

Biodegradation of Xenobiotic Organic Compounds in Wastewater Treatment Plants

Kåre Press-Kristensen

Ph.D. Thesis

July 2007

Institute of Environment & Resources

Technical University of Denmark



The project is partly financed by Interreg IIIA Øresund Region

***Biodegradation of Xenobiotic Organic Compounds in
Wastewater Treatment Plants***

Cover: Torben Dolin & Julie Camilla Middleton
Printed by: Vester Kopi, DTU
Institute of Environment & Resources
ISBN 978-87-91855-38-2

The thesis will be available as a pdf-file for downloading from the institute homepage on: www.er.dtu.dk

Institute of Environment & Resources
Library
Bygningstorvet, Building 115,
Technical University of Denmark
DK-2800 Kgs. Lyngby

Phone:

Direct: (+45) 45 25 16 10
(+45) 45 25 16 00
Fax: (+45) 45 93 28 50
E-mail: library@er.dtu.dk

Table of contents

Preface.....	iii
Acknowledgements.....	iv
Abstract	v
Resumé (abstract in Danish)	vi
Abbreviations	vii
1. Introduction	1
1.1 Objectives.....	1
1.2 Structure of the thesis.....	2
2. Removal mechanisms	3
2.1 Biodegradation	3
2.2 Abiotic processes	4
2.2.1 Sorption	4
2.2.2 Stripping and volatilization	5
2.2.3 Abiotic hydrolysis	5
2.2.4 Abiotic oxidation	6
2.2.5 Tertiary methods	6
3. Selection of model pollutants	7
3.1 Screening tool	7
3.1.1 XOCs in WWTP effluents	7
3.1.2 Hazardous XOCs	7
3.1.3 Biodegradable XOCs	9
3.2 Identifying model pollutants	9
3.2.1 Risk screening and biodegradability	12
3.2.2 Limitations	14
4. Model pollutants	15
4.1 Bisphenol A	15
4.2 Nonylphenol	16
5. Development of analytical methods	19
5.1 Validation	20
5.1.1 Detection and quantification limit	20
5.1.2 Repeatability and recovery	20
5.1.3 External quality assurance	21

6. Biodegradation experiments	23
6.1 Experimental set-up	24
6.1.1 Experimental considerations	25
6.2 Results and discussion	26
6.2.1 Background concentrations	26
6.2.2 Step-additions	27
6.2.3 Mass-balances	28
6.2.4 Batch experiments	29
6.2.5 Optimization experiment	30
7. Model results	33
7.1 Model development	33
7.2 Modelling as a tool	35
8. Conclusions	37
9. Perspectives	39
9.1 Further investigations.....	33
References	41
Appendixes	51

Preface

This Ph.D. thesis is based upon research performed from 2003 to 2007 at Institute of Environment & Resources (E&R) at the Technical University of Denmark (DTU).

The main supervisor was Professor Mogens Henze and co-supervisors were Professor Anna Ledin and Associate Professor Jens Ejbye Schmidt (all from E&R). Furthermore, Associate Research Professor Nina Christensen (E&R) has contributed with vital input to the analytical work and my colleague Erik Lindblom (E&R) has been of great value when planning and evaluating the experimental work.

The thesis comprises a summary of five journal papers:

- I Press-Kristensen K, Ledin A, Schmidt JE, Henze M. Identifying model pollutants to investigate biodegradation of hazardous XOCs in WWTPs. *Science of the Total Environment*, 2007, 373, 122-130.
- II Press-Kristensen K, Lindblom E, Schmidt JE, Henze M. Examining the biodegradation of endocrine disrupting bisphenol A and nonylphenol in WWTPs. *Water Science and Technology*, 2007, submitted.
- III Press-Kristensen K, Lindblom E, Henze M. Modelling as a tool when interpreting biodegradation of micro pollutants in activated sludge systems. *Water Science and Technology*, 2007, submitted.
- IV Press-Kristensen K, Christensen N, Ledin A. Simultaneous extraction and quantification of bisphenol A and nonylphenol in complex wastewater matrixes. *International Journal of Environmental Analytical Chemistry*, 2007, submitted.
- V Lindblom E, Press-Kristensen K, Vanrolleghem P, Mikkelsen PS, Henze M. Modelling the fate of bisphenol A in activated sludge systems. *Environmental Science and Technology*, submitted, 2007.

The journal papers are not included in this www-version but can be obtained from the Library at the Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Lyngby (library@er.dtu.dk).

Other publications related to the thesis:

- a) Press-Kristensen K. Book chapter: *Removal mechanisms*. In press, 2007. Coordinator: Raghida Lepistö, Dr. Tech., Docent, Institute of Environmental Engineering & Biotechnology, Tampere University of Technology. P.O. Box 541, 33101 Tampere, Finland.
- b) Press-Kristensen K, Thirsing C. Biodegradation of micro pollutants in wastewater (In Danish: Biologisk nedbrydning af miljøfremmede stoffer i spildevand). *Dansk Kemi*, 2005, 86, 16-20.

Acknowledgements

I would like to acknowledge the following organizations and persons:

- DTU, Lynettefællesskabet I/S, Avedore Wastewater Services I/S, and the EU INTERREG project *Kildesamarbejdet* for funding the Ph.D. study.
- My supervisors Mogens Henze, Anna Ledin, and Jens Ejbye Schmidt for their direct (and on-line) support and pep talks when needed as well as their endless attempts to make me spend more time with my family. Especially, I would like to thank Mogens Henze because he has been very open minded to most of my crazy ideas/suggestions (and because he managed to freeze many of them ;-).
- Carsten Thirsing and Kim Rindel from Lynettefællesskabet I/S, Bo Neergaard Jacobsen from Avedore Wastewater Services I/S, Gert Holm Kristensen from DHI Group, Erik Lindblom and Henrik Andersen from E&R, and others who have contributed with scientific input and constructive support during the Ph.D. study.
- Nina Christensen, Hector Garcia, Babak Diznabi, Nana Hartmann, Stine Reimer Pedersen from E&R, Lene Vig from Lynettefællesskabet I/S, and others who have been directly involved in the laboratory work and/or the experiments.
- My colleagues at E&R - especially Stefan Trapp, Jens Christian Tjell, Anders Fredenslund, and my fellow Ph.D. students as well as security guards and other lab rats that helped me look at the bright sides during the early morning hours.
- My family and friends for keeping in touch.

Abstract

Xenobiotic organic compounds (XOCs) in wastewater treatment plant (WWTP) effluents have been shown to result in adverse environmental effects on a global scale. In this study the removal of two key XOCs in an activated sludge system was investigated at a pilot WWTP receiving urban and industrial wastewater from Copenhagen, Denmark.

The main objective was to generate operational and chemical data which can be used to develop process models for removal of XOCs in WWTPs. To meet the objective a brief study of removal mechanisms was conducted and a screening tool to identify model pollutants was devised. Subsequently a method for analysing model pollutants was developed and biodegradation experiments in a pilot WWTP were performed. The perspective of the process model is to induce new concepts for designing and operating WWTPs with efficient removal of XOCs.

Bisphenol A (BPA) and nonylphenol (NP) were selected as model pollutants in this study since the two XOCs have been detected in WWTP effluents on a global scale in potential ecotoxic concentrations and were expected to biodegrade and behave quite different in activated sludge systems. A robust method for simultaneously extraction and GC-MS quantification of BPA and NP in complex wastewater matrixes was developed, validated, and used in the study.

The BPA concentration was reduced 50-80 % and NP concentration was reduced 10-65 % during standard operation of the pilot plant. Step-addition experiments where the BPA and NP influent concentrations were increased 100-200 times showed that the effluent concentrations were independent of the influent concentrations after an adaption time of 10 to more than 40 days depending on temperature, hydraulic retention time, and pre-exposure of the biomass. Mass-balance experiments suggested that more than 99 % of BPA was removed by biodegradation at steady-state when the influent concentration was increased in step-addition experiments. Batch experiments showed that BPA degradation only occurred under aerobic conditions in the pilot plant. In an optimization experiment with increased aerobic phase-time the effluent concentrations of BPA and NP dropped to the lowest observed levels in this study.

Based on biological key parameters identified from the performed background and step-addition experiments a process model for removal of XOCs in WWTPs was developed by extending the *Activated Sludge Model no. 1* to include sorption and biodegradation of BPA. The process model confirmed that effluent concentrations of BPA from the pilot plant are independent of influent concentrations at steady-state and proved its success as an interpretation tool when analysing data from an optimization experiment. The process model is believed to improve the fate assessment of XOCs in WWTPs and thereby the possibility to design and operate WWTPs to remove key XOCs.

Resumé

Miljøfremmede stoffer i afløbet fra renselanlæg har vist sig at forårsage negative effekter i miljøet på globalt plan. I nærværende studie undersøges fjernelsen af to vigtige miljøfremmede stoffer i et aktivt slamanlæg på et pilotrenseanlæg, der modtager blandet husholdnings- og industrispildevand fra København, Danmark.

Hovedformålet med dette studie var at generere driftsparametre og kemiske data til brug for udvikling af en procesmodel for fjernelse af miljøfremmede stoffer i renselanlæg. For at opfylde formålet blev der udført et kort studie af fjernelsesmekanismer og opstillet et screeningværktøj til at identificere modelstoffer. Dernæst blev udviklet en analysemetode for modelstofferne og udført en række biologiske nedbrydningsforsøg i et pilotrenseanlæg. Perspektivet for procesmodellen er at indføre nye koncepter for design og styring af renselanlæg mhp. fjernelse af miljøfremmede stoffer.

Bisphenol A (BPA) og nonylphenol (NP) blev udvalgt som modelstoffer i studiet fordi de to miljøfremmede stoffer var målt i afløbet fra renselanlæg på globalt plan i potentielt økotoksikologiske koncentrationer og forventes at være bionedbrydelige og opføre sig markant forskelligt i aktive slamsystemer. En robust metode til simultan ekstraktion og GC-MS kvantifikation af BPA og NP i komplekse spildevandsprøver blev udviklet, valideret og anvendt i studiet.

BPA koncentrationen blev reduceret 50-80 % og NP koncentrationen 10-65 % ved standard drift af pilotanlægget. Step-doseringsforsøg, hvor indløbskoncentrationen af BPA og NP blev øget 100-200 gange viste, at udløbskoncentrationen var uafhængig af indløbskoncentrationen efter en adaptationstid fra 10 til mere end 40 dage afhængig af temperatur, hydraulisk opholdstid og pre-eksponering af biomassen. Masse-balance forsøg viste, at over 99 % af BPA blev fjernet ved bionedbrydning ved steady-state, når indløbskoncentrationen blev øget ved step-doseringsforsøg. Batchforsøg illustrerede, at nedbrydning af BPA kun skete under aerobe forhold i pilotrenseanlægget. I et optimeringsforsøg med 40 % øget aerob fase-tid faldt udløbskoncentrationen af BPA og NP til de laveste niveauer observeret i dette studie.

Baseret på biologiske nøgleparametre identificeret i baggrundseksperimenterne og de udførte step-doseringsforsøg blev udviklet en procesmodel for fjernelse af miljøfremmede stoffer i renselanlæg ved at udvide den eksisterende *Activated Sludge Model nr. 1* til at omfatte sorption og bionedbrydning af BPA. Procesmodellen bekræftede at udløbskoncentrationerne af BPA fra pilotanlægget er uafhængige af indløbskoncentrationerne ved steady-state og viste dens succes som fortolkningsværktøj ved analyse af resultaterne fra optimeringsforsøget. Derved vurderes procesmodellen at bidrage til en bedre skæbnevurdering for miljøfremmede stoffer i renselanlæg og med væsentlige informationer til design og drift mhp. fjernelse af miljøfremmede stoffer.

Abbreviations

BPA:	Bisphenol A
CBZ:	Carbamazepine
DEHP:	Di(2ethylhexyl)-phthalate
DL:	Detection limit
E1:	Estrone
E2:	17 β -estradiol
EC ₅₀ :	Effect concentration (50 % effect)
ED ₅₀ :	Effect dose (50 % effect)
EDC:	Endocrine disrupting compound
EE2:	17 α -ethinyloestradiol
DIPE:	Di-iso-propyl-ether
HRT:	Hydraulic retention time
LC ₅₀ :	Lethal concentration (50 % lethality)
LD ₅₀ :	Lethal dose (50 % lethality)
LOAEL:	Lowest observed adverse effect level
LOEC:	Lowest observed effect concentration
NP:	Nonylphenol
NPnEC	Nonylphenol carboxylates
NPnEO:	Nonylphenol ethoxylates
OP:	Octylphenol
PEC:	Predicted environmental concentration
PNEC:	Predicted no-effect concentration
QL:	Quantification limit
SD:	Standard deviation
SRT:	Solid retention time
TDI:	Tolerable daily intake
XOC:	Xenobiotic organic compound
WSG:	Potential water supply guideline
WWTP:	Wastewater treatment plant

1. Introduction

Municipal wastewater contains a complex mixture of xenobiotic organic compounds (XOCs) originating from personal care products, pharmaceuticals, excreted hormones, household and industrial chemicals etc. Main sources are households, institutions, the industry (Eriksson et al., 2003; Douzinas, 2001), and rain that collects pollutants from air and surfaces before entering the sewer (Eriksson et al., 2002). Press-Kristensen et al. (2007a) have estimated that wastewater could contain many thousand XOCs.

Wastewater treatment plants (WWTPs) mainly remove XOCs from the water phase by sorption, biological and/or chemical degradation, volatilization, and/or stripping. Conventional wastewater treatment is not directly designed for removing XOCs but is known to reduce concentrations of several key XOCs (DEPA, 2002a; Fauser et al., 2003; Tanaka et al., 2003; Jacobsen and Guildal, 2000).

