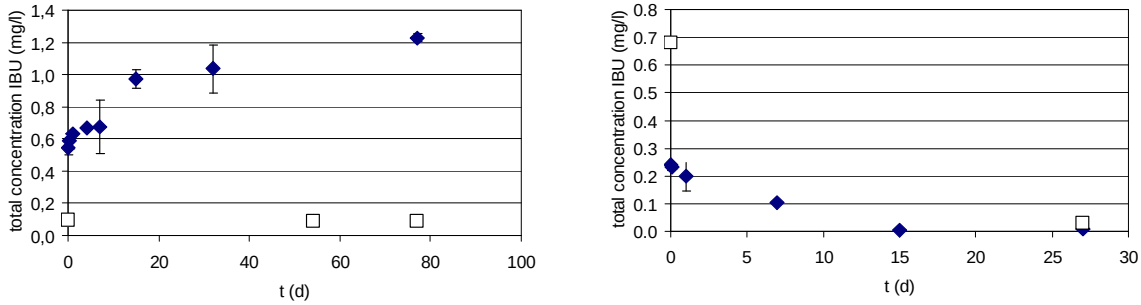


Figure 5.22: Ibuprofen (IBU) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

In the ANAER-2, ASA and FNF were 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 unclear, it can not be explained by the difference in anaerobic sludge characteristics. 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. However, IBU concentration in ANAER-2 test decreases in both, controls and tests, making the distinction between sorption and biodegradation impossible.
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, ASA, FNF, IBU decreased by more than 90%.
Unfortunately for the eliminated pharmaceuticals, also the control concentration decreased in both tests. In ANAER-2 this was for ASA, IBU and FNF >99%, 95% and 90% respectively. The decrease in concentration in the biodegradation tests, can therefore not be only assigned to biodegradation processes. For ASA and FNF the decrease in concentration in the controls was also present in the aerobic tests and slightly in the ANOX-20 test for IBU after continuation of the tests to 30 days.

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.
A more careful look to the results of ASA and FNF reveals that for these compounds the expected initial concentration was very different in all batch tests in contrary to the other selected pharmaceuticals. The much lower measured concentration can be due to a fast transformation or sorption of the substances. Perhaps a higher operational temperature (30°C) played here also an important role. It could also be a matter of improper mixing at the start, but than the same phenomena could have been observed for all other pharmaceuticals, what was not the case.

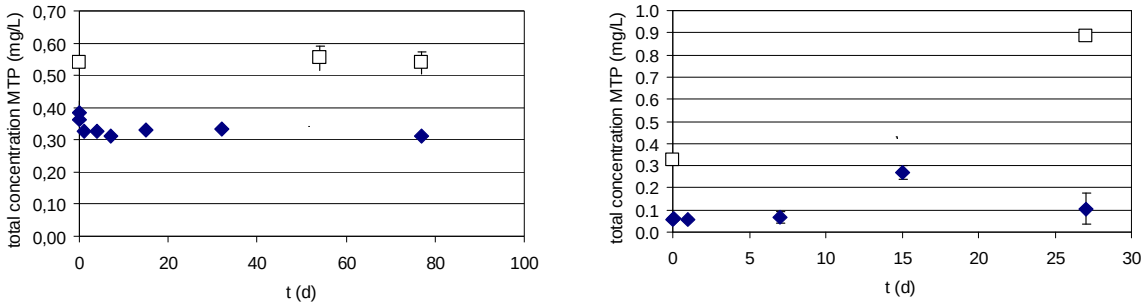


Figure 5.23: Metoprolol (MTP) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

