Modelling Illicit Drug Fate in Sewers for Wastewater-Based Epidemiology

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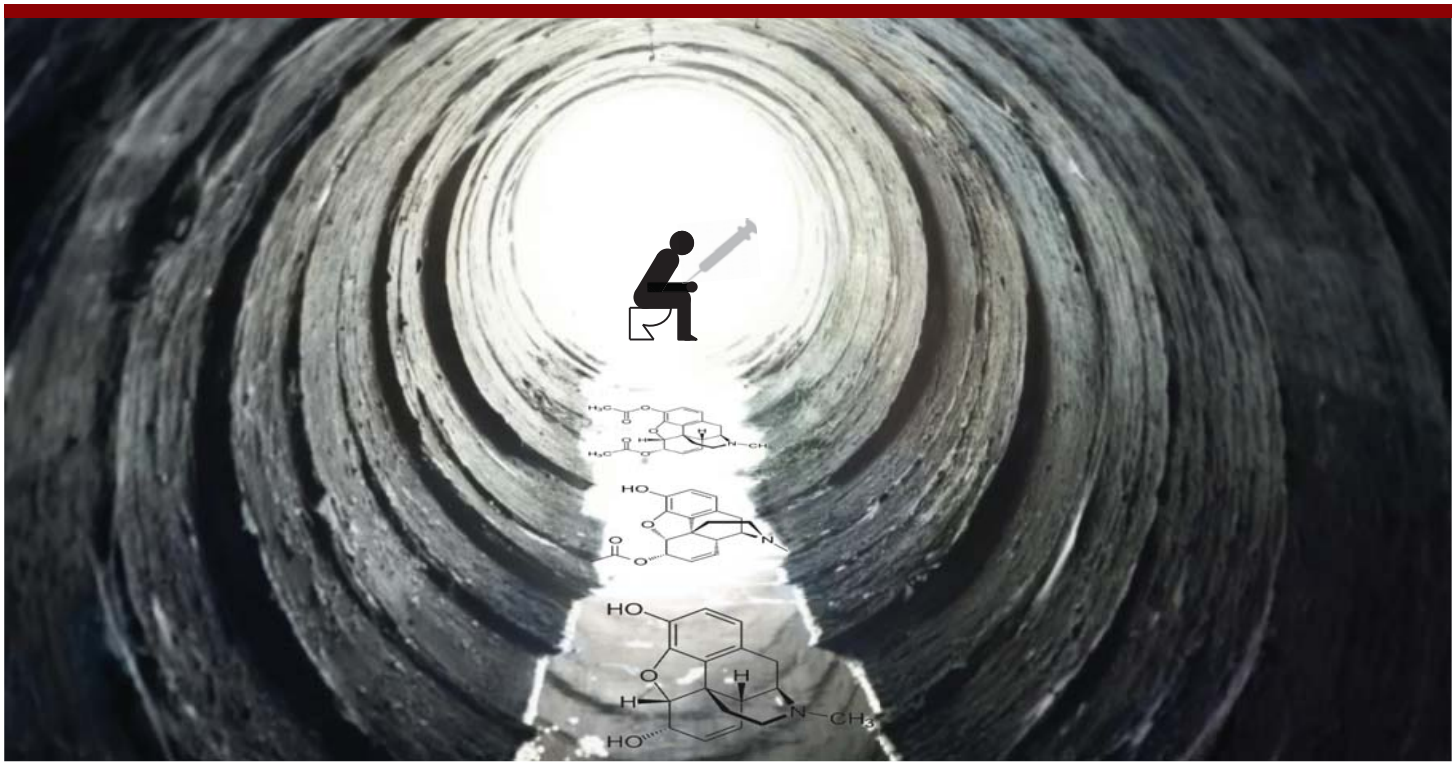

Pedram Ramin

PhD Thesis
December 2016

DTU Environment
Department of Environmental Engineering

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Preface

The PhD thesis is based on the work carried out at the Technical University of Denmark, Department of Environmental Engineering, from February 2013 to December 2016. The project was carried out under the main supervision of Assoc. Prof. Benedek Gy. Plósz with Prof. Peter Steen Mikkelsen, as co-supervisor. The project funding was provided by the Marie Curie Initial Training Networks (ITN) in a European project "SEWPROF" (https://sewprof-itn.eu/).