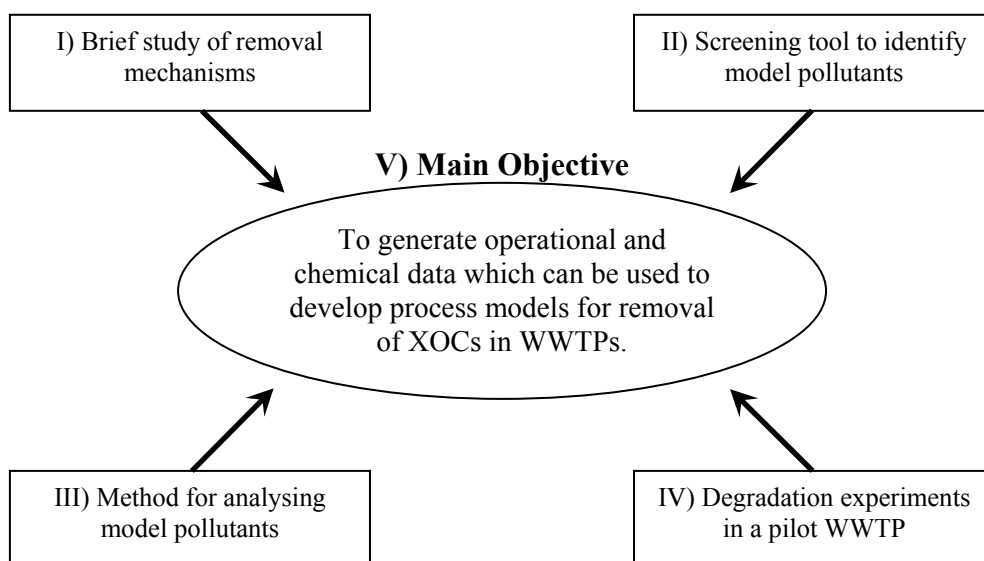
However, critical XOCs are frequently reported in WWTP effluents in potential toxic concentrations (Andersen, 2004; Knacker, 2002) and are known to cause toxic effects in different recipients (Schäfer and Waite, 2002) on different species (DEPA, 2002a) and might cause long-term changes in aquatic ecosystems (Daughton and Ternes, 1999). Feminisation of male fish in different species is a well-known effect observed in recipients loaded with treated wastewater. Cage experiments with fish have confirmed that wastewater containing endocrine disrupting compounds (EDC) have the capability to cause feminization. EDCs originate from several sources e.g. natural and pharmaceutical hormones as well as industrial chemicals (DEPA, 2002a). Furthermore, XOCs in freshwater may constitute a risk or an aesthetic problem in drinking water e.g. pharmaceuticals have been detected in German tap water (Heberer and Stan, 1996).

Improved wastewater treatment may be an efficient way to reduce pollution with XOCs. Several studies have identified biodegradation of XOCs as a vital elimination process in WWTPs (DEPA, 2004; Fauser et al., 2003; Buser et al., 1999; Poseidon, 2005). But our ability to utilize and control parameters and processes governing the biodegradation of XOCs in activated sludge systems remains a challenge. Therefore, models developed and calibrated from experiments with carefully selected model pollutants should be constructed to support scientists to reveal and realise possible optimisation potentials.

1.1 Objectives

The main objective was to generate operational and chemical data which can be used to develop process models for removal of XOCs in WWTPs. To meet the objective a brief study of removal mechanisms was conducted and a screening tool to identify model pollutants was devised. Subsequently a method for analysing model pollutants was developed and biodegradation experiments in a pilot WWTP were performed (Figure 1.1). The perspective of the process model is to induce new concepts for designing and operating WWTPs with efficient removal of XOCs.

Figure 1.1: Main objective and the milestones of the study



1.2 Structure of the thesis

This thesis is introduced by an overview of important removal mechanisms for XOCs in WWTPs intended to give a quick overview of the main removal mechanisms available for process models simulating the removal of XOCs in WWTPs (milestone I).

Then a systematic screening tool to identify model pollutants is developed and tested with ten XOCs detected in WWTP effluents. From the screening tool and the limitations of the study model pollutants are selected for further investigation of biological removal mechanisms in activated sludge systems at WWTPs (milestone II).

Then basic physical-chemical data of the model pollutants are mapped and a short study concerning the expected fate of the model pollutants in WWTPs are conducted. Based upon this information an analytical method is developed (milestone III) and degradation experiments in the pilot WWTP is performed (milestone IV).

Finally, a short description of the model development, validation, and use based upon the experimental results is included to present the process model developed from the generated operational and chemical data in this study (milestone V).

2. Removal mechanisms

The main removal mechanisms for XOCs in activated sludge systems in conventional WWTPs are biodegradation, sorption, stripping and volatilization, abiotic hydrolysis, and abiotic oxidation. Furthermore, tertiary methods exist e.g. filtration, UV-radiation, ozonation etc.

2.1 Biodegradation

Biodegradation of XOCs can either be *mineralization* i.e. transformation to CO₂, H₂O, and inorganic molecules, or *metabolization* i.e. transformation of XOCs to one or more organic metabolites. Biodegradation of a XOC is expected if the wastewater influent concentrations are above the threshold concentration for biodegradation of the XOC, the XOC is bioavailable, no higher prioritized carbon source is available, and bacteria producing specific enzymes are active and constitute a significant biomass. Co-metabolism is an exception where the XOC is biodegraded in the presence of a primary carbon source that serves as growth substance for the bacteria (Connell, 1997).

Biological wastewater treatment has been shown to reduce the concentration of several XOCs (Poseidon, 2005), key EDCs (DEAP, 2004; Fauser et al., 2003) as well as the estrogenic effect of wastewater (Svenson et al., 2003). Biodegradation mainly occurs in activated sludge systems where XOCs are exposed to a dense heterogeneous biomass under aerobic (nitrification), anoxic (denitrification), and anaerobic (Bio-P) conditions. Furthermore, biodegradation during anaerobic digestion (mesophilic or thermophilic) stimulates degradation of key XOCs e.g. di-*n*-butyl phthalate and di-ethyl phthalate (Gavala et al., 2003).

Biodegradation of key XOCs has been found to increase when the hydraulic retention time (HRT) and solid retention time (SRT) increase (Johnson et al., 2005; Tanaka et al., 2002). Especially the SRT seems to be an important parameter for biodegradation (Kreuzinger et al., 2004). A SRT above 10 days (nutrient removal) is crucial for biodegradation of several XOCs, probably due to the development of higher biodiversity in the biomass (Poseidon, 2005; Clara et al., 2005).

Furthermore, the kinetics of biodegradation is important for the removal.

Due to relatively low concentrations of XOCs (often in the ng to µg per litre level) a pseudo first order degradation is usually used (see Box 2.1) in models. In most WWTPs this results in insignificant biodegradation when the rate constant is below 0.1 m³/kgSS·d and substantial biodegradation if the rate constant is above 10 m³/kgSS·d. Inside this interval, biodegradation depends on plant and operation design. However, biodegradation of XOCs in WWTPs is difficult to predict due to unknown specific rate constants and should therefore be determined experimentally (Poseidon, 2005).

Box 2.1: Biodegradation

Hydraulic Retention Time:	HRT	=	$V/Q_{\text{Inf}} = V / (Q_{\text{Eff}} + Q_{\text{WS}})$
Solid Retention Time:	SRT	=	$(V \cdot X) / (Q_{\text{Eff}} \cdot X_{\text{Eff}} + Q_{\text{WS}} \cdot X_{\text{WS}})$
First order kinetics:	dC_w/dt	=	$k \cdot SS \cdot C_w$
Half life of XOC:	$t_{1/2}$	=	$\ln 2 / (k \cdot SS)$

Symbols:

V:	Volume of reactor	X_{WS}:	Waste sludge concentration
Q_{Inf}:	Inflow to reactor	C_w:	Concentration of XOC in water
Q_{Eff}:	Outflow from reactor	t:	Time
X:	Sludge concentration in reactor	k:	First order rate constant
X_{Eff}:	Effluent sludge concentration	Q_{WS}:	Flow of waste sludge
SS:	Suspended solid in reactor		

Suggested units:

V in m³, Q in m³/d, X in kg VSS/m³, C in mg/m³, t in d, k in m³/kgSS·d, and SS in kg/m³)

2.2 Abiotic processes

Sorption, stripping, and volatilization are mainly governed by phase partition coefficients that usually applies for the non-dissociated fraction of a XOC (See box 2.2).

Box 2.2: Dissociation

Fraction undissociated acid, $f_{\text{acid}} = (1 + 10^{\text{pH} - \text{pK}_a})^{-1}$

Fraction undissociated base, $f_{\text{base}} = (1 + 10^{14 - \text{pK}_b - \text{pH}})^{-1}$

K_a and K_b are respectively the acid and base dissociation constant

2.2.1 Sorption

Several XOCs bind to sludge and are thereby removed with waste sludge (see box 2.3). The binding can be *adsorption* (on the surface) or *absorption* (diffusing into the solid phase). Adsorption is caused by electrostatic interactions and absorption by hydrophobic properties. Sorbed XOCs are unavailable for biodegradation, stripping, and volatilization. Nevertheless, most sorption processes are reversible through desorption. Consequently, sorbed XOCs can be released if the concentration in the water phase is reduced e.g. due to biodegradation. Sorption ability can be estimated from the sorption coefficient (K_d) that mainly depends on the properties of the XOC and the sludge (See box 2.3). Several studies report expressions where K_d is estimated directly from the octanol-water partition coefficient (K_{ow}). However, measured K_d -values under relevant conditions should always be preferred over estimated coefficients (Poseidon, 2005).

Box 2.3: Sorption

Equilibrium expression: $C_s = K_d \cdot C_w$

Removed by sorption: $M = Q_{ws} \cdot X_{ws} \cdot C_s$

Symbols and suggested units:

C_s: Concentration sorbed to sludge (mg/kgSS)

K_d: Sorption coefficient for the XOC (m³/kgSS)

M: Sorbed to and removed with waste sludge (mg/d)

In most WWTPs sorption will be an insignificant removal mechanism for XOCs with a $K_d \leq 0.5 \text{ m}^3/\text{kgSS}$ (Poseidon, 2005).

2.2.2 Stripping and volatilization

Volatile XOCs are transferred from the water phase to the air by stripping and volatilization. *Stripping* is an active process due to mechanical aeration whereas *volatilization* is a passive process governed by diffusion (see box 2.4).

Box 2.4: Stripping and volatilization

Equilibrium expression: $K_H^* = C_{\text{Air}} / C_{\text{Water}}$ (Henry's law)

$$K_H = R \cdot T \cdot K_H^*$$

K_H may be estimated: $K_H \approx p_a/S$ (measured K_H -values are preferred)

Symbols and suggested units:

K_H^{*}: Dimensionless K_H

R: Gas constant ($8.21 \cdot 10^{-5} \text{ m}^3 \cdot \text{atm} / \text{mol} \cdot \text{K}$)

C_{Air}: Concentration in air (mg/m³)

T: Temperature (K)

C_{Water}: Concentration in water (mg/m³)

p_a: Partial pressure in the gas phase (atm)

K_H: Henry's constant (atm·m³/mol)

S: Water solubility (mol/m³)

Stripping and volatilization are governed by Henry's law but stripping increases the rate of the phase transfer due to increased contact surface between the water and air phase. The stripping capacity thereby depends on K_H and the properties of the aeration unit. K_H usually increases with increasing temperature. In general, a $K_H^* > 3 \cdot 10^{-3}$ is required to make stripping a significant removal mechanism in WWTPs (Poseidon, 2005).

2.2.3 Abiotic hydrolysis

Abiotic hydrolysis is a reaction between water and a XOC that often results in fragmentation of the XOC into metabolites containing hydrogen and/or oxygen from the water molecule. XOCs susceptible to hydrolysis are e.g. alkyl halides, amides, carbamates, and esters (Connell, 1997).

2.2.4 Abiotic oxidation

Abiotic oxidation in wastewater is a reaction between an oxidant (O_2 , NO_3^- etc.) and a XOC that typically involves addition of oxygen to or splitting the XOC into oxygen-containing metabolites. The process depends on the concentration of dissolved oxygen, nitrate and the properties of the XOC (Connell, 1997). Incineration of waste sludge is an efficient chemical oxidation at high temperatures by which most XOCs are mineralized.

2.2.5 Tertiary methods

Important tertiary removal mechanisms are activated carbon or membrane filtration, UV-radiation, and ozonation. Filtration removes XOCs from the water phase whereas UV-radiation and ozonation often transform XOCs to metabolites. The mechanisms are powerful removal processes for many XOCs but only ozonation is emphasized as a potential tertiary removal mechanism in WWTPs (Poseidon, 2005). However, attention should be paid to formation of toxic metabolites from ozonation (Wert et al., 2007).

3. Selection of model pollutants

Initially, a screening tool was developed to select model pollutants. The screening tool was based on three criteria (appendix I):

- 1) The XOC is present in WWTP effluents.
- 2) The XOC is hazardous i.e. an intolerable risk in drinking water or in the environment.
- 3) The XOC is expected to be biodegradable in WWTPs.

3.1 Screening tool

If a criterion was not fulfilled the XOC was rejected as a model pollutant whereas compounds passing the screening tool were suitable as model pollutants investigating biodegradation of hazardous XOCs in WWTPs. In case of insufficient data the XOCs were transferred to a list for further investigations.

3.1.1 XOCs in WWTP effluents

The first criterion was evaluated by reviewing scientific journals, conference proceedings, and literature containing data on XOCs in WWTP effluents. XOCs detected in at least three different investigations within the last ten years fulfilled the criterion. Effluent data consisting of a median, mean, and maximum concentration from each investigation were collected if possible. Non detected concentrations were assigned as below (<) the detection limit and to zero when calculating the median and mean respectively.

3.1.2 Hazardous XOCs

The second criterion was evaluated by a brief risk screening of the XOCs observed in WWTP effluents (Table 3.1). The method was developed inspired by TGD (2003), WHO (2004), and EMEA (2005). Toxicological data were collected from literature and databases. Databases for mammal toxicity were NIOSH (2005), Toxnet (2005), Rxlist (2005), and Rippen (2005). Databases for ecotoxicity were ECOTOX (2005), ESIS (2005), and Toxnet (2005). Search words used were CAS no. and chemical name combined with the words: Toxicity, g/kg, daily intake, LC₅₀, or EC₅₀. The lowest observed adverse effect level (LOAEL) or concentration (LOEC) and lethal or effect dose or concentration (LD₅₀/LC₅₀) or (ED₅₀/EC₅₀) were used to estimate the tolerable daily intake (TDI) for humans or a predicted no-effect concentration (PNEC) for aquatic organisms. TDI was estimated from the highest mammal toxicity divided by an uncertainty factor of 1,000 while PNEC was estimated from the highest ecotoxicity divided with an uncertainty factor of 100.

Table 3.1: Risk screening (Press-Kristensen et al., 2007a)

1) Officially estimated WSG based on an excess lifetime cancer risk of one out of 100,000.

Drinking water			Does the XOC constitute an intolerable risk ?	
Mammal toxicity		Potential water supply guideline		Yes
Non-carcinogenic	TDI = LOAEL, LD ₅₀ or ED ₅₀ / 1,000	WSG = TDI · 60 kg bw · 0.1 / 2 litres		?
Carcinogenic	Lowest WSG based on TDI from non-carcinogenic toxicity or an excess lifetime cancer risk ¹⁾			
Environment				PEC > (WSG and/or PNEC)
Ecotoxicity		Predicted no-effect concentration		
Data from acute and chronic ecotoxicity tests		PNEC = LOEC, LC ₅₀ or EC ₅₀ / 100		
Exposure				Insufficient data
Effluent concentrations		Predicted environmental concentration		
Median, mean, and maximum effluent concentration		PEC = Effluent concentration / 10		Sufficient data and PEC ≤ (WSG and PNEC)
			No	

A potential water supply guideline (WSG) was estimated by multiplying the TDI by a body weight of 60 kg, an allocation factor of 0.1 (assuming 10 % of the daily XOC-intake from drinking water), divided by a daily intake of 2 litres of water. For carcinogens were used the lowest of a WSG estimated from a TDI based on non-carcinogenic properties and a WSG based on an excess cancer risk of 1:100,000 i.e. one additional cancer case due to water intake per 100,000 individuals during a lifetime of 70 years (WHO, 2004).