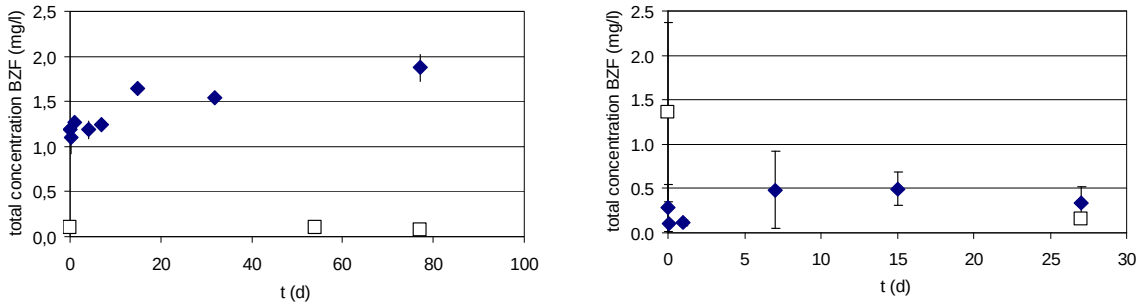


Figure 5.24: Bezafibrate (BZF) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

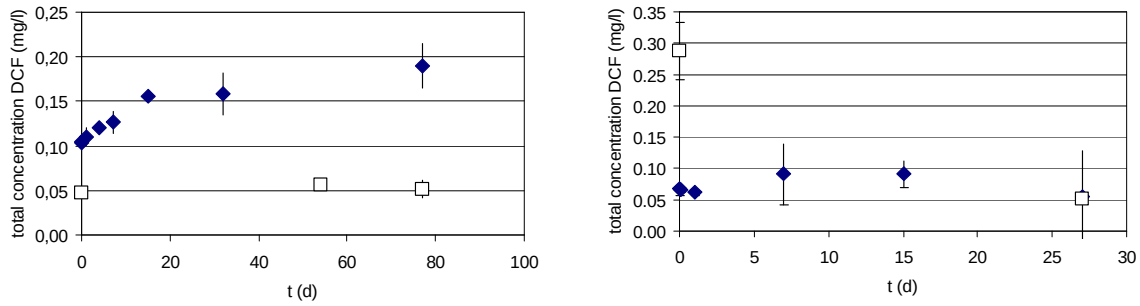


Figure 5.25: Diclofenac (DCF) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

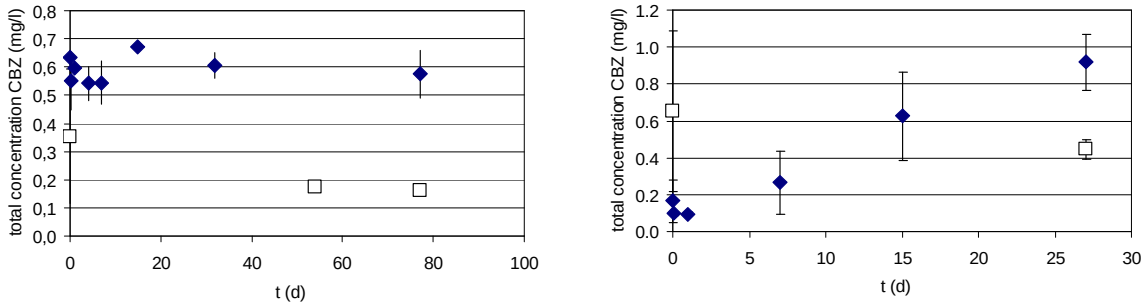


Figure 5.26. Carbamazepine (CBZ) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