I **Ramin, P.**, Brock, A.L., Polesel, F., Causanilles, A., Emke, E., de Voogt, P., Plósz, B.G. (2016). Transformation and sorption of illicit drug biomarkers in sewer systems : understanding the role of suspended solids in raw wastewater. Environmental Science and Technology. doi:10.1021 /acs.est.6b03049

II **Ramin, P.**, Valverde-Pérez, B., Polesel, F., Locatelli, L., Plósz, B.G. (2016). A systematic model calibration method for chemical and biochemical transformation pathway models – The case of transformation of heroin biomarkers in wastewater. *Submitted manuscript*

III **Ramin, P.**, Brock, A.L., Causanilles, A., Valverde-Pérez, B., Emke, E., de Voogt, P., Polesel, F., Plósz, B.G. (2016). Transformation and sorption of illicit drug biomarkers in sewer biofilms. *Submitted manuscript*

IV **Ramin, P.**, Vezzaro, L., Mikkelsen, P.S., Plósz, B.G. (2016). Back-calculating illicit drug abuse rates in urban areas – Quantifying uncertainties associated with in-sewer transformation and samplin. *Submitted manuscript*

In addition, the following publications, not included in this thesis, were also concluded during this PhD study

- Mardal, M., Kinyua, J., **Ramin, P.**, Miserez, B., van Nuijs, A.L.N., Covaci, A., Meyer, M.R. (2016). Screening for illicit drugs in pooled human urine and urinated soil samples and studies on the stability of urinary excretion products of cocaine, MDMA, and MDEA in wastewater by hyphenated mass spectrometry techniques. Drug Testing and Analysis. doi:10.1002/dta.1957.
- Bade, R., Bijlsma, L., Sancho, J. V, Baz-Lomba, J.A., Castiglioni, S., Castrignanò, E., Causanilles, A., Gracia-Lor, E., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K.,van Nuijs, A.L.N., Ort, C., Plósz, B.G., **Ramin, P**., Rousis, N.I., Ryu, Y., Thomas, K.V., de Voogt, P., Zuccato, E., Hernandez., F. (2016). Chemosphere Liquid chromatography-tandem mass spectrometry determination of synthetic cathinones and phenethylamines in fluent wastewater of eight European cities. Chemosphere. doi:10.1016/j.chemosphere.2016.10.107
- Baz-Lomba, J.A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignanò, E., Causanilles, A., Hernandez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., van Nuijs, A., Ort, C., Plósz, B.G., **Ramin, P.**, Reid, M., Rousis, N.I., Ryu, Y., de Voogt, P., Bramness, J., Thomas, K. (2016). Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16: 1035. doi:10.1186/s12889-016-3686-5
- Ryu, Y., Garcia-lor, E., Bade, R., Bramness, J.G., Castiglioni, S., Causanilles, A., Covaci, A., de Voogt, P., Hernandez, F., Kasprzyk-hordern, B., Mccall, A., Ort, C., Plósz, B.G., **Ramin, P.**, Rousis, N.I., Reid, M.J., Thomas, K. V. (2016). Increased levels of the oxidative stress biomarker 8-iso-prostaglandin F2α in a city´s wastewater related to tobacco use, Scientific Reports, *Accepted Manuscript*
- Gracia-lor, E., Castiglioni, S., Bade, R., Béen, F., Castrignanò, E., Covaci, A., González-, I., Hapeshi, E., Kasprzyk-hordern, B., Kinyua, J., Lai, F.Y., Letzel, T., Lopardo, L., Meyer, M.R., O'Brien, J., **Ramin, P.**, Rousis, N.I., Rydevik, A., Ryu, Y., Santos, M., Senta, I., Thomaidis, N., Veloutsou, S., Yang, Z., Bijlsma, L. (2016). Measuring excretion biomarkers in wastewater as a new source of information for epidemiological studies. *Submitted Manuscript*

- Causanilles, A., Emke, E., Bade, R., Baz-Lomb, J.A., Castiglioni, S., Castrignanò, E., Hernández, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., Nuijs, A. van, Ort, C., Plósz, B.G., **Ramin, P.**, Rousis, N.I., Ryu, Y., Thomas, K., de Voogt, P. (2016). Application of wastewater-based epidemiology to assess erectile dysfunction pharmaceuticals usage in 10 European citiesin. *Manuscript in preparation*