Predicted environmental concentrations (PECs) were estimated by dividing the median, mean, and the maximum effluent concentration by a recipient dilution factor of 10. Medians given as below ($<$) the detection limit were set to half the detection limit when calculating the PEC.

PECs calculated from each median, mean, and maximum concentration were compared to the WSG and the PNEC. Effluent concentrations constitute an intolerable risk if $PEC > WSG$ or $PEC > PNEC$ while a tolerable risk was assumed (with the present knowledge) in case of sufficient data if $PEC \leq WSG$ and $PEC \leq PNEC$. Sufficient data were defined as data on acute and specific toxicity i.e. lethality, endocrine disruptions, reproduction damages, cancer, and behavioural changes.

To limit the impact of non-representative effluent concentrations, XOCs were only considered as hazardous if more than 10 %, 20 %, or 40 % of respectively the median, mean, or maximum PECs for each XOC was an intolerable risk.

3.1.3 Biodegradable XOCs

The third criterion was evaluated from information about biodegradation in conference proceedings, reports, and databases: ESIS (2005) and Toxnet (2005). Search words used were chemical name and CAS no. combined with the words: Biodegradation and sludge. XOCs reported biodegradable were expected biodegradable in WWTPs and thereby fulfilled the third criterion.

3.2 Identifying model pollutants

Ten XOCs detected in WWTP effluents on a global scale were selected from the review (first criterion) to identify model pollutants. Four industrial chemicals (Table 3.2): Bisphenol A (BPA), di(2ethylhexyl)-phthalate (DEHP), nonylphenol (NP), and octylphenol (OP). Six hormones/pharmaceuticals (Table 3.3): Carbamazepine (CBZ), 17 β -estradiol (E2), estrone (E1), 17 α -ethinyloestradiol (EE2), ibuprofen, and naproxen.

Table 3.2: WWTP effluent concentrations of selected industrial chemicals (ng/l)

Modified from Press-Kristensen et al. (2007a).

1) 95 % percentile used as maximum concentration.

2) Median based upon samples above the detection limit.

	CAS no.	Application	Median/Mean/Max.	Reference
Bisphenol A (BPA)	80-05-7	Intermediate in the production of and a residual in epoxy resins, flame retardants, polycarbonate etc. (Staples et al., 1998)	--- / 511 / ---	(Snyder et al., 2002)
			190 / 571 / 2,700	(Barber et al., 2000)
			30 / 30 / 40	(Kobuke et al., 2002)
			--- / 200 / 700 ¹⁾	(NOVA, 2005)
			< 100 / 270 / 1,400	(DEPA, 2003)
			118 / --- / 4,090 ²⁾	(DGPWWM, 2002)
			20 / --- / 520	(Tanaka et al., 2003)
--- / 38 / 55	(Rudel et al., 1998)			
Di(2ethylhexyl)-phthalate (DEHP)	117-81-7	Plasticizer. (DEHP-IC, 2005)	760 / 960 / 2,650	(Fauser et al., 2003)
			400 / 500 / 800	(Kobuke et al., 2002)
			--- / 1,800 / 6,100 ¹⁾	(NOVA, 2005)
			1,500 / --- / 2,400 ²⁾	(DGPWWM, 2002)
			400 / --- / 6,200	(Tanaka et al., 2003)
Nonylphenol (NP)	84852-15-3	Metabolite of alkyl phenol ethoxylates widely used in cleaning agents. (Lee and Peart, 1998)	310 / 329 / 690	(Fauser et al., 2003)
			--- / 1,060 / ---	(Snyder, 2002)
			170 / 100 / 400	(Kobuke et al., 2002)
			1,650 / 3,837 / 19,000	(Barber et al., 2000)
			120 / 121 / 310	(DEPA, 2003)
			1,500 / --- / 1,500 ²⁾	(DGPWWM, 2002)
			200 / --- / 1,000	(Tanaka et al., 2003)
			1,205 / 1,165 / 2,120	(Lee and Peart, 1998)
			--- / 16,000 / ---	(Rudel et al., 1998)
600 / 988 / 2,700	(R-Gray et al., 2000)			
Octylphenol (OP)	27193-28-8	Metabolite of alkyl phenol ethoxylates widely used in cleaning agents. (Lee and Peart, 1998)	--- / 15 / ---	(Snyder, 2002)
			700 / --- / 1,300 ²⁾	(DGPWWM, 2002)
			140 / 200 / 660	(Lee and Peart, 1998)
			--- / 150 / ---	(Rudel et al., 1998)

Table 3.3: WWTP effluent concentrations of selected hormones/pharmaceuticals (ng/l)

Modified from Press-Kristensen et al. (2007a).

1) Median based upon samples above the detection limit.

	CAS no.	Application	Median/Mean/Max.	Reference
Carbamazepine (CBZ)	298-46-4	Antiepileptic. (Poseidon, 2005)	--- / 471 / ---	(Snyder et al., 2002)
			300 / 405 / 950	(Tixier et al., 2003)
			480 / 713 / 6,300	(Poseidon, 2005)
			440 / 596 / 1,200	(Rempharmawater, 2003)
17β-estradiol (E2)	50-28-2	Natural steroid hormone. (DEPA, 2004)	10 / 11 / 14	(Kobuke et al., 2002)
			6 / --- / 64	(Ternes et. al, 1999)
			< 1 / 0.6 / 4.5	(DEPA, 2003)
			10 / --- / 66	(Tanaka et al., 2003)
			7.9 / 30 / 88	(R-Gray et al., 2000)
			6 / --- / 50	(Desbrow et al., 1998)
Estrone (E1)	53-16-7	Natural steroid hormone. (DEPA, 2004)	--- / 38.5 / ---	(Snyder et al., 2002)
			< 2 / 3.3 / 11	(DEPA, 2003)
			5.4 / --- / ---	(Kobuke et al., 2002)
			3 / --- / 48	(Ternes et. al, 1999)
			3.4 / --- / 11¹⁾	(DGPWWM, 2002)
			5.4 / --- / 63	(Tanaka et al., 2003)
			45 / 68 / 220	(R-Gray et al., 2000)
17α-ethinyloestradiol (EE2)	57-63-6	Pharmaceutical steroid hormone. (DEPA, 2004)	9 / --- / 42	(Ternes et. al, 1999)
			< 1 / 1.1 / 5.2	(DEPA, 2003)
			2.6 / --- / 2.6¹⁾	(DGPWWM, 2002)
			0.25 / --- / 7	(Desbrow et al., 1998)
Ibuprofen	15687-27-1	Anti-inflammatory and analgesic. (Stumpf et al., 1999)	< 100 / 63 / 300	(DEPA, 2003)
			190 / 190 / 280	(Stumpf et al., 1999)
			40 / 155 / 1,350	(Tixier et al., 2003)
			92 / 400 / 3,900	(Poseidon, 2005)
			7.5 / 24.5 / 81	(Buser et al., 1999)
			110 / 318 / 1,960	(Rempharmawater, 2003)
Naproxen	22204-53-1	Anti-inflammatory and analgesic. (Stumpf et al., 1999)	335 / 335 / 500	(Stumpf et al., 1999)
			250 / 530 / 3,300	(Tixier et al., 2003)
			410 / 527 / 1,510	(Rempharmawater, 2003)

3.2.1 Risk screening and biodegradability

Table 3.2 and table 3.3 present XOCs all fulfilling the first criterion i.e. the XOCs were detected in WWTP effluents in three or more investigations carried out during the last ten years. Decisive variations in the data quality were observed between investigations varying from data based on a few grab samples in one WWTP to data consisting of median concentrations from extensive monitoring programmes that include several WWTPs. Therefore it was not possible to conduct a reliable comparison of quantitative effluent concentrations in the included investigations. Comparing the median and mean concentration to the maximum concentration it appears that concentrations vary significantly in most investigations. Furthermore, steroid hormones (E2, E1, and EE2) seem to be present in concentrations some decades below other tested XOCs.

Table 3.4 contains results from evaluating the second and third criterion. BPA disregarded, WSGs for E2 and EE2 are decades below WSGs for other tested XOCs. This may indicate that the steroid hormones are more toxic because they are designed to activate specific sites in organisms. Comparable trends are observed when comparing the ecotoxicity of steroid hormones to the endocrine disruptive effects from industrial XOCs (BPA, DEHP, NP, or OP). This is consistent with observations from other studies e.g. DEPA (2003) and DEPA (2002a). The WSG for BPA is estimated from toxic levels that are significant lower than toxic levels used in official risk assessments e.g. ECB (2003a). Consequently, the WSG for BPA should be used with care. A WSG based on carcinogenicity was used for DEHP only.

The second criterion was fulfilled by BPA, DEHP, E2, E1, EE2, and NP since these constitute an intolerable risk to the environment. BPA was found to be an intolerable risk in drinking water as well. OP constitutes a tolerable risk (the data taken into account). CBZ, ibuprofen, and naproxen were added to a list for further investigations.

The third criterion was fulfilled by BPA, DEHP, E2, E1, EE2, and NP. The criterion was not evaluated for XOCs not fulfilling the second criterion. NP is characterized as inherently biodegradable and fulfils the third criterion on this basis but conflicting observations exist (ECB, 2002).

Thereby BPA, DEHP, E2, E1, EE2, and NP pass the screening tool because the XOCs are present in WWTP effluents in concentrations constituting an intolerable risk in drinking water and/or the environment and the XOCs are expected biodegradable in WWTPs. These six XOCs are thereby suitable as model pollutants to investigate biodegradation in WWTPs. OP was rejected as model pollutant. CBZ, ibuprofen, and naproxen were added to a list for further investigations due to insufficient data and should be reassessed when sufficient data are available.

Table 3.4: Risk screening and biodegradability assessment of XOCs (Press-Kristensen et al., 2007a)
 1) Insufficient data concerning specific toxicity. 2) Inherently biodegradable.

Criterion 2: Risk screening							Criterion 3
XOCs	Mammal toxicity	Ecotoxicity	WSG (ng/l)	PNEC (ng/l)	Is the XOC an intolerable risk ?		Is the XOC expected to be biodegradable in WWTPs ? (Yes / No / ?)
					No. of: Median / Mean / Max. (total)		
					PEC > WSG	PEC > PNEC	
BPA	2 µg/kg/d (LOAEL) (Kawai et al., 2002)	10 µg/l (LOEC) (Tabata et al., 2001)	6	100	Yes / Yes / Yes 2 (5) / 4 (6) / 5 (7)	No / No / Yes 0 (5) / 0 (6) / 3 (7)	Yes (Lobos et al., 1992)
CBZ	200 mg/kg (LOAEL) (Novartis, 2003)	Insufficient data ¹⁾	6 · 10 ⁵	---	No / No / No 0 (3) / 0 (4) / 0 (3)	?	Not evaluated
DEHP	30 µg/l (Carcinogenic) (USEPA, 2004)	4 µg/l (LOEC) (Mayer et al., 1977)	30,000	40	No / No / No 0 (4) / 0 (3) / 0 (5)	Yes / Yes / Yes 2 (4) / 3 (3) / 5 (5)	Yes (Brooke et al., 1991)
E2	50 µg/kg/d (LOAEL) (Delongas et al., 1994)	4 ng/l (LOEC) (Metcalfé et al., 2001)	150	0.04	No / No / No 0 (7) / 0 (3) / 0 (7)	Yes / Yes / Yes 7 (7) / 3 (3) / 7 (7)	Yes (Ternes et al., 1999)
E1	Insufficient data ¹⁾	8 ng/l (LOEC) (Metcalfé et al., 2001)	---	0.08	?	Yes / Yes / Yes 6 (6) / 3 (3) / 5 (5)	Yes (Ternes et al., 1999)
EE2	2 µg/kg/d (LOAEL) (Grote et al., 2002)	0,03 ng/l (LOEC) (Metcalfé et al., 2001)	6	0.0003	No / No / No 0 (4) / 0 (1) / 0 (4)	Yes / Yes / Yes 4 (4) / 1 (1) / 4 (4)	Yes (Andersen., 2004)
Ibuprofen	600 mg/kg/d (LOAEL) (Burdan et al., 2004)	Insufficient data ¹⁾	18 · 10 ⁵	---	No / No / No 0 (6) / 0 (6) / 0 (6)	?	Not evaluated
Naproxen	543 mg/kg (LD ₅₀) (Rxlist, 2005)	Insufficient data ¹⁾	16 · 10 ⁵	---	No / No / No 0 (3) / 0 (3) / 0 (3)	?	Not evaluated
NP	15 mg/kg/d (LOAEL) (NTP, 1997)	10 µg/l (LOEC) (Shurin and Dodson, 1997)	45,000	100	No / No / No 0 (8) / 0 (8) / 0 (8)	Yes / Yes / Yes 3 (8) / 4 (8) / 5 (8)	Yes ²⁾ (ECB, 2002)
OP	1 mg/kg/d (LOAEL) (Wright et al., 1999)	39 µg/l (EC ₅₀) (Jobling et al., 1999)	3,000	390	No / No / No 0 (2) / 0 (3) / 0 (2)	No / No / No 0 (2) / 0 (3) / 0 (2)	Not evaluated

3.2.2 Limitations

The screening tool does not directly take bioaccumulation into account. However, bioaccumulation is to a certain extent included in the stated requirements for long-term toxicity tests. Biomagnification, additive, and synergistic effects, bioavailability, and toxic metabolites formed from discharged XOCs are not included. Finally, removal of XOCs during water treatment in water works is not integrated and the PECs do not include background concentrations in recipients. These limitations should be kept in mind below but is not believed to influence the results of this study.

4. Model pollutants

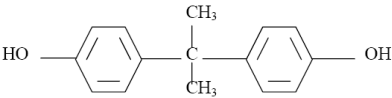
Due to analytical, financial, and time limitations it was only possible to consider BPA, DEHP, and/or NP as model pollutants in this study. However, due to predictable experimental difficulties connected to the extremely low water solubility of DEHP (3 µg/l) and its carcinogenic properties (ECB, 2003b) it was decided not to include DEHP in this study. Since it was expected that a method for simultaneous extraction and quantification of BPA and NP in complex wastewater samples could be developed (chapter 5) and the two compounds were expected to behave quite different with respect to sorption and biodegradation in WWTPs (section 4.1 and 4.2) both compounds were selected as model compounds in this study.

4.1 Bisphenol A

In EU are produced and used around 700,000 tonnes BPA per year of which more than 95 % are used for the production of polycarbonate and epoxy resins (ECB, 2003a). The annual Danish mass of BPA in untreated wastewater has been estimated to 750 kg (DEPA, 2002a).

Table 4.1 gives basic physical-chemical data for BPA.

Table 4.1: Physical-chemical data for Bisphenol A (ECB, 2003a)
1) Average value calculated from Staples et al. (1998).