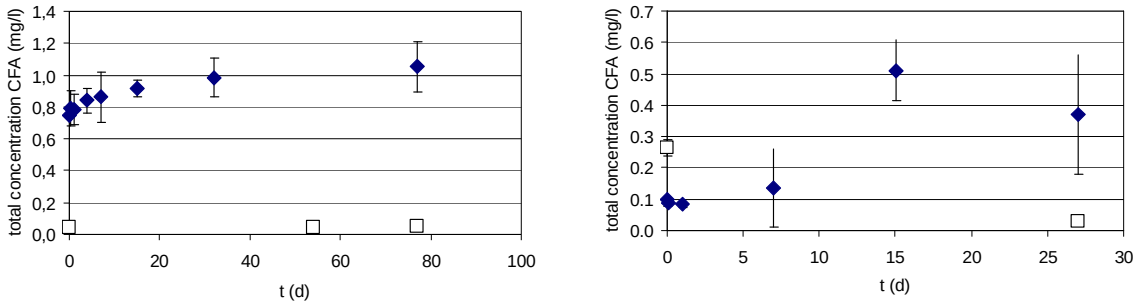


Figure 5.27: Clofbric acid (CFA) concentration in time of the anaerobic experiments ANAER-1 (left) and ANAER-2 (right) (♦ with sludge, □ without sludge).

For MTP, BZF, DCF, CBZ and CFA, no decrease in concentrations 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 significant decrease in controls was measured and a constant concentration or even increase in the batches with sludge.
For CBZ and CFA a high increase in concentration in the batches was measured in ANAER-2. Difference between concentrations and controls would indicate that a strong sorption took place and in the course of the test desorption. The end concentrations in some of the tests with CBZ and CFA are similar to the initial (demanded) concentrations in controls. Another possibility would be the presence of conjugated pharmaceuticals in the anaerobic sludge. Then deconjugation of the conjugates of the mentioned compounds, already present in the used sludge, during the treatment could have also taken place. However, since the increase is very high, this seems not be likely to explain the whole difference.

(Carballa, Omil et al. 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 general, the anaerobic samples seem to be 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/removed than under aerobic and anoxic conditions.

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.
A more careful look to the results of ASA and FNF show that for these compounds the expected initial concentration was very different in all batch tests in contrary to the other selected pharmaceuticals. The much lower measured concentration can be due to a fast transformation or sorption of the substances. For FNF absorbance to glass and other material used can be an important factor to contribute to this effect because of its highly hydrophobic character. It could also be a matter of improper mixing at the start, but than the same phenomena could have been observed for all other pharmaceuticals, what was not the case.
Another explanation would be that in the stock solution both pharmaceuticals were already present in lower amounts than expected (e.g. weighting error, sorption to glassware, hydrolysis). Some stock solutions have been analysed for there pharmaceutical concentration. From the stock solution used in the AER-20-2 test, it turns out that the concentration ASA is indeed lower than expected (0.033 mg/L instead of 2 mg/L) but that FNF concentration is comparable with the expected concentration (1.8 mg/L instead of 2 m/L).
The batch tests showed a clear potential for some pharmaceuticals to be biotransformed (in some cases significantly). The degradation rate for the pharmaceuticals differed per compound. Under various environmental conditions different rates were obtained. The exponential elimination rates (degradation kinetics) is discussed in the following section.
The continuation of the most of the batch tests for 30 days provided more information. The, at the first sight, persistent DCF was eliminated for 90% in the aerobic tests after 1 month. The pharmaceuticals partially eliminated during 2 days, like MTP and BZF, were completely removed to levels under or close to the detection limit of 0.005 ug/l when the aerobic and anoxic test was prolonged to 30 days.
It should be kept in mind that only the removal of the original pharmaceutical was analyzed. Whether a pharmaceutical degraded/mineralised and if the subsequent produced metabolites are degraded is at this moment unclear. Regarding IBU and ASA the produced metabolites are not likely to be persistent to biodegradation. According to results of (Quintana 2005) the metabolites of BZF are also degradable. The possible produced metabolite fenofibric acid of FNF can be transformed most likely too although not much is known about other metabolites produced. The biodegradability of metabolites of MTP and DCF are unknown.