This PhD study also contributed to international conferences with the following proceeding and conference papers:

- **Ramin, P.**, Polesel, F., Andrésson, G., Vezzaro L., Sharma, A.K., Reid, M., Thomas, K.V., Mikkelsen, P.S., Plósz, B.G. (2014). Impacts of Hydraulic Residence Time Prediction and Diurnal Loading Pattern on the Estimation of Drug Abuse in Urban Areas. 13th International Conference on Urban Drainage, Kuching, Malaysia, 7-11 September 2014.
- Mardal, M., **Ramin, P.**, Plósz, B.G., Maurer H.H., Meyer, M.R. (2014). Analysis of Drugs of Abuse in Anonymously Collected Urine and Soil samples from a Music Festival in Scandinavia. 52nd Annual meeting of the International Association of Forensic Toxicologists (TIAFT), Buenos Aires, Argentina, 9-11 November 2014.
- **Ramin, P.**, Causanilles, A., Polesel, F., Emke, E., de Voogt P., Plósz, B.G. (2015). Abiotic and biofilm-mediated transformation of heroin biomarkers in wastewater under aerobic and anaerobic conditions. 2nd International Conference on "Wastewater-based drug epidemiology", Ascona, Switzerland, 11-15 October 2015.
- **Ramin, P.**, Baz-Lomba, J.A., Reid, M., Thomas, K.V., Plósz, B.G. (2015). Impact of sampling resolution on estimation of community-wide daily illicit drug use. 9th IWA Specialist Conference on Assessment and Control of Micropollutants and Hazardous Substances in Water, 2015, Singapore, 22-25 November.
- **Ramin, P.**, Brock, A.L., Polesel, F., Causanilles, A., Emke, E., de Voogt P., Plósz, B.G. (2016). Fate of cocaine drug biomarkers in sewer system: the role of suspended solids in biotransformation and sorption. 18th International EWA Symposium, Munich, Germany, 1-2 June 2016.

- **Ramin, P.**, Polesel, F., Brock, A.L., Torresi, E., Plósz, B.G. (2016). Removal of primary and secondary trace organic substrates in aerobic and anaerobic sewer biofilm. IWA Microbial Ecology in Water Engineering & Biofilms, Copenhagen, Denmark, 4-7 September 2016.
- **Ramin, P.**, Brock, A.L., Polesel, F., Torresi, E., Plósz, B.G. (2016). Improving the prediction of in-sewer transformation of illicit drug biomarkers by identifying a new modelling framework. 5th International Conference on Emerging Contaminants (EmCon 2016) and Micropollutants (WiOW 2016) in the Environment, Sidney, Australia, 20-23 September 2016.

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My final thanks goes to my mum and dad, just for being there, even on the phone. They are truly the co-authors of this thesis.

The end

The reverse story of
Wastewater-based epidemiology

Summary

With increasing consumption of illicit drugs, in particular cocaine and cannabis, in recent decades, the negative social and public health impact has also propagated. Following drug consumption and human metabolism, fractions of unchanged parent drugs and metabolites are excreted into toilets. After transport in sewers, these chemicals enter wastewater treatment plants (WWTPs). Monitoring campaigns are normally performed at WWTP influent to collect representative samples. Following quantitative chemical analysis, measured drug loads are used to estimate population-normalized parent drug consumption based on a candidate *biomarker* (the parent drug itself or one of the human metabolites). This approach has gained increasing attention in the past decade and is termed *wastewater-based epidemiology*. It has been shown that this emerging approach can improve and complement survey-based methods.

Sewer systems can be considered as biological reactors, in which the concentration of organic chemicals present in wastewater can be impacted by in-sewer processes during hydraulic residence time. Illicit drug biomarkers, as trace organic chemicals in the range of nanograms to micrograms per liter, are subject to physical, chemical or biological processes in sewers (fate processes). The occurrence of these processes may lead to significant change of drug loads at WWTP influent compared to source release points. Therefore, not accounting for these variations may negatively affect drug use estimates. However, due to a lack of sufficient evidence on potential in-sewer sorption and transformation of drug biomarkers, these processes are often neglected by wastewater-based epidemiologists. The motivation of this thesis was to overcome this substantial knowledge gap by: (i) providing new evidence on sorption and transformation of drug biomarkers in raw wastewater and sewer biofilms; and (ii) developing modelling tools – by combining and extending existing modelling frameworks – to predict such processes. To achieve this goal, a substantial part of this thesis was dedicated to the experimental assessment and modelling of in-sewer processes by means of laboratory scale studies under the conditions representative to sewer systems. Eventually, the prediction of in-sewer processes at the catchment level was carried out and back-calculation of drug consumption was performed using measured data from a monitoring campaign.