Chemical formula	$C_{15}H_{16}O_2$
Chemical structure	
Molar weight	228 g/mol
Melting point	155 °C
Boiling point	360 °C
Water solubility	300 mg/l
Dissociation constant (pK _a)	10.4 ¹⁾
Sorption coefficient (K _D)	0.07 m ³ /kg SS
Henry's constant (K _H [*])	1.7 · 10 ⁻⁹

Dissociation is neglected since less than 10 % will be dissociated even if pH reaches 9 in WWTPs (section 2.2). From the sorption coefficient it is seen that BPA is expected to sorp poorly in WWTPs (cf. section 2.2.1). However, Clara et al. (2004) find a sorption coefficient about 1 m³/kg SS and conclude that sorption may cause significant removal. Henry's constant shows that stripping and volatilization are irrelevant (cf. section 2.2.2).

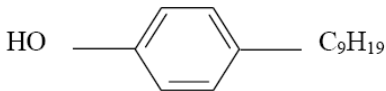
Several full-scale studies have shown a removal of 70-90 % for BPA in large WWTPs (Gómez et al., 2006; Fuerhacker, 2003; ECB, 2003a; Snyder, 2002). However, removal efficiencies down to around 40 % have been reported (DGPWWM, 2002; ECB, 2002) as well as more than 95 % removal (Tanaka et al., 2002). In Denmark the average influent concentration in nine routine monitored WWTPs was 2.35 µg BPA per litre and the removal was around 80 % (NOVA, 2005).

DEPA (2002a) concludes that BPA is readily biodegradable under aerobic conditions and that biodegradation under anoxic and anaerobic conditions is unlikely. DEPA (2002a) estimates the fate of BPA in Danish WWTPs to be: 12 % lost to recipients with the effluents, 6 % sorped to the sludge, and 82 % biodegraded in WWTPs.

4.2 Nonylphenol

In EU are produced and used around 75,000 tonnes NP per year of which more than 95 % are used for the production of detergents (nonylphenol ethoxylates), resins, plastics, stabilisers etc. (ECB 2002). The annual Danish mass of nonylphenolic compounds in untreated wastewater has been estimated to 50-1,000 tons (DEPA, 2002a). Table 4.2 gives physical-chemical data.

Table 4.2: Physical-chemical data for nonylphenol (ECB, 2002)

Chemical formula	$C_{15}H_{24}O$
Chemical structure	
Molar weight	220 g/mol
Density	0.95 kg/l
Melting point	- 8 °C
Boiling point	300 °C
Water solubility	6 mg/l
Dissociation constant (pK _a)	10
Sorption coefficient (K _D)	0.55 m ³ /kgSS
Henry's constant (K _H [*])	4,7 · 10 ⁻³

Dissociation is neglected since less than 10 % will be dissociated even if pH reaches 9 in WWTPs (section 2.2). From the sorption coefficient and Henry's constant is seen that to some extent NP is expected to sorp in WWTPs (cf. section 2.2.1) and be stripped to the air (cf. section 2.2.2). However, ECB (2002) does not see stripping as an important removal mechanism in WWTPs. This conclusion is in direct contradiction to Porter and Hayden (2001) that find volatilization to be a significant removal processes in WWTPs.

In WWTPs NP are formed from the degradation of nonylphenol ethoxylates (NPnEOs) with n ethoxylates groups. NPnEOs are present in WWTP influents due to industrial use and discharge. NPnEOs are often rapidly metabolized to NP1EO or NP2EO in WWTPs. Under aerobic conditions NP1EO and NP2EO can be further oxidised to nonylphenol carboxylates (NP1EC and NP2EC respectively), NP or completely mineralized. NP1EC and NP2EC seem to be favoured over NP under aerobic conditions whereas NP seems to be the primarily metabolite from NP1EO and NP2EO under anaerobic conditions (ECB, 2002; Komori et al., 2006; Fujita et al., 2000).

Table 4.3 gives an overview from fate studies of NPnEOs in WWTPs.

Table 4.3: Fate of NP and NPnEOs in WWTPs (mole basis)

Keller et al (2003) did not measure NPnEC which introduces an uncertainty.
 1) Combination of biodegradation and removal with biological waste sludge.
 2) Only sludge from primary clarifier.
 3) Only Plant 1 from this study (only plant with nitrification/denitrification).

	(ECB, 2002)	(Ahel et al., 1994)	(Keller et al., 2003) ³⁾
Mineralized or aromatic ring broken	45 %	< 40 % ¹⁾	5 %
Effluent as NPnEO/NPnEC (n = 1,2)	25 %	30 %	51.5 %
Effluent as NPnEOs (n ≥ 3)	8 %	8 %	
Effluent as NP	2.5 %	2 %	
Accumulated in sludge	19.5 %	> 20 % ²⁾	43.5 %

NP in form of NPnEOs have been found to account for the main part of NP (mole basis) in WWTP influents (Komori et al., 2006; González et al., 2004; Shao et al., 2003; Fujita et al., 2000; Lee and Peat, 1998; Ahel et al., 1994). Therefore NPnEO can contribute significantly to the formation of NP in WWTPs and thereby to the concentration of NP in the effluents. The formation of NP thereby becomes a key parameter when estimation the removal of NP in WWTPs. In Denmark the sum of NP and NP1EO has been found to account for more than 60 % of the total nonylphenolic compounds (mole basis) in WWTP influents (DEPA, 2002b) indicating significant degradation before the WWTPs. Similar results was reported by Shao et al. (2003). In Denmark the average influent concentration in nine routine monitored WWTPs was 1.35 µg NP per litre and the effluent concentration was around 0.52 µg NP per litre (NOVA, 2005).

ECB (2002) estimates the fate of NP (exclusive NPnEOs) in WWTPs to be: 35 % in the effluent, 34 % in the sludge, 24 % biodegraded, and 7 % removed by volatilization, if NP can be considered to be inherently biodegradable. If NP is considered to be persistent the fate of NP in WWTPs is estimated to be: 53 % in the effluent, 37 % in the sludge, 0 % biodegraded, and 10 % removed by volatilization.

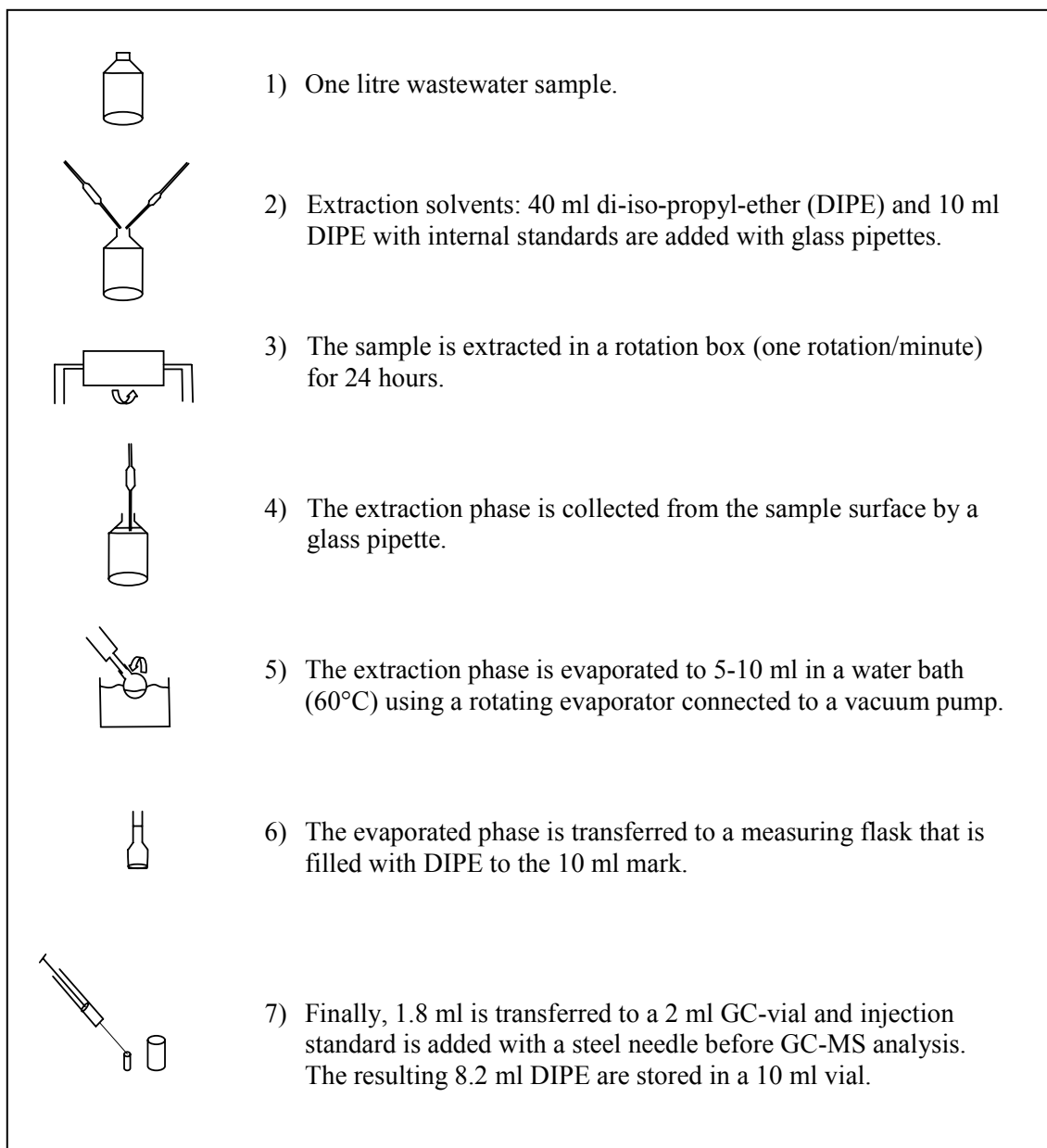
In experiments performed in this study it was not possible to measure NPnEO (n ≥ 3) and NPnEC. The resulting consequences are further discussed below (section 6.1.1).

5. Development of analytical methods

Only a few studies describe simultaneous extraction and quantification of BPA and NP in complex wastewater matrices (Meesters and Schröder, 2002) and the methods and/or apparatus used were not available in this study. Therefore a robust and simple method for simultaneous extraction and GC-MS quantification of BPA and NP was developed and validated with influent, effluent, and sludge from a WWTP (appendix IV).

Figure 5.1 illustrates the extraction principle of the method.

Figure 5.1: The extraction includes seven basic steps (Press-Kristensen et al., 2007d)
To reach recoveries of the internal standards within 100 ± 25 % in sludge samples were used a di-iso-propyl-ether to dichloromethane ratio of 5 to 2.



5.1 Validation

The detection limit (DL) and quantification limit (QL) were determined in wastewater influent as well as the repeatability of the GC-MS and the recovery of BPA and NP in different wastewater matrixes. Finally, the method was subjected to external quality assurance by comparing the results with results from replicate analysis obtained from an external accredited laboratory.

5.1.1 Detection and quantification limit

The obtained DLs/QLs limits in WWTP influent were 0.6/1.4 µg/l and 0.6/1.7 µg/l for BPA and NP, respectively. DLs and QLs of this study are a factor 100 lower than the results reported by (Meesters and Schröder, 2002). This could be due to the fact that Meesters and Schröder (2002) investigates wastewater sludge and not wastewater influent. However, the obtained DLs and QLs are higher than results reported by Kuklenyik et al. (2003) and Cai et al. (2003). The explanation is probably that Kuklenyik et al. (2003) analyse urine and Cai et al. (2003) analyse natural waters i.e. less complex matrixes than wastewater influent. Specialized analytical methods targeting either BPA or NP often result in significant lower DLs and QLs (Kawaguchi et al., 2004; Gómez et al., 2006; Liu et al., 2003; Xinmei et al., 2006).

The DLs and QLs may improve using larger sample volumes, further condensing of the extract, a larger injection volume, derivatization, and different clean-up steps. In theory, extracting two litre wastewater samples, condensing the extract to 1 ml, and injecting a volume of 8 µl would improve the DL and QL a factor 80. This would make the method competitive to the mentioned specialized analytical methods targeting either BPA or NP. However, since the aim was to develop a robust and simple method and the obtained DLs and QLs were sufficient for the experiments (Chapter 5) no further optimization of the method was performed.

5.1.2 Repeatability and recovery

The repeatability of the GC-MS including data quantification were robust i.e. 95 % of the observations from ten runs in a row of BPA, NP and standards were contained in an interval of ± 10 % of the mean. Thereby the apparatus and data quantification only induced a minor uncertainty.

The recovery from standard addition to different wastewater matrixes were measured and calculated in absolute concentrations and percentage (Table 5.1). The recovery of the injection standard was within the calculated uncertainty of the GC-MS described above i.e. ± 10 %. The recovery of the internal standards was in general 100 ± 10 % in the influent and effluent samples, 100 ± 25 % in sludge samples extracted with di-isopropyl-ether / dichloromethane, and 100 ± 40 % in sludge samples extracted with DIPE.

The final concentration of BPA and NP in sludge samples adjusted by internal standards did not seem to vary significantly between the two extraction methods.

Table 5.1: Recovery (standard addition) in wastewater (Press-Kristensen et al., 2007d)

1) Trace concentration i.e. above the DL but below the QL.

DL: Detection limit. QL: Quantification limit. SD: Standard Deviation

	No. of replicates	Addition of BPA/ NP (+ µg/l)	Measured in µg/l (SD)		Recovery in µg/l (%)	
			BPA	NP	BPA	NP
Mean influent	5	0	6.0	9.0	---	---
Influent (high)	5	100 / 100	98 (11)	118 (4)	92 (92)	109 (109)
Mean influent	2	0	13.6 (1.0)	4.0 (0.5)	---	---
Influent (low)	2	8.0 / 8.5	18.9 (0.9)	13.1 (1.3)	5.3 (66)	9.1 (107)
Influent (low)	2	16.0 / 17.0	26.5 (0.6)	20.1 (0.8)	12.9 (80)	16.1 (95)
Mean effluent	3	0	1.0 ¹⁾ (0.1)	3.1 (0.5)	---	---
Effluent (low)	1	5.0 / 5.0	6.1	8.1	5.1 (102)	5.0 (100)
Effluent (low)	1	5.0 / 5.0	6.8	7.0	5.8 (116)	3.9 (78)
Active sludge	3	0	1,084 (55)	318 (15)	---	---
Active sludge	5	140 / 113	1,208 (39)	434 (72)	124 (88)	116 (102)
Active sludge	5	280 / 226	1,380 (105)	429 (65)	296 (106)	111 (49)

The standard deviation was generally 5-10 % which is satisfying in wastewater samples. The recovery of BPA and NP in the high spike experiment was within the uncertainty margin of ± 10 % pointed out above. The same was the case in half of the influent and effluent samples in low spike experiments (Table 5.1). Seven of eight influent and effluent samples in low spike experiments had recoveries of 100 ± 25 %. The reduced recovery might be explained by the general uncertainty arising when working in the µg-area with wastewater samples not being completely homogeneous.

The recovery in influent, effluent, and sludge samples are comparable to the recoveries reported elsewhere (Meesters, 2002; Cai et al., 2004; Gómez et al., 2006; Xinmei et al., 2006). However, the poor recovery for NP (49 %) in sludge samples when adding 226 µg/l could not be explained but could arise from uncertainty related to the high background concentrations in the sludge samples.