5.6 Assessment of biodegradation kinetics

The results, in which exponential decrease of pharmaceuticals in the course of a given test was obtained, were used to calculate, with a pseudo first-order reaction rate, the degradation rate constant (k) and the specific biological degradation rate (k_{biol}) according to equations in chapter 2. 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 values is attempted to be expressed. However in this interval deviation total solids concentration is not included. The influence of the TS concentration on the k_{biol} is estimated by using an average TS concentration of both duplicates in a given batch tests. The specific degradation constant is calculated based on the 95% confidence interval of the k_{biol} calculated for both TS concentrations.
The degradation rate of FNF and ASA was also determined, but as in all the tests it was demonstrated that their removal is due to biological processes, the assessed values may therefore represent the disappearance rate as indicated in the table. Moreover, because of the fast decrease of ASA in the tests, obtained reduction curves could not be fitted. In this case, where possible, k-values for ASA have been 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'.

Obviously, the kinetics in the aerobic, anoxic and anaerobic tests differed. Comparing the aerobic and the anaerobic tests, the degradation rates were 20 to 200 higher for ASA, FNF and IBU for aerobic conditions. A difference between aerobic and anoxic tests was of a factor 2 to 4 for IBU and BZF respectively. For other compounds, no exponential curve could be fitted, so no comparison could be made between the different rates.

For aerobic conditions, the literature values are reported for some of the selected pharmaceutical. In this research the specific degradation rate were lower than these found in literature. The differences between these experiments and the experiments reported in literature is the high concentration of pharmaceuticals. Also in (Mes and 2007) in where the biodegradation kinetics of estrogens in concentrated waste streams was investigated, lower kinetic constants were reported compared to those observed when using lower pharmaceutical concentration (as in sewage). The high concentration and the mixture of pharmaceuticals perhaps inhibit the activity of bacteria to a certain extent. The activated sludge used in this research was also not adapted to such high concentrations of pharmaceutical compounds. It is indicated in literature (Joss, Zabczynski et al. 2006) that co-metabolism may enhance the degradation of persistent micro-pollutants. As in the biodegradation test no external (easily biodegradable) substrate was added, the biodegradation proceeded slower or did not proceed.
The often observed differences between controls and test concentrations, indicating strong sorption of the certain compounds to the sludge (if only), causes that the assessed kinetic parameters are indicative.

Table 5.2: The degradation rate constant k, its 95% confidence interval, the range of specific degradation rate constant kbiol based on the TS concentration and related the 95% confidence interval and the R2 of the regression model for tested pharmaceuticals under various environmental conditions (assessed where possible)

Pharmaceutical	Test	k-value (1/d)	95% confidence interval of k		R^2	k_{biol} (L/gTS/d) range
Acetylsalicylic acid	AER-20-1	104	103	106	0.986	25.5 -26.4
Acetylsalicylic acid (best scenario)	AER-20-2	218	217	219	0.999	37.3 -43.9
Acetylsalicylic acid (best scenario)	AER-10	74	72	76	0.830	15.9 -17.5
Acetylsalicylic acid	ANAER-2	1.9	1.3	1.4	0.932	0.111- 0.127*
Bezafibrate	AER-20-1	0.24	0.22	0.24	0.960	0.054 - 0.060
Bezafibrate	AER-20-2	0.19	0.19	0.22	0.871	0.038 - 0.043
Bezafibrate	ANOX-20	0.58	0.55	0.58	0.922	0.111 - 0.120
Fenofibrate	AER-20-2	22.0	21.8	22.3	0.960	3.74 - 4.46*
Fenofibrate	ANAER-2	0.38	0.36	0.40	0.930	0.031- 0.035*
Ibuprofen	AER-20-1	5.6	5.4	5.9	0.980	1.47 - 1.35
Ibuprofen	AER-20-2	5.2	5.1	5.4	0.937	0.874 - 1.07
Ibuprofen	AER-10	4.4	4.3	4.6	0.900	0.952 - 1.06
Ibuprofen	ANOX-10	0.9	0.8	0.9	0.903	0.103 - 0.119
Ibuprofen	ANAER-2	0.29	0.28	0.30	0.942	0.024 - 0.026
Metoprolol	AER-20-1	3.46	3.4	3.6	0.963	0.840 - 0.887
Metoprolol	AER-20-2	3.38	3.3	3.5	0.954	0.569 - 0.691
Metoprolol	AER-10	0.86	0.86	0.89	0.980	0.192 - 0.205

* the specific *disappearance/degradation* rate constant, since it is not elucidated that the elimination is due to biological processes.