Overall, the methodology used in this thesis combined different aspects, namely: (i) optimal experimental design; (ii) mathematical formulation of processes; (iii) model calibration; (iv) uncertainty analysis and model param-

eters identifiability; (v) model validation; and (vi) model application for back-calculation at catchment level. In this thesis, 16 drug biomarkers were selected based on their ubiquitous occurrence in wastewater, and include cocaine, mephedrone, methadone, heroin, codeine and tetrahydrocannabinol (THC) and their respective major human metabolites.

In-sewer processes, namely, sorption and transformation of these chemicals were assessed in raw wastewater (suspended biomass) and sewer biofilms in targeted batch experiments. These experiments were conducted under aerobic and anaerobic conditions. Annular rotating biofilm reactors were used to simulate shear conditions prevailing in sewers and were operated over 14 months. Abiotic transformation (e.g., hydrolysis) was also evaluated using mineral water and sorption to suspended solids and biofilms were additionally assessed. Overall, two sets of experiments were performed and used for model calibration and model validation purposes.

To predict the fate of drug biomarkers in raw wastewater, simultaneous evaluation and modelling of substrate utilization and microbial growth processes was performed. It was hypothesized that active biomass dynamics during batch experiments (due to high substrate availability and significant microbial growth) can significantly impact the prediction of biotransformation rates. For this purpose, the Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) model was combined with the Activated Sludge Model for Xenobiotics (ASM-X) to predict the fate of drug biomarkers together with the primary metabolic processes. Two new processes were considered, namely sorption-desorption to reactor wall and abiotic transformation. As for sewer biofilms, the extended ASM-X model was further modified by accounting for diffusive mass transfer limitation of biomarkers from the bulk phase into the biofilms and within the biofilm matrix.

Selected model parameters were estimated with the Bayesian optimization method DREAM$_{(ZS)}$. A calibration methodology was developed with focus on uncertainty propagation among model parameters, e.g. from abiotic transformation rates to biotransformation rates. Subsequently, uncertainty analysis was performed to assess the impact of variability of model parameters on model output. Moreover, different transformation pathways were tested for the selected biomarkers and new pathways were identified based on mass balance, uncertainty analysis, and feasibility of transformations (according to an existing pathway database). Results from the experimental and modelling assessment indicated that by ignoring primary metabolic processes in raw wastewater would impose significant overestimation (up to 385%) of trans-

formation rates under aerobic conditions, whereas no difference was found under anaerobic conditions. Abiotic transformation processes were the dominant removal mechanism for many of the selected chemicals (e.g., cocaine: 80-100%, batch experiments with raw wastewater) under both aerobic and anaerobic conditions. Several biomarkers underwent substantial biotransformation e.g., almost complete removal of heroin and morphine-3-glucuronide after 12 h in batch experiments with raw wastewater. It was further observed that sewer biofilms can enhance biotransformation of a number of selected chemicals, such as benzoylecgonine and 6-monoacetylmorphine. Overall, redox conditions were found to have an influence on biotransformation rates (especially for methadone) and, to a lesser extent, on abiotic transformation rates. Only a few chemicals, such as 11-hydroxy-THC, were found to sorb onto suspended solids and sewer biofilms. Validation of calibrated models with an independent dataset was successful for most compounds, the main exception being methadone under aerobic conditions.

To demonstrate the impact of in-sewer processes on estimation of daily drug use at catchment level, a generic scenario analysis was performed to assess the uncertainties associated with in-sewer processes and sampling. It was found that ignoring in-sewer processes for cocaine and its metabolite benzoylecgonine can add up to 11% (median value for a *large* catchment) error in daily cocaine consumption estimates. This error was 43% and 11% for estimates of daily heroin use with 6-monoacetylmorphine and morphine as candidate biomarkers, respectively. In contrary, sampling error (flow-proportional sampling mode) was the highest in the *smallest* catchment – up to 17% for cocaine.