On basis of the recovery the method was considered as robust and the uncertainty as acceptable. However, results from NP analysis in sludge should be used with care until the applicability has been clearly defined from more recovery experiments.

5.1.3 External quality assurance

Results obtained from an external accredited laboratory were compared to the results gained from the method described above (Table 5.2). Generally, internal recovery was closer to 100 % than the accredited laboratory. But neither the internal recoveries nor the recoveries of the external laboratory were impressive.

The NP measurements show that the accredited laboratory measured significant lower concentrations than us. The same was the case in three out of four BPA measurements. However, the deviation can not be explained by a factor-difference in the standards rows since the deviation is not a fixed factor and the results do not indicate any systematic errors in the standard addition.

Table 5.2: Internal versus external laboratory results (Press-Kristensen et al., 2007d)

	Addition of BPA and NP (+ µg/l) ¹⁾	Measured in µg/l		Recovery in µg/l (%)	
		BPA	NP	BPA	NP
Effluent	0	2	4	---	---
Accredited lab.	10	12	7.4	10 (100)	3.4 (34)
Own results	10	11	13	9 (90)	9 (90)
Effluent	0	3	3	---	---
Accredited lab.	20	18	15	15 (75)	12 (60)
Own results	20	27	23	24 (120)	20 (100)
Influent	0	8	5	---	---
Accredited lab.	20	22	15	14 (70)	10 (50)
Own results	20	36	31	28 (140)	26 (130)
Bio-P sludge	0	---	---	---	---
Accredited lab.	0	340	479	---	---
Own results	0	550	703	---	---

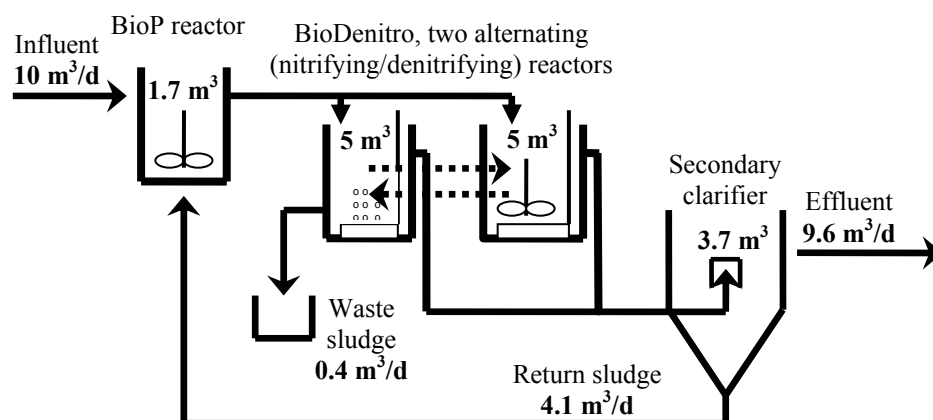
On basis of the external quality assurance the method was considered as applicable for the planned experiments.

6. Biodegradation experiments

Experiments were performed in a pilot plant (Figure 6.1) placed next to Lynetten wastewater treatment plant that receives a mixture of urban and industrial wastewater from Copenhagen in Denmark (appendix II and appendix III).

The pilot plant is operated as the activated sludge system of the full scale plant (scale 1:14,700) and includes an anaerobic BioP reactor, two alternating aerobic/anoxic BioN reactors (BioDenitro principle) and one secondary clarifier. The plant treats pre-clarified wastewater from the full scale plant. Figure 6.1 shows dimensions and typical flow settings which yield a HRT of 1.2 days and a SRT of 29 days. The aerobic time among the two BioDenitro reactors is around 50 % under standard operation. The influent contains about 370 mg total COD/l, 50 mg total N/l, and 9 mg total P/l. Removal efficiencies are around 90 % for total COD and 80 % for nitrogen and phosphorus.

Figure 6.1: Pilot plant dimensions and typical flow rates (Press-Kristensen et al., 2007b)



Grab samples were taken with a two-litre jug made of polypropylene. The jug was filled with wastewater and emptied before sampling. One litre blue cap bottles were used as containers. The bottles were flushed and cleaned with 500 ml of tap water five times before use. Sodium azide (2.5 g) was added as preserving agent. One litre of wastewater was added to each bottle when sampling. The samples were stored at $15 \text{ }^\circ\text{C}$ less than three weeks before extraction.

Composite 24h samples from the influent and effluent were collected by an auto-sampler (50 ml sample every 25 min.) in 2 five litre blue cap bottles placed in a refrigerator ($4 \text{ }^\circ\text{C}$). The hoses to and from the auto-sampler were non-sorbing Teflon hoses. Samples from the five litre blue cap bottles were transferred to one litre blue cap bottles and conserved as described above.

6.1 Experimental set-up

Five types of experiments were performed:

- 1) Background concentrations
- 2) Step-addition experiments
- 3) Mass-balance experiments
- 4) Batch experiments
- 5) An optimization experiment

In the background experiments were characterized influent and effluent concentrations of BPA and NP to find the removal during standard operation and to determine how much BPA and NP to dose in the further experiments to avoid noise from fluctuating background concentrations.

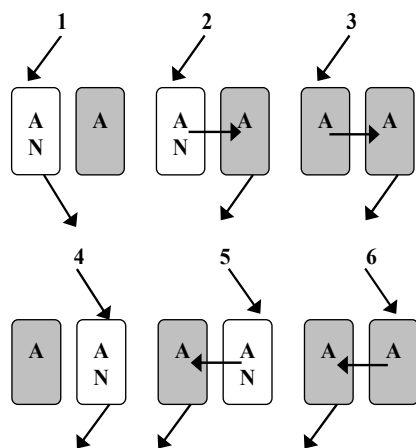
Step-addition experiments were conducted during different operational conditions to investigate the required adaptation time and to identify model parameters. BPA and NP were continuously dosed from an acetone solution by a hose pump to the influent and samples were taken in the effluent.

Mass-balance experiments were conducted in the end of the step-addition experiments by measuring BPA in flows to identify and quantify removal processes.

Batch experiments were performed to investigate BPA removal during different redox (aerobic, anoxic, and anaerobic) conditions with unadapted and preadapted biomass i.e. the three pilot plant reactors operated in batch mode.

An optimization experiment with increased aerobic phase time (Figure 6.2) was performed on basis of the results from the batch experiments.

Figure 6.2: Phases used during optimization experiment (Press-Kristensen et al., 2007c).
A: Aerobic conditions. AN: Anoxic conditions.



Phase times during optimization experiment

	Time per reactor	
	Standard	Optimized
Phase 1 and 4	18 min.	18 min.
Phase 2 and 5	72 min.	36 min.
Phase 3 and 6	0 min.	36 min.
Aerobic time	50 %	70 %

Table 6.1 lists operational data for the pilot plant during the performed experiments. The aerobic hydraulic retention time (HRT_{aer}) varies due to varying influent flow rates and the current BioDenitro settings.

Table 6.1: Operational settings during experiments

Modified from Press-Kristensen et al. (2007b). 1) Prior to start of experiment.

Experiments	Period	Dosed BPA and NP (g/d)	Inflow (m^3/d)	HRT_{aer} (days)	SRT (days)	Temp. ($^{\circ}C$)
Background	Winter 2005	0.00	10	0.50	27	13-16
Step-addition	Spring 2005	1.00	10	0.50	27	16-18
	Summer 2005	10.00	10	0.50	27	19-22
	Autumn 2006	10.00	12	0.38	26-52	15-16
Mass-balance	Winter 2005	15.00	12	0.33	26-52 ¹⁾	15
	Winter 2006	10.00	12	0.38	26-52 ¹⁾	16
Batch	Autumn 2006	0.00 ¹⁾	12 ¹⁾	0.38 ¹⁾	26 ¹⁾	20
	Winter 2006	10.00 ¹⁾	12 ¹⁾	0.38 ¹⁾	26-52 ¹⁾	16
Optimization	Winter 2007	0.00	12	0.38	33	13

6.1.1 Experimental considerations

It was not possible to measure NPnEO and NPnEC in this study and thereby it was not possible to determine the formation of NP in the pilot plant (section 4.2). But due to dosage of NP to the influent (Table 6.1) the formation of NP from NPnEO was not believed influence results from the step-addition, mass-balance, and batch experiments. Even if NPnEO accounted for 95 % of the nonylphenolic compounds (mole basis) in the influent (reasonable worst case) the formation of NP would not contribute notable to the NP concentration in experiments with NP dosage. However, the formation of NP in the plant did probably increase the measured effluent concentrations of NP when mapping the background concentrations and could have influenced the results of the optimization experiment (section 7.2).

Significant volatilization of NP could influence the results dramatically. According to ECB (2002) can generally be assumed that 7-10 % NP is removed due to volatilization in WWTPs. However, this will depend on design and operation of the specific WWTPs. Furthermore, this is in direct contradiction to Porter and Hayden (2001) that predict up to 75 % NP removal due to volatilization in WWTPs. Since a significant volatilization has not been reported by other studies was assumed a removal of 7-10 % due to volatilization in this study. Furthermore, Porter and Hayden (2001) could overestimate the volatilization since they do not consider biodegradation and sorption as competitive processes in WWTPs. However, significant volatilization could be stimulated by the aberrant high concentrations of NP due to dosage (Table 6.1) in this study and may explain some of the findings for NP in the step-addition experiment (section 6.2.2).

6.2 Results and discussion

All analyses were performed using the method described in chapter 5. However, in experiments from 2005 phenanthrene-d₁₀ was used as internal standard for BPA instead of bisphenol A-d₆ (appendix IV). This is not believed to influence significantly on the results and conclusions in this study since most experiments from 2005 included influent and effluent samples with good recoveries (section 5.1.2). However, sludge analysis in the mass-balance experiment performed in the winter 2005 could be affected but not in a degree that is expected to cause notable changes in the results.

6.2.1 Background concentrations

Background concentrations of BPA and NP in the pilot plant were measured in influent and effluent samples during the winter 2005 (Table 6.2).

Table 6.2 Background concentrations in µg/l

1) Trace concentration i.e. above the DL but below the QL.

DL: Detection limit. QL: Quantification limit. SD: Standard Deviation.

		Median	Mean	Minimum	Maximum	SD
BPA	Influent	7.9	8.8	5.5	19.0	3.3
	Effluent	3.4	4.0	1.0 ¹⁾	11.1	2.8
NP	Influent	4.2	6.7	1.9	34.5	7.0
	Effluent	4.2	5.8	1.0 ¹⁾	24.5	5.7

The background concentrations are based upon analysis of 26 influent and 19 effluent samples covering both grab (morning, lunch, and afternoon) as well as composite 24h samples. No significant differences were observed between grab samples and composite samples and no clear tendencies were observed between different week days or times.

The measured influent concentrations of BPA are significantly higher than reported elsewhere (Gómez et al., 2006; NOVA, 2005; Tanaka et al., 2003; Snyder et al., 2002) even though the influent to the pilot plant was clarified. This could reflect the impact of local industries. NP concentrations were comparable to concentrations found in other studies (Tanaka et al., 2003; DEPA, 2002b). The measured effluent BPA and NP concentrations from the pilot plant are significantly above concentrations reported in other studies (Table 3.2).

Measured median and mean influent concentrations can not be directly linked to the measured effluent concentrations. However, the median and mean concentrations indicate that the BPA concentration is reduced about 50 % and the NP concentration is almost unchanged during treatment. The observed reduction of BPA is probably due to biodegradation since BPA is biodegradable, non-volatile, poorly sorbing, and hydrolysis or chemical degradation have not been reported. BPA removal is comparable to results from other studies (section 4.1). NP concentrations can not be compared to other studies since concentrations of NPnEOs and NPnECs were not measured (section 6.1.1).

However, the unchanged NP concentration is probably a result of NP formation from NPnEOs in the pilot plant (section 4.2).

The background influent concentrations were monitored continuously during the experiments described below and did only change significantly in the optimization experiment (section 6.2.5).

6.2.2 Step-additions

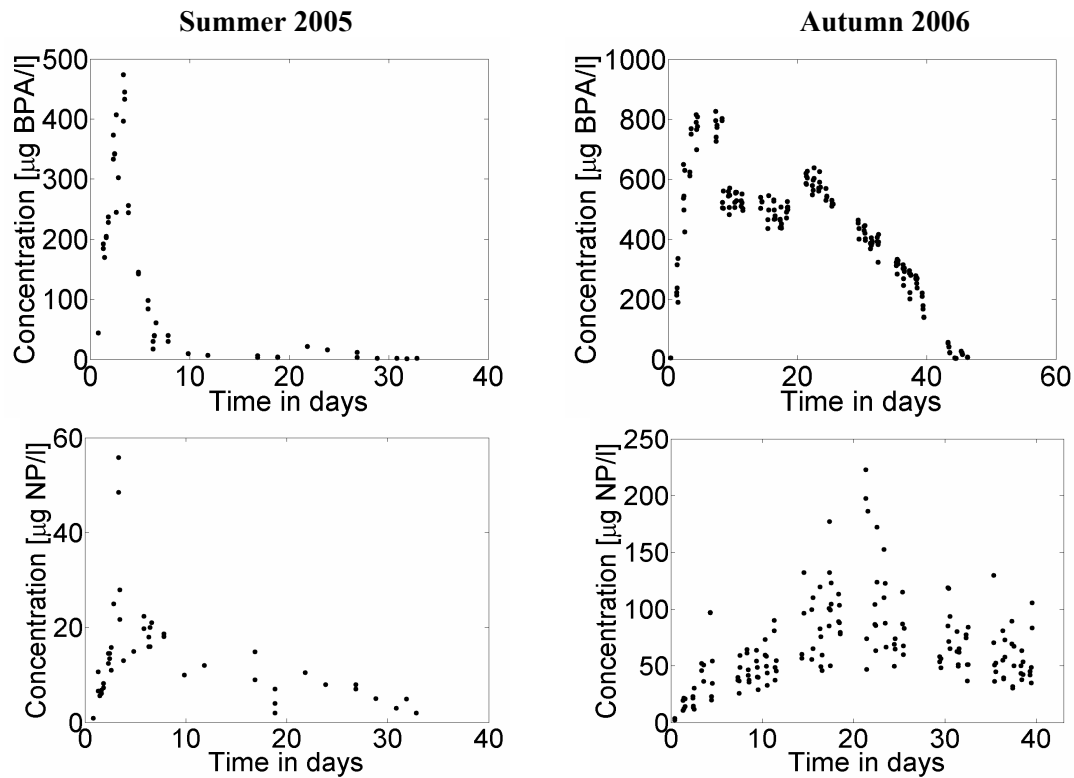
Three step-addition experiments where BPA and NP were dosed continuously to the influent were performed during 2005 and 2006 (Table 6.1). The effluent concentrations were measured as grab and composite 24h samples. No significant breakthrough peaks were observed in the effluent during the spring experiment when dosing 1 g BPA and NP per day to the influent. However, there was a clear tendency to increased BPA concentrations in the effluent during the first week of dosage (results not shown).