Next to variation between aerobic, anoxic and anaerobic 'response', the differences in kinetics in relation to the operational temperature were also observed. The temperature coefficient κ of the Arrhenius equation was calculated for MTP, IBU and ASA (Table 5.3). The κ is expected to be in the range of 0.03-0.09 for these compounds (Ternes 2006).

Table 5.3: The influence of temperature on biodegradation rate. A κ (coefficient, see eq. 3.5) was calculated based on kbiol range of AER-20-1, AER-20-2 and the AER-10 results.

Pharmaceutical	Test results	κ (-)
Metoprolol	AER-20-1 / AER-10	0.17-0.16
	AER-20-2 / AER-10	0.14-0.11
Acetylsalicylic acid	AER-20-1 / AER-10	0.06-0.04
	AER-20-2 / AER-10	0.11-0.08
Ibuprofen	AER-20-1 / AER-10	0.03-0.05
	AER-20-2 / AER-10	No sig. difference

Ibuprofen showed not a significant decrease in biodegradation rate when temperature decreased from 20 to 10°C. Comparing two tests: AER-20-1 and AER-10 gives κ- values (two k-values) ranging from 0.03-0.05 which is similar to the expected range.
Kinetics of MTP resulted in the highest temperature coefficient. Still, it was similar to the expected range. For ASA the κ value was in the range as reported in literature.

5.7 Sorption onto the sludge

This section elaborates the sorption behavior of the selected pharmaceuticals in the biodegradation tests. In all samples, the concentration of pharmaceutical compounds present in water and solid phase was separately analyzed. The contribution of the solid phase to the total concentration is presented in figure 5.28. In this figure a sorption of selected pharmaceutical compounds to activated sludge is shown for ANOX-10 as there it was the most significant. The results of ANOX-10 are used because not only disposable centrifuge tubes were used for the anoxic tests (enabling optimal separation between liquid and solids), but also in the anox-10 the least biotransformation of pharmaceuticals was observed. For the anaerobic sludge the sorption results of ANAER-2 were used, since sampling of the solid phase in this experiment was improved in relation to ANAER-1. Sorption equilibrium was assumed only in the sampling times at which a pharmaceutical concentration did not decrease; these values were used to calculate average sorption values. Also concentrations at the start of the experiment (t=0) were left out of the calculation for this purpose.
Figure 5-28 shows clearly that the pharmaceutical fraction in the liquid phase prevailed. Only a small part of the total amount of the selected pharmaceuticals was present in the solid phase. Differences between the pharmaceuticals and between the to different types of sludge were observed. Sorption seems to be most relevant for CBZ, MTP and FNF. Regarding ASA, IBU, DCF, BZF and CFA, less than 10% of the total concentration was absorbed to the biological sludge.

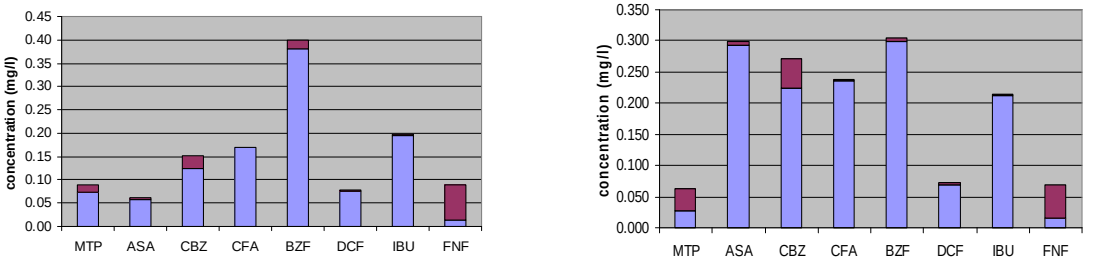


Figure 5.28: Average concentration of pharmaceuticals in both liquid (blue) and solid (red) phase during the time period in which the pharmaceutical were constant in concentration. Results from the ANOX-10 test (left) and ANAER-2 test (right).