Subsequently, measured cocaine and benzoylecgonine loads from a 2-week monitoring campaign at the Lynetten WWTP influent (Copenhagen, Denmark) was used to estimate cocaine consumption in two upstream catchments by accounting for in-sewer fate processes. Significant differences in consumption trends were observed between weekdays, weekends, holidays and a street music festival. On average, twice as high cocaine consumption was found during festival period as compared to normal weekdays. Wastewater-based epidemiology is a truly interdisciplinary approach in which engineering tools, including models developed and tested in this thesis, can be beneficial for the accurate estimation of drug consumption in urban areas.

Dansk sammenfatning

Med et stigende narkotikaforbrug gennem de seneste årtier, særligt af kokain og cannabis, er også fulgt en stigning i negative sociale og folkesundheds påvirkninger. Efter indtagelse af et narkotikum og metabolisme i kroppen vil rester af stoffet blive udskilt, delvist som det uændrede aktivstof og delvist i form af forskellige nedbrydningsprodukter. Stofferne udskilles typisk via urinen og transporteres derefter via kloaksystemet til et rensningsanlæg. Overvågningskampagner tager typisk udgangspunkt i målinger ved indløbet til rensningsanlægget, hvor man efter kvantitativ kemisk analyse kan estimere forbruget af et givent narkotikum i oplandet. Forbruget vil da være baseret på koncentrationen af en biomarkør (aktivstoffet selv eller dets nedbrydningsprodukter) i det urensede spildevand. Denne metode kaldes *spildevandsbaseret epidemiologi* og har opnået stigende opmærksomhed i det seneste årti. Det er blevet påvist, at metoden kan forbedre og supplere spørgeundersøgelser.

Kloaksystemer kan ses som biologiske reaktorer, hvor koncentrationsniveauet af spildevandets organiske kemikalier påvirkes af forskellige processer i løbet af den hydrauliske opholdstid. Biomarkører fra ulovlig narkotika, såsom sporstoffer fra organiske kemikalier i nanogram/L og microgram/L niveau, undergår fysiske, kemiske og biologiske processer i kloaksystemet. Disse processer kan medføre, at der er signifikant forskel på koncentrationsniveauet i indløbet til rensningsanlægget sammenlignet med koncentrationsniveauet ved udledningspunktet. Hvis der ikke tages højde for disse variationer, vil det betyde at forbruget af stoffer i oplandet underestimeres. På grund af manglende viden overses potentielle sorptions- og omdannelsesprocesser i kloaksystemer ofte af eksperter indenfor spildevandsbaseret epidemiologi. Motivationen for denne afhandling var derfor at dække en del af dette store videnshul ved (i) at finde nye vidnesbyrd om sorption og transformation af narkotikum-biomarkører i råt spildevand samt i kloakkernes biofilm; og (ii) at udvikle modelværktøjer til at forudsige sådanne processer ved at kombinere og udvide eksisterende modelleringsprogrammer. En væsentlig del af denne afhandling var dedikeret til eksperimentel undersøgelse og modellering af processer i kloaksystemer ved hjælp af laboratorieskala forsøg under forhold der er repræsentative for kloaksystemer. Endeligt blev bestemmelsen af processer i kloaksystemet på oplands-niveau og beregningen af narkotikaforbrug udført og evalueret ved brug af måledata fra en overvågningskampagne.

Den metode, der anvendes i denne afhandling, kombinerer forskellige aspekter: (i) optimalt eksperimentelt design; (ii) matematisk formulering af processer; (iii) model kalibrering; (iv) statistisk analyse, såsom usikkerhedsanalyse

og identifikation af modelparametre; (v) modelvalidering; og (vi) anvendelse af de udviklede modeller til beregning af narkotika forbrug på oplandsniveau. I denne afhandling blev seksten narkotika-biomarkører valgt baseret på deres udbredte brug og deraf følgende høje forekomst i spildevand. Blandt stofferne var kokain, mephedron, metadon, heroin, kodein og tetrahydrocannabinol (THC) samt deres respektive nedbrydningsprodukter.

Processer i kloakken, især sorption og transformation af disse kemikalier blev vurderet i råt spildevand (suspenderet biomasse) og kloak biofilm i målrettede batch eksperimenter. Disse eksperimenter blev udført under aerobe og anaerobe forhold. Ringformede roterende biofilm reaktorer blev brugt til at simulere forskydnings forhold i kloakker og eksperimentet kørte