When increasing the dosage to 10 g BPA and NP per day (summer 2005) breakthrough peaks were observed in the effluent (Figure 6.3, left). Breakthrough peaks were most significant in the grab samples compared to composite 24h samples and were quickest observed and most clear for BPA. The summer experiment breakthrough curves peaked after 3 days for BPA and NP and reached background levels after 10 days for BPA and 20 days for NP. In autumn 2006 breakthrough curves were more significant compared to the summer experiment in 2005 and peaked after 4 days for BPA and 23 days for NP (Figure 6.3, right). Concentrations reached the background after approximately 43 days for BPA. NP did not reach the background concentrations during the experiment. Since the abiotic removal mechanisms did not change during the experiment biodegradation is believed to have caused the post-peak concentration decrease in the effluent.

The less significant breakthrough peaks for NP may be explained by a significant volatilization of NP (section 6.1.1).

The rapid removal in the summer experiment compared to the autumn experiment is probably caused by preadaptation of the biomass in the summer experiment due to the previous dosage in the spring experiment, which was finalized 14 days before the summer experiment. Furthermore, a higher sludge temperature and aerobic hydraulic retention time in the summer experiment have probably stimulated the biodegradation (table 6.1). This hypothesis was confirmed for BPA by the process model (section 7.1).

Figure 6.3 Breakthrough curves during step-addition (Press-Kristensen et al., 2007b)
Step-addition with dosage of 10 g BPA and NP per day to the influent.



Finally, the results show that effluent concentrations of BPA and NP at steady-state in the pilot plant are comparable to the background concentrations even when the influent concentrations are increased around a factor 100-200 due to dosage. This observation was later confirmed for BPA by calculations in the process model (section 7.1) and underlines the potential of biodegradation in activated sludge systems.

6.2.3 Mass-balances

Mass-balance experiments were performed during the winter 2005 and 2006 (Table 6.1). BPA concentrations were measured (five replicates) at various locations in the pilot plant under assumed steady-state conditions (Table 6.3) which together with flow data were used to calculate mass balances (Table 6.4). Steady-state conditions were assumed when effluent concentrations stabilized around the background concentrations after a step-addition experiment.

Table 6.3: BPA (µg/l) in pilot plant flows

- 1) Calculated on basis of flows, concentrations, and dosage of BPA.
 - 2) Trace concentration i.e. above the DL but below the QL.
- DL: Detection limit. QL: Quantification limit. SD: Standard Deviation.

	Influent	After dosage	Waste sludge	Recirculation	Effluent
Winter 2005 (SD)	16 (5.2)	932 ¹⁾	95 (9)	118 (39)	1.3 ²⁾ (0.2)
Winter 2006 (SD)	5.0 (1.2)	607 ¹⁾	15 (1)	44 (19)	9.4 (2.4)

Table 6.4: Mass-balance for BPA in mg/d

BPA (mg/d)	Winter 2005	Winter 2006
Influent	192	60
Addition	15,000	10,000
Total input	15,192	10,060
Effluent	15	110
Waste sludge	23	4
Total output	38	114
Biodegradation (%)	15,154 (99.7)	9,946 (98.9)

As suggested above, biodegradation is believed to cause the BPA degradation in the pilot plant. Similar experiments showed that around 90 % of dosed NP was biodegraded (results not shown due to analytical uncertainties c.f. section 5.1.2) assuming a removal of 7 % due to volatilization (ECB, 2002). A removal/biodegradation above 99.5 % for BPA is a vast improvement compared to other studies (section 4.1) and the removal estimated during the measurements of the background concentrations (section 6.2.1). However, the biodegradation is enhanced by the dosage of BPA resulting in an aberrant high influent concentration.

6.2.4 Batch experiments

Removal of BPA under aerobic, anoxic, and anaerobic conditions with unadapted and preadapted biomass was studied by operating the three plant reactors in batch mode before (unadapted) and after (adapted) a step-addition experiment. However, unadapted biomass had been exposed to background BPA concentrations but the biomass was not adapted to the high concentrations investigated in the batch experiments.

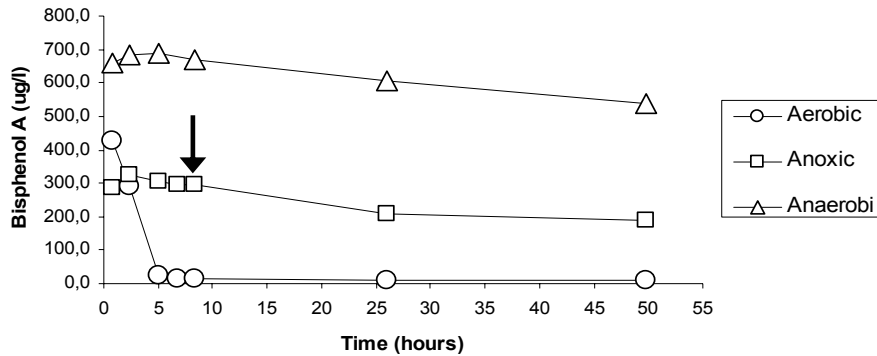
In the anoxic experiments nitrate concentration was kept between 5 and 30 mg/l by adding KNO_3 and NaNO_3 . After 8.5 hours nitrate addition was stopped and the anoxic reactor probably became anaerobic.

In the experiment with unadapted biomass no significant removal of BPA in any of the three reactors was seen (results not shown).

The experiment with preadapted biomass (Figure 6.4) shows a significant removal of BPA under aerobic conditions and no removal during anoxic and anaerobic conditions. These observations are in accordance with conclusions drawn by Cousins et al. (2002) and DEPA (2002a). The fastest observed degradation rate was around 1.6 μg per litre per minute (assuming 0. order kinetics due to high concentrations) which would be sufficient to remove the mean background concentration within 5-10 minutes if the rate was transferable. However, the adaption to an aberrant high influent concentration prior to the batch experiment did probably enhance the biodegradation as reflected by the poor aerobic degradation from 5 to 26 hours and since no further degradation was seen.

However, the poor degradation could, in theory, be due to other parameters affecting the biodegradation e.g. the remaining BPA could be strongly sorbed and thereby not bioavailable or the biomass could be inhibited by the irregular operation i.e. constant aeration in batch for several days without new substrate addition.

Figure 6.4: Removal of BPA under different redox conditions with preadapted biomass
After 8.5 hours the nitrate addition was stopped and the anoxic reactor probably became anaerobic (arrow).



6.2.5 Optimization experiment

The batch experiments showed that BPA only seemed to be degraded under aerobic conditions and since NP is degraded aerobic (section 4.2) as well was performed an optimization experiment with increased aerobic phase time (Figure 6.2). Two weeks before the optimized operation the influent and effluent concentrations were monitored during standard operation. Composite 24h samples (two replicates) were used to reduce the influence of fluctuating concentrations. Table 6.5 sums up the results.

During standard operation the median and mean influent concentrations of BPA were much higher than earlier measured whereas effluent concentrations were close to previously findings (section 6.2.1). Unfortunately, vital influent data from the week just before the optimized operation were lost which complicated the later interpretation and modelling of the results (section 7.2).

During standard operation the median and mean influent concentrations of NP were in the same range as earlier observed whereas effluent concentrations were lower. Unfortunately, influent data from the week just before the optimized operation were lost. During optimized operation the median and mean influent and effluent concentrations were lower than previously observed (section 6.2.1). However, since NPnEO and NPnEC were not measured it was not possible to determine if the removal of NP was increased during optimization (section 4.2).

Table 6.5: Results ($\mu\text{g/l}$) from an optimization experiment (Press-Kristensen et al., 2007c).
 1) Trace concentration i.e. above the DL but below the QL. DL: Detection limit.
 QL: Quantification limit. SD: Standard Deviation. No: Number of samples.

Operational conditions			Median	Mean	Minimum	Maximum	SD	No
BPA	Standard	Influent	21	19	2.5	33	8.7	15
		Effluent	2.6	3.8	< 0.6	13	3.1	28
	Optimized	Influent	5.6	6.3	2.7	14	2.9	14
		Effluent	< 0.6	< 0.6	< 0.6	1.3 ¹⁾	< 0.6	16
NP	Standard	Influent	5.2	5.9	2.0	11.2	2.6	15
		Effluent	1.9	2.0	< 0.6	3.7	0.8	28
	Optimized	Influent	3.0	3.1	2.1	5.6	0.9	14
		Effluent	< 0.6	0.7 ¹⁾	< 0.6	1.2 ¹⁾	< 0.6	16

Due to the higher influent concentrations measured before the optimized operation and the lost influent data it was impossible to state from the measurements if the lower effluent concentrations under optimized operation were caused by increased aerobic phase time, a larger specific biomass to BPA and NP ratio, or a combination. This is further discussed for BPA using the process model (section 7.2).

Influent and effluent concentrations of COD, nitrogen, and phosphorus were monitored in composite 24h samples in the weeks before, during, and after the optimization. The results from before and after are given as one number in standard operation (Table 6.6).

Table 6.6: Mean concentrations of COD, nitrogen, and phosphorus in mg/l
 SD: Standard Deviation. No: Number of samples.

Operation	Influent				Effluent			
	Standard	No.	Optimized	No.	Standard	No.	Optimized	No.
COD in mg/l (SD)	350 (99)	5	280 (14)	2	27 (5)	5	25 (0)	2
Total N in mg/l (SD)	36 (11)	4	29 (2.8)	2	7.5 (1.8)	4	11.5 (1.5)	2
Total P in mg/l (SD)	7 (2.8)	4	6 (0.2)	2	1.1 (0.4)	4	1.9 (0.4)	2

Removal of COD is unchanged during optimization whereas the removal of nitrogen and phosphorus are reduced and the limit values, 8 mg total N per litre and 1.5 mg total P per litre in the effluent, are no longer fulfilled. This may be expected since decreased anoxic time for denitrification theoretically can reduce removal of nitrogen and disturb accumulation of P in the biomass. Increased nitrate concentrations in the effluent during optimized operation confirmed the hypothesis of reduced denitrification. The results did not indicate an adaption time from standard to optimized operation or vice versa.

This underlines the importance of monitoring traditional wastewater parameters during optimization experiments with XOCs to take other changes in efficiency into account. The challenge is an increased removal of XOCs without spoiling the removal of organic matter and nutrients i.e. a systemic optimization.

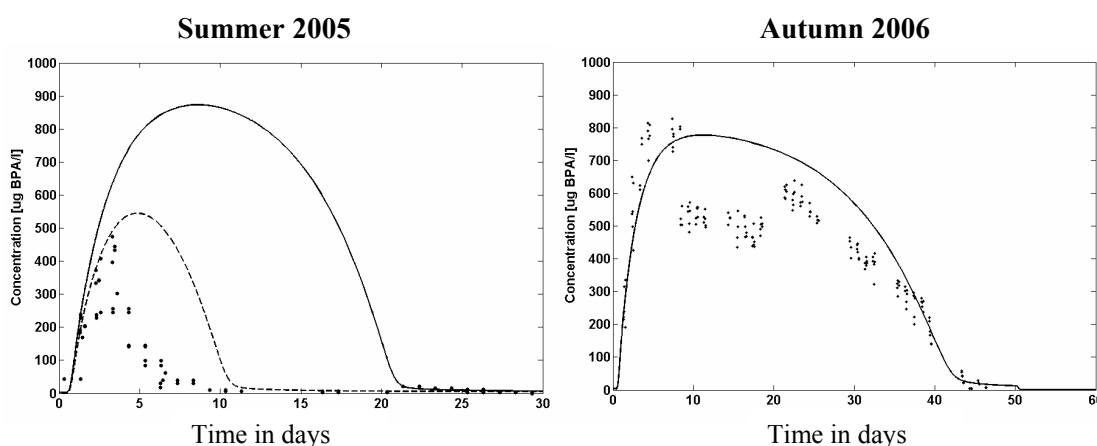
7. Model results

On basis of the data generated from the experiments (chapter 6) were estimated model parameters and developed a growth-based biological/physical/chemical process model for removal of BPA in WWTPs (appendix V). A similar process model (including volatilization) for NP has not yet been developed.

7.1 Model development

The model was developed from the *Activated Sludge Model no. 1* (Henze et al., 2000) extended to include sorption and biodegradation of BPA. The model was constructed by comparing simulations with measurements from the background and step-addition experiments (Lindblom et al., 2007). Figure 7.1 illustrates the principle.

Figure 7.1: Breakthrough curves for BPA in step-addition experiments (Lindblom, 2007) Measurements (points) and simulations (lines) in $\mu\text{g/l}$. Dosage 10 g/d starting at time 0. Solid lines: Unadapted biomass. Dashed line: Preadapted biomass.



In 2005 there was a gap of 14 days without dosage between the two performed step-addition experiments. The rapid removal in the summer experiment indicates that the biomass was preadapted due to dosage in the spring although there was a gap between the two experiments. The SRT of 27 days supports this as a plausible explanation.

However, external conditions did probably affect the outcome in the two experiments as well. The summer experiment was performed with a mean activated sludge temperature around 21 °C whereas the mean sludge temperature was approximately 16 °C in the autumn. A 5 °C increase can induce significant changes in biomass properties which in the *Activated Sludge Model no. 1* are coped with increasing the heterotrophic maximum growth rate and decay rate with 40 % (Lindblom, 2007). The experiments were, as well, conducted under different operational conditions. The autumn experiment had a 20 % higher inflow (12 m³/d) compared to the summer experiment (10 m³/d) which together with slightly different phase operation resulted in 25 % less nitrifying time. In the summer experiment the SRT was 27 days while the autumn experiment was initiated at a SRT of 26 days changed to 52 days two days within the experiment (Table 6.1).

The simulations in Figure 7.1 show the effluent BPA concentrations in the step-addition experiments (summer 2005 and autumn 2006). The parameters and constants used in the process model in the two step-addition experiments are given in Lindblom et al. (2007). The variations are caused by different sets of kinetics and operational parameters as well as different initial conditions (unadapted or preadapted biomass) mentioned above.

The model approach followed three steps according to Lindblom (2007):

1) Figure 7.1 (right)

The model was initialised by simulating steady-state conditions with a constant input according to the measured background concentrations. Operational conditions were as defined for the autumn experiment (Table 6.1). Subsequently a step increase of 10 g BPA per day in the influent followed. The parameters were varied manually to an acceptable fit between measured and simulated effluent concentrations. Background removal of BPA was set to 50 % at time 0 (section 6.2.1).

2) Figure 7.1 (left, solid line)

The operational parameters were adjusted to reflect the summer conditions (Table 6.1) and the effluent concentrations were simulated (solid line) as explained in 1) above.

3. Figure 7.1 (left, dashed line)

The same parameters as in 2) were applied but the model was initialised by simulating the steady-state with a constant input according to background concentrations followed by a step increase of 1 g BPA per day in the influent lasting 60 days (spring 2005). Subsequently, the dosage was stopped for 14 days and then resumed with 10 g BPA/d (summer 2005). The result is shown as the dashed line in Figure 7.1 (left).