The concentration of pharmaceuticals present in the anaerobic sludge seemed to be in general higher than the concentration of pharmaceutical sorbed to activated sludge. However, also the concentration TS in the anaerobic test was much higher. To compare the differences in sorption affinity of a given compound in relation to the sludge, the concentration of absorbed pharmaceutical per g TS is presented in figure 5.29. Fenofibrate, bezafibrate and clofibric acid concentrations in activated sludge were distinctly higher than in anaerobic sludge. Metoprolol and carbamazepine fractions located in sludge were slightly higher for anaerobic sludge. For other compound similar fractions were measured in both types of sludge.

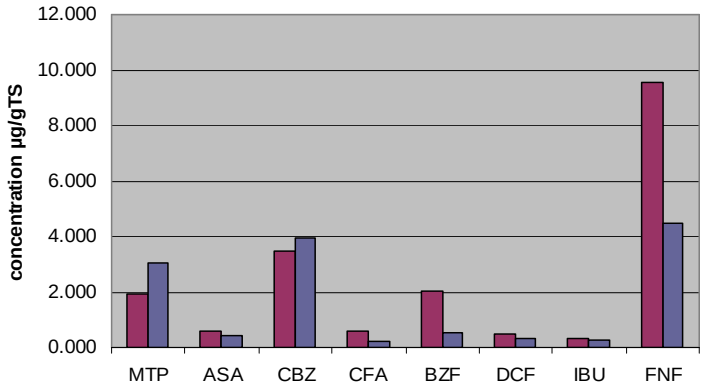


Figure 5.29: Pharmaceutical concentration in solid phase per g of TS (total solids) in ANOX-10 test (red) and in ANAER-2 (blue) test.

To compare the results with literature, the concentration in the solid is divided by the concentration in the liquid, since it influences the sorption equilibrium too. 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. The K_d values of activated sludge were obtained from the average sorption results of ANOX-20 and ANOX-10 test.

Table 5.4: A comparison of the assessed observed solid distribution coefficients (K_d) with literature values for activated sludge and anaerobic sludge. The observed K_d for activated sludge were determined based on the concentrations of pharmaceuticals in the ANOX-10 and ANOX-20 tests. Literature values are from (Ternes 2004); n.a. = not available;

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

A high K_d value for fenofibrate (FNF) is remarkable, but this compound is also the most hydrophobic. Sorption of FNF is one or two magnitudes of order higher than for the other pharmaceuticals.
The K_d values of metoprolol (MTP) differ a lot between the anaerobic and activated sludge, this is in contrast to figure 5.29. However, the large K_d in the anaerobic test can be caused by very low concentrations in the liquid phase. Since some of the concentration values of the anaerobic test are uncertain, the concentration of MTP in the water phase might be higher, causing overestimation of Kd value.
For all other pharmaceuticals the difference between the anaerobic and activated sludge is not so high (factor 2-6).

For activated sludge in municipal WWTP, K_d values are reported in literature for some of the selected pharmaceuticals. Values for clofibric acid (CFA) obtained in this research are very similar to those reported by Ternes (1998). The K_d value of ibuprofen (IBU) and diclofenac (DCF) are somewhat lower; CBZ value was on contrary higher in this research.
Differences between the K_d values can be explained by different sludge characteristics as this play an important role in sorption behaviour.