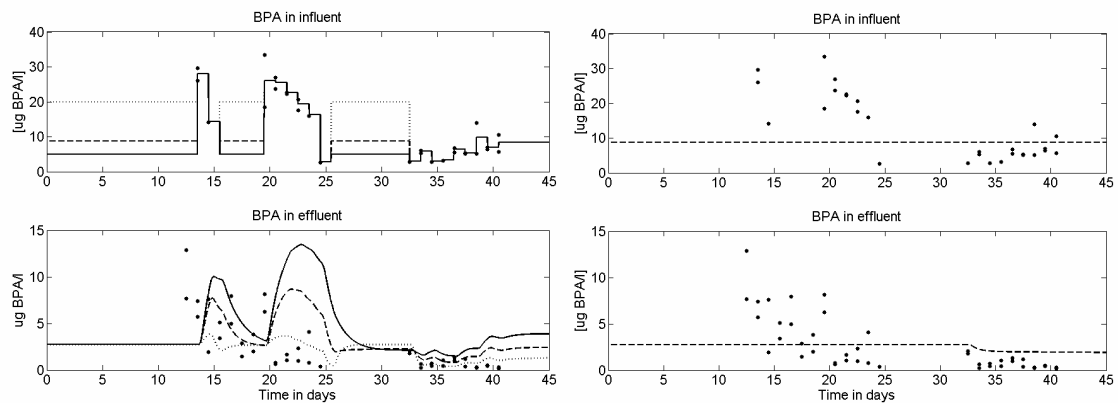
In summary, results from the step-addition experiment during the autumn 2006 were used to select optimum model parameters. These were then modified to reflect the conditions in the step-addition experiment during the summer 2005. The results indicate that the model seems to capture important features of the observations. The simulations show that the faster degradation in the summer experiment only partly can be described by varying temperature and operational conditions (Figure 7.1, left, solid line). A significantly better model fit is obtained if the history (preadaptation) of the activated sludge is taken into account (Figure 7.1, left, dashed line).

Finally, both results and simulations underline that effluent concentrations of BPA at steady-state were comparable to the background concentrations even when the influent concentrations were increased 100-200 times due to dosage. Biodegradation thereby becomes a powerful removal mechanism in activated sludge systems at WWTPs.

7.2 Modelling as a tool

The model was used to investigate and analyse the results from the optimization experiment for BPA (appendix III). Due to different influent concentrations measured during standard and optimized operation it was difficult to state if the increased aerobic phase time resulted in improved biodegradation of BPA as expected (section 6.2.5). The lost influent data further complicated the process and necessitated different scenarios for the influent concentration in the time before the optimized operation (Figure 7.2).

Figure 7.2: Modelling the optimization experiment for BPA (Press-Kristensen et al., 2007c) Dots show measured influent and effluent BPA concentrations. *Left:* Simulated effluent concentrations (bottom) done with dynamic influent concentrations (top) and no operational changes. The model was initiated ($t < 14$ days) with an influent concentration of respectively 20 (dotted), 8.8 (dashed), and 5.0 (solid) $\mu\text{g BPA/l}$. *Right:* Simulated effluent concentrations (bottom) done with constant influent concentration (top) and by modelling the operational change at $t = 32.5$ days.



The left windows show the three simulations (different influent concentrations) that are considered whereas all other parameters and inputs are kept constant. The measured BPA concentrations were thereby used as a dynamic model input. For the days without measurements as well as for the period prior to the experiment (required to initialise the model) three different influent BPA concentrations were assumed. The dashed line scenario is based on background concentrations (section 6.2.1) with a relatively stable mean influent BPA concentration (8.8 $\mu\text{g/l}$). However, the influent concentrations measured before the optimized operation illustrate the need for considering other BPA influent scenarios: The solid line (5 $\mu\text{g/l}$) and dotted line (20 $\mu\text{g/l}$). Higher influent BPA concentrations increase the simulated concentration of specific BPA degrading biomass. The simulation results illustrate that for all three scenarios the effluent concentration was independent of the influent concentration at steady-state as found above (section 7.1). Furthermore, the lower effluent concentration observed during optimized operation could be a result of increased specific biomass to influent BPA ratios caused by a drop in influent concentrations during optimized operation (Press-Kristensen et al., 2007c).

The right windows show a model simulation where the influent BPA concentration is assumed to be stable (8.8 µg/l). At $t = 32.5$ days the optimized operation is simulated. The rather small model response in the effluent was caused by a relatively high applied value of the half-saturation constant for BPA ($K_{\text{BPA}} = 10$ µg/l) determined from other experiments (Lindblom et al., submitted). However, these limited and fluctuating input data cannot motivate a modification of K_{BPA} (Press-Kristensen et al., 2007c).

To sum up, since the drop in the influent concentrations of BPA coincides with the shift between standard and optimized operation and due to the lost influent data it was not possible from the results to determine if the reduced effluent concentrations during optimized operation were caused by a sudden drop in influent concentrations, by more efficient biodegradation, or a combination of the two. However, by using the process model as an interpretation tool it was possible to show that the lower effluent concentrations under optimized operation could be caused by both a combination of increased aerobic phase time and a larger specific biomass to BPA ratio, or alone by a larger specific biomass to BPA ratio. A similar conclusion might be drawn for NP but was further complicated because the formation of NP in the plant and the volatilization of NP were unknown (section 6.1.1).

8. Conclusions

BPA and NP were selected as model pollutants in this study since the two XOCs have been detected in WWTP effluents on a global scale in potential ecotoxic concentrations, are biodegradable, and expected to behave quite differently in activated sludge systems. A robust method for simultaneously extraction and GC-MS quantification of BPA and NP in complex wastewater matrixes was developed, validated, and used in the study.

The BPA concentration was reduced 50-80 % and NP concentration was reduced 10-65 % during standard operation of the pilot plant. Step-addition experiments where the BPA and NP influent concentrations were increased 100-200 times showed that the effluent concentrations were independent of the influent concentrations after an adaption time of 10 to more than 40 days depending on temperature, hydraulic retention time, and pre-exposure of the biomass. Mass-balance experiments suggested that more than 99 % of BPA was removed by biodegradation at steady-state when the influent concentration was increased during step-addition experiments. Batch experiments showed that BPA degradation only occurs under aerobic conditions in the pilot plant. In an optimization experiment with 40 % increased aerobic phase-time the effluent concentrations of BPA and NP dropped to the lowest observed levels in this study.

Based on biological key parameters identified from the performed background and step-addition experiments a process model for removal of XOCs in WWTPs was developed by extending the *Activated Sludge Model no. 1* to include sorption and biodegradation of BPA. The process model confirmed that effluent concentrations of BPA from the pilot plant are independent of influent concentrations at steady-state and proved its success as an interpretation tool when analysing data from the optimization experiment. Thereby the process model is believed to improve the fate assessment of XOCs in WWTPs and thereby the possibility to design and operate WWTPs to remove key XOCs.

9. Perspectives

The findings and results in this study underline the need for:

- 1) Methods to reveal specific ecotoxicity of pharmaceuticals to evaluate the XOCs with insufficient ecotoxicity data (section 3.2.1).
- 2) Investigating the volatilization of NP from aerated activated sludge systems at WWTP (section 6.1.1).
- 3) Investigating if extracellular enzymes remove BPA and NP in WWTPs. Some of the experiments (results not shown) could support such a hypothesis.
- 4) Generalizing the process model to other key XOCs by inserting their specific parameters into the model and performing validation experiments in the pilot WWTP e.g. XOCs with similar and/or different expected fate than BPA and NP.
- 5) Integrating the process model into the existing design and operation models for WWTPs and to determine if optimal removal of XOCs is consistent with other important optimization parameters e.g. removal of organic matter, nutrients, and energy consumption.

9.1 Further investigations

- 1) Further testing and validation of the analytical method for NP in sludge samples (section 5.1.2).
- 2) Measuring the fate of NPnEO and NPnEC during optimization experiments to identify the fate of NP and NPnEO (section 6.1.1).
- 3) Explaining why the degradation of BPA stopped during the batch experiment (section 6.2.4).
- 4) More experiments to identify biological and physical-chemical input parameters to process models for BPA for NP (especially the volatilization of NP).
- 5) A new optimization experiment where background concentrations are monitored one month before the optimized operation and where the operation is shifted between standard and optimized operation (section 6.2.5 and 7.2).

References

Ahel M, Giger W, Koch M. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment - I. Occurrence and transformation in sewage treatment. *Water Research*, 1994, 28, 1131-1142.

Andersen RH. Pollution of the aquatic environment by steroid estrogens (In Danish: Forurening af vandmiljøet med steroidøstrogener), Ph.D. thesis, Department of Analytical Chemistry, The Danish University of Pharmaceutical Sciences, Denmark, 2004, 94 pp.

Barber LB, Brown GK, Zaugg SD. Potential Endocrine Disrupting Organic Chemicals in Treated Municipal Wastewater and River Water. ACS Symposium Series, American Chemical Society, 2000, 747, 97-124.

Belfroid AC, Van der HA, Vethaak AD, Schäfer AJ, Rijs GBJ, Wegener J, Cofino WP. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. *Science of the Total Environment*, 1999, 225, 101-108.

Brooke DN, Dobson S, Howe PD, Nielsen IR. Environmental hazard assessment: Di-(2-ethylhexyl)phthalate, Toxic Substances Division, Department of the Environment, London, 1991.

Burdan F, Szumilo J, Klepacz R, Dudka J, Korobowicz A, Tokarska E. Prenatal Effect Of Repeated Daily Doses Of Ibuprofen – A Nonselective COX Inhibitor. Birth Defects Research Part A: Clinical and Molecular Teratology, Poster Sessions Abstracts, 2004, 70, 299.

Buser HR, Poiger T, Muller MD. Occurrence and Environmental Behaviour of the Chiral Pharmaceutical Drug Ibuprofen in Surface Waters and in Wastewater. *Environmental Science and Technology*, 1999, 33, 2529-2535.

Cai Y, Jiang G, Liu J, Liang X, Yao Z, Liu J, Liu J, Zhou Q. Solid-phase microextraction coupled with high performance liquid chromatography-fluorimetric detection for the determination of bisphenol A, 4-n-nonylphenol, and 4-tert-octylphenol in environmental water samples. *Analytical Letters*, 2003, 37, 739-753.

Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H. The solids retention time - a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, 2005, 39, 97-106.

Clara M, Strenn B, Saracevic E, Kreuzinger N. Adsorption of bisphenol-A, 17 β -estradiol and 17 α -ethinylestradiol to sewage sludge. *Chemosphere*, 2004, 56, 843-851.

Connell D.W. Basic concepts of Environmental Chemistry. CRC Press LLC, 1997, ISBN 0-87371-998-0, 506 pp.

Cousins IT, Staples CA, Klecka GM, Mackay D. A Multimedia Assessment of the Environmental Fate of Bisphenol A. *Human and Ecological Risk Assessment*, 2002, 8, 1107-1135.

Danish Environmental Protection Agency (DEPA). Degradation of estrogens in Sewage Treatment Processes. Environmental project No. 899, Denmark, 2004, 61 pp.

Danish Environmental Protection Agency (DEPA). Endocrine disrupting compounds and pharmaceuticals in wastewater (In Danish: Hormonforstyrrende stoffer og lægemidler i spildevand), Environmental project No. 799, Denmark, 2003, 46 pp.

Danish Environmental Protection Agency (DEPA). Feminisation of fish. The effect of estrogenic compounds and their fate in sewage treatment plants and nature. Environmental project No. 729, Denmark, 2002a, 184 pp.

Danish Environmental Protection Agency (DEPA). Nonylphenol and nonylphenoethoxylates in wastewater and sludge (In Danish: Nonylphenol og nonylphenoethoxylater i spildevand og slam), Environmental project No. 704, Denmark, 2002b, 86 pp.

Daughton CG, Ternes TA. Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change? Special report. *Environmental health perspectives*, 1999, 107, 907-938.

DEHP Information Centre (DEHP-IC). DEHP - one of the world's most commonly used plasticisers. September, 2005: <http://www.dehp-facts.com/>

Delongas JL, Trabarel C, Guittin P, Nohynek G. Milk collection in lactating rats during regulatory studies: effects after estradiol administration. Abstract, *Teratology* 1994, 50, 37A.

Desbrow C, Routledge EJ, Brighty GC, Sumpter JP, Waldock M. Identification of Estrogenic Chemicals in STW Effluents. 1. Chemical Fraction and in Vitro Biological Screening. *Environmental Science and Technology*, 1998, 32, 1549-1557.

Directorate-General for Public Works and Water Management (DGPWWM). Estrogens and xeno-estrogens in the aquatic environment of the Netherlands, Occurrence, Potency and Biological Effects., RIKZ-report No. 2002.001, The Netherlands, 2002, 297 pp.

Douzinis R. Literature Review on Substances of Potential Concern in Reclaimed Water. Urban Water Resources Management Center, University of Washington, 2001, 53 pp.

ECOTOX database. US Environmental Protection Agency, 2005: http://www.epa.gov/ecotox/ecotox_home.htm

EMEA. Guideline on the environmental risk assessment of medicinal products for human use. Committee for medicinal products for human use, European Medicines Agency, UK, 2005, 21 pp.

Eriksson E, Auffarth K, Eilersen AM, Henze H, Ledin A. Household chemicals and personal care products as sources for xenobiotic organic compounds in grey wastewater. *Water SA*, 2003, 29, 135-146.

Eriksson E, Albrechtsen HJ, Baun A, Boe-Hansen R, Mikkelsen PS, Ledin A. Hazard identification of rainwater collected for non-potable reuse in households. Discharged urban waters - Resource or risk? First World Wide Workshop for Junior Environmental Scientists, France, 2002.

European Chemical Bureau (ECB). 4,4'-Isopropylidenediphenol (Bisphenol-A). European Union Risk Assessment Report, Institute for Health and Consumer Protection, European Commission, 2003a, 302 pp.

European Chemical Bureau (ECB). Bis(2-ethylhexyl) phthalate (DEHP). European Union Draft Risk Assessment Report, Institute for Health and Consumer Protection, European Commission, 2003b, 582 pp.

European Chemical Bureau (ECB). 4-Nonylphenol (Branched) and Nonylphenol. European Union Risk Assessment Report, Institute for Health and Consumer Protection, European Commission, 2002, 244 pp.

European chemical Substances Information System (ESIS), 2005: <http://ecb.jrc.it/>

Fauser P, Vikelsøe J, Sørensen PB, Carlsen L. Phthalates, nonylphenols and LAS in an alternately operated wastewater treatment plant – fate modelling based on measured concentrations in wastewater and sludge. *Water Resources*, 2003, 37, 1288-1295.

Fuerhacker M. Bisphenol A emission factors from industrial sources and elimination rates in a sewage treatment plant. *Water Science and Technology*, 2003, 47, 117-122.

Fujita M, Ike M, Mori K, Kaku H, Sakaguchi Y, Asano M, Maki H, Nishihara T. Behavior of nonylphenol ethoxylates in sewage treatment plants in Japan - biotransformation and ecotoxicity. *Water Science and Technology*, 2000, 42, 23-30.

Gavala HN, Alatríste-Mondragón F, Iranpour R, Ahring BK. Biodegradation of phthalate esters during the mesophilic anaerobic digestion of sludge. *Chemosphere*, 2003, 52, 673-68.

Gómez M.J., Mezcua M., Martínez M.J., Fernández-alba A.R. and Agüera A. A new method for monitoring oestrogens, N-octylphenol, and bisphenol A in wastewater treatment plants by solid-phase extraction-gas chromatography-tandem mass spectrometry. *International Journal of Environmental Analytical Chemistry*, 2006, 86, 3-13.

Gonzalez S, Petrovic M, Barcelo D. Simultaneous extraction and fate of linear alkylbenzene sulfonates, coconut diethanol amides, nonylphenol ethoxylates and their degradation products in wastewater treatment plants, receiving coastal waters and sediments in the Catalanian area (NE Spain). *Journal of Chromatography*, 1052, 111-120.

Grote K, Talsness CE, Kuriyama S, Nowak J, Chahoud I. Effect of ethinylestradiol on follicle maturation of female rat offspring after prenatal exposure. *Reproductive Toxicology*, 2002, 16, 411-412.

Heberer T, Stan HJ. Occurrence of polar organic contaminants in Berlin drinking water (In German: Vorkommen von polaren organischen Kontaminanten im Berliner Trinkwasser). *Vom Wasser*, 1996, 86, 19-32.

Henze M, Gujer W, Mino T, Van Loosdrecht M. Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. Scientific and Technical Report, No 9, 2000, London, IWA Publishing.

Jacobsen BN, Guildal T. Novel aspects for management of xenobiotic compounds in wastewater treatment plants – linking theory, field studies, regulation engineering, and experience. *Water Science and Technology*, 2000, 42, 315-322.

Jobling S, Sheahan D, Osborne JA, Matthiessen P, Sumpter JP. Inhibition of Testicular Growth in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Estrogenic Alkylphenolic Chemicals. *Environmental toxicology and chemistry*, 1996, 15, 194-202.

Johnson AC, Aerni HR, Gerritsen A, Gibert M, Giger W, Hylland K, Jurgens M, Nakari T, Pickering A, Suter MJF, Svenson A, Wettstein FE. Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices. *Water Research*, 2005, 39, 47-58.

Kawaguchi M, Inoue K, Yoshimura M, Ito R, Sakui N, Nakazawa H. Determination of 4-nonylphenol and 4-tert.-octylphenol in water samples by stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry. *Analytica Chimica Acta*, 2004, 505, 217-222.

Kawai K, Nozaki T, Nishikata H, Aou S, Takii M, Kubo C. Aggressive Behavior and Serum Testosterone Concentration during the Maturation Process of Male Mice: The Effects of Fetal Exposure to Bisphenol A. *Environmental Health Perspectives*, 2002, 111, 175-179.

Keller H, Xia K, Bhandari A. Occurrence and degradation of estrogenic nonylphenol and its precursors in Northeast Kansas wastewater treatment plants. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*, 2003, 7, 203-213.

Knacker T. POSEIDON - Environmental Risk Assessment. IWA World Water Congress Melbourne 2002, Workshop on Endocrine Disruptors Proceedings, conference proceedings, 2002, 21-30.

Kobuke Y, Tanaka H, Magara T. Nationwide and Regional River Monitoring Studies as well as Bioassays and Treatment of EDs in Waterworks. IWA World Water Congress Melbourne 2002, Workshop on Endocrine Disruptors Proceedings, conference proceedings, 2002, 53-62.

Komori K, Okayasu Y, Yasojima M, Suzuki Y, Tanaka H. Occurrence of nonylphenol, nonylphenol ethoxylate surfactants and nonylphenol carboxylic acids in wastewater in Japan. *Water Science and Technology*, 2006, 53, 27-33.

Kreuzinger N, Clara M, Strenn B, Kroiss H. Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater. *Water Science and Technology*, 2004, 50, 149-156.

Kuklenyik Z, Ekong J, Cutchins CD, Needham LL. Simultaneous Measurement of Urinary Bisphenol A and Alkylphenols by Automated Solid-Phase Extractive Derivatization Gas Chromatography/Mass Spectrometry. *Analytical Chemistry*, 2003, 75, 6820-6825.

Lee HB, Peart TE. Occurrence and Elimination of Nonylphenol Ethoxylates and Metabolites in Municipal Wastewater and Effluents. *Water Quality Research Journal of Canada*, 1998, 33, 398-402.

Lindblom E. Personal communication and plotted process model figures. E-mail: eul@er.dtu.dk and phone: (+45) 45 25 21 82. Institute of Environment and Resources, Technical University of Denmark, 2007.

Lindblom E, Press-Kristensen K, Mikkelsen PS, Henze M. Modelling the fate of bisphenol A in activated sludge systems. *Environmental Science and Technology*, submitted, 2007 (appendix V).

Liu JF, Liang X, Jiang GB, Cai YQ, Zhou QX, Liu GG. High performance liquid chromatography determination of 4-nonylphenol, 4-tert-octylphenol, and their short ethoxyl chain polyethoxylates in water samples using a microporous membrane liquid-liquid extraction sample pretreatment technique. *Journal of Separation Science*, 26, 823-828.

Lobos JH, Leib TK, Su TM. Biodegradation of bisphenol A and other bisphenols by a gram-negative aerobic bacterium. *Applied Environmental Microbiology*, 1992, 58, 1823-1831.

Mayer FL, Mehrle PM, Schoettger RA. Collagen metabolism in fish exposed to organic chemicals: Recent Advances in Fish Toxicology: A Symposium. U.S. Environmental Protection Agency, Corvallis, OR. 1977, 205, 31-54.

Meesters RJW, Schröder HF. Simultaneous Determination of 4-Nonylphenol and Bisphenol A in Sewage Sludge. *Analytical Chemistry*, 2002, 74, 3566-3574.

Metcalf CD, Metcalfe TL, Kiparissis Y, Koenig BG, Khan C, Hughes RJ, Croley TR, March RE, Potter T. Environmental Toxicology - Estrogenic Potency of Chemicals Detected in Sewage Treatment Plant Effluents as Determined by In Vivo Assays with Japanese Medaka (*Oryzias latipes*). *Environmental toxicology and chemistry*, 2001, 20, 297-308.

National Institute for Occupational Safety and Health (NIOSH), 2005: <http://www.cdc.gov/niosh/homepage.html>

National Toxicology Program (NTP). Nonylphenol: multigenerational reproductive effects in Sprague-Dawley rats when exposed to nonylphenol in the diet. R.O.W. Sciences study no. 8989-30, 1997.

Novartis. Prescribing Information, Carbamazepine USP, Novartis Pharmaceutical Corporation, East Hanover, New Jersey, USA, 2003, 17 pp.

NOVA. Point sources 2003 - Revised edition (In Danish: Punktkilder 2003 - revideret udgave). Danish Environmental Protection Agency, Orientation paper No. 1, Denmark, 2005, 167 pp.

Porter A and Hayden N. Processes Affecting the Fate of Nonylphenol During Wastewater Treatment. Conference Proceedings from the 2nd International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Minneapolis, Minnesota, 2001, 112-125.

Poseidon. Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse. Final report, EU's Fifth Framework Programme, European Commission, 2005, 58 pp.

Press-Kristensen K, Ledin A, Schmidt JE, Henze M. Identifying model pollutants to investigate biodegradation of hazardous XOCs in WWTPs. *Science of the Total Environment*, 2007a, 373, 122-130 (appendix I).

Press-Kristensen K, Lindblom E, Schmidt JE, Henze M. Examining the biodegradation of endocrine disrupting bisphenol A and nonylphenol in WWTPs. *Water Science and Technology*, 2007b, submitted (appendix II).

Press-Kristensen K, Lindblom E, Henze M. Modelling as a tool when interpreting biodegradation of micro pollutants in activated sludge systems. *Water Science and Technology*, 2007c, submitted (appendix III).

Press-Kristensen K, Christensen N, Ledin A. Simultaneous extraction and quantification of bisphenol A and nonylphenol in complex wastewater matrixes. *International Journal of Environmental Analytical Chemistry*, 2007d, submitted (appendix IV).

Rempharmawater. Ecotoxicological Assessments and Removal Technologies for Pharmaceuticals in Wastewater. Final report, EU's Fifth Framework Programme, European Commission, 2004, 50 pp.

R-Gray TP, Jobling S, Morris S, Kelly C, Kirby S, Janbakhsh A, Harries JE, Waldock MJ, Sumpter JP, Tyler CR. Long-Term Temporal Changes in the Estrogenic Composition of Treated Sewage Effluent and Its Biological Effects on Fish. *Environmental Science and Technology*, 2000, 34, 1521-1528.

Rippen, G. Handbook in environment chemicals (In German: Handbuch Umwelt-chemikalien). Third edition 1990 (last updated 22.03.05), 2005, ISBN 3-609-73210-5.

Rudel RA, Melly SJ, Geno PW, Sun G, Brody JG. Identification of Alkylphenols and Other Estrogenic Phenolic Compounds in Wastewater, Septage, and Groundwater on Cape Cod, Massachusetts. *Environmental Science and Technology*, 1998, 32, 861-869.

Rxlist. The internet drug index, 2005: <http://www.rxlist.com/>

Schäfer AI and Waite TD. Removal of endocrine disrupters in advanced treatment - the Australian approach. IWA World Water Congress Melbourne 2002, Workshop on Endocrine Disruptors Proceedings, conference proceedings, 2002, 37-51.

Shao B, Hu J, Yang M. Nonylphenol Ethoxylates and Their Biodegradation Intermediates in Water and Sludge of a Sewage Treatment Plant. *Bulletin of Environmental Contamination and Toxicology*, 2003, 70, 527-532.

Shurin JB and Dodson SI. Sublethal toxic effects of cyanobacteria and nonylphenol on environmental sex determination and development in *Daphnia*. *Environmental toxicology and chemistry*, 1997, 16, 1269-1276.

Snyder S. Endocrine Disruptors and Pharmaceutically Active Compounds: US Regulations and Research. IWA World Water Congress Melbourne 2002, Workshop on Endocrine Disruptors Proceedings, conference proceedings, 2002, 1-10.

Staples CA, Dorn PB, Klecka GM, O'Block ST, and Harris LR. A review of the environmental fate, effects, and exposure of Bisphenol A. *Chemosphere*, 1998, 36, 2149-2173.

Stumpf M, Ternes TA, Wilken R-D, Rodrigues SV, Baumann W. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *Science of the Total Environment*, 1999, 225, 135-141.

Svenson A, Allard A & Ek M. Removal of estrogenicity in Swedish municipal sewage treatment plants. *Water Research*, 2003, 37, 4433-4443.

Tabata A, Kashiwada S, Ohnishi Y, Ishikawa H, Miyamoto N, Itoh M, Magara Y. Estrogenic influences of estradiol-17 β , p-nonylphenol and bisphenol-A on Japanese Medaka (*Oryzias latipes*) at detected environmental concentrations. *Water Science and Technology*, 2001, 42, 109-116.

Tanaka H, Sato C, Komori K, Yakou Y, Tamamoto H, Miyamoto N, Higashitani T. Occurrence of Endocrine Disruptors in Sewage and Their Behaviour in Sewage Treatment Plants in Japan. *Environmental Science and Technology*, 2003, 10, 1-024.

Tanaka H, Komori K, Tamamoto H, Miyamoto N, Higashitani T, Sato C. Endocrine disruptors in sewage treatment plants. Summary of a three-year nationwide survey and recent research at PWRI. Report from the Public Works Research Institute in Japan, Japan, 2002, 26 pp.

Technical Guidance Document on Risk Assessment (TGD). Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission, EU, 2003, 304 pp.

Ternes TA, Mueller J, Wilken RD. Behaviour and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. *Science of the Total Environment*, 1999, 225, 81-90.

Tixier C, Singer HP, Oellers S, Müller SR. Occurrence and Fate of Carbamazepine, Clofibrilic Acid, Diclofenac, Ibuprofen, Ketoprofen, and Naproxen in Surface Waters. *Environmental Science and Technology*, 2003, 37, 1061-1068.

Toxnet. Abstracts from TOXLINE Special. National Institutes of Health, Department of Health and Human Services, U.S. National Library of Medicine, 2005: <http://toxnet.nlm.nih.gov>.

United States Environmental Protection Agency (USEPA). Edition of the Drinking Water Standards and Health Advisories. EPA 822-R-04-005, Office of Water, Washington, DC, 2004, 20 pp.

Wert EC, Rosario-Ortiz FL, Drury DD, and Snyder SA. Formation of oxidation byproducts from ozonation of wastewater. *Water Research*, 2007, 41, 1481-1490.

World Health Organization (WHO). WHO Guidelines for drinking-water quality, Vol. 1 - Recommendations, 2004, ISBN 9241546387, 540 pp.

Wright C, Fox J, Duffy P, Boland MP, Roche JF, Evans NP, Sweeney T. Exposure to the endocrine disrupting compound - octylphenol in-utero and in postnatal life influences the onset of puberty and duration of first breeding season in the ewe. *Journal of Reproduction and Fertility*, Abstract series 1999, 0, 16-17.

Xinmei F, Dai S, Zhang Y. 1-Butyl-3-methylimidazolium hexafluorophosphate ionic liquid-based liquid-liquid microextraction for the determination of 4-nonylphenol and 4-tert-octylphenol in environmental waters. *International Journal of Environmental Analytical Chemistry*, 2006, 86, 985-993.

Appendixes

The following appendixes contain the five journal papers written in relation to this study.

Appendix I

Press-Kristensen K, Ledin A, Schmidt JE, Henze M. Identifying model pollutants to investigate biodegradation of hazardous XOCs in WWTPs. *Science of the Total Environment*, 373 (1), 122-130, 2007.

Appendix II

Press-Kristensen K, Lindblom E, Schmidt JE, Henze M. Examining the biodegradation of endocrine disrupting bisphenol A and nonylphenol in WWTPs. *Water Science and Technology*, submitted, 2007.

Appendix III

Press-Kristensen K, Lindblom E, Henze M. Modelling as a tool when interpreting biodegradation of micro pollutants in activated sludge systems. *Water Science and Technology*, submitted, 2007.

Appendix IV

Press-Kristensen K, Christensen N, Ledin A. Simultaneous extraction and quantification of bisphenol A and nonylphenol in complex wastewater matrixes. *International Journal of Environmental Analytical Chemistry*, submitted, 2007.

Appendix V

Lindblom E, Press-Kristensen K, Vanrolleghem P, Mikkelsen PS, Henze M. Modelling the fate of bisphenol A in activated sludge systems. *Environmental Science and Technology*, submitted, 2007.

The journal papers are not included in this www-version but can be obtained from the Library at the Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Lyngby (library@er.dtu.dk).