The results point out that the electrostatic interactions between pharmaceuticals and sludge are relevant processes. Both, metoprolol (MTP) and carbamazepine (CBZ), which are not acidic compounds, showed compared to clofibric acid, bezafibrate and ibuprofen (CFA, BZF and IBU) a higher sorption although the log Kow value are similar or lower.
Moreover, the calculated distribution coefficient K_d for 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, it disappears very fast; its concentration in the sludge drops from 85 to less than 5 µg/L 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 waste water.

6 Conclusions

The fate of pharmaceuticals was researched in biological treatment systems under various environmental conditions. A summary of biotransformation behaviour for all selected pharmaceuticals together with the influence of different environmental conditions is given in Table 6.1.

Of all the selected pharmaceuticals ASA and FNF were eliminated at the highest rate. Biological processes played an important role, but not solely since abiotic processes were observed as well. The acetyl salicylic acid and fenofibrate can be eliminated well at aerobic, anoxic and anaerobic conditions.
Ibuprofen could be biotransformed under the all applied redox conditions. Metoprolol can be biotransformed under aerobic and anoxic conditions but at a slower rate than acetyl salicylic acid, fenofibrate and ibuprofen (ASA, FNF, IBU). Under anaerobic conditions no biodegradation of metoprolol (MTP) was observed.
Bezafibrate can be slowly eliminated under aerobic and anoxic conditions. Diclofenac (DCF) can be potentially biotransformed under aerobic conditions, but relatively much time is required for this; more days than a typical HRT of a municipal WWTPs. At anoxic and anaerobic conditions, diclofenac (DCF) is not eliminated at all. Clofibric acid and carbamazepine are not eliminated biologically at all. They show under aerobic, anoxic and anaerobic a persistency to biodegradation. Different behaviour of selected pharmaceuticals in biological systems suggests to classify them into 3 groups: group 1 (easily biodegradable), group 2 (degradable under optimal conditions) and group 3 (persistent).
The biotransformation of pharmaceuticals follows a (pseudo) first order kinetics.
Aerobic conditions result, in general, in the highest biotransformation rates, followed by the anoxic and anaerobic conditions. A temperature decrease from 20^{o}C to 10^{o}C influences the biotransformation rate in aerobic and anoxic conditions. It varies from no significant to a distinct difference.

Compared to pharmaceuticals present in conventional sanitation systems, biotransformation rate of pharmaceuticals in concentrated waste streams was slower. The activated sludge used however, was not used to these high concentrations of pharmaceuticals.

The fraction absorbed to sludge is for the selected pharmaceuticals of a minor importance. For most pharmaceuticals concentration in the solid phase is <10%. For the non-acidic pharmaceuticals MTP and CBZ sorption is somewhat higher. The very hydrophobic but fast eliminated FNF is highly absorbed (up to 80% in anaerobic systems). Sorption turns out to be the highest for non-acidic pharmaceuticals and pharmaceuticals with a very high hydrophobic character. Of all selected pharmaceuticals sorption to sludge might be the most relevant process for CBZ, since this pharmaceutical is not biotransformed and it is absorbed to activated sludge and anaerobic for about 20%.

Table 6.1: Comparison of biotransformation rate of the selected pharmaceuticals at different environmental conditions. Biotransformability: +++ = very well, ++ = well, + = moderately, +/- = only at retention time > 2 days, - = not biodegradable

	Aerobic 20°C	Aerobic 10°C	Anoxic 20°C	Anoxic 10°C	Anaerobic 30°C
Group 1 (easily biodegradable)					
Acetyl salicylic acid (ASA)	+++	+++	++	++	+
Fenofibrate (FNF)	+++	++	++	++	+
Ibuprofen (IBU)	++	++	+	+*	+
Group 2 (biodegradable under optimal conditions)					
Metoprolol (MTP)	++	+	+	-	-
Bezafibrate (BZF)	+/-	+/-	+	-	-
Diclofenac (DCF)	+/-	+/-	-	-	-
Group 3 (persistent)					
Carbamazepine (CBZ)	-	-	-	-	-
Clofibric acid (CFA)	-	-	-	-	-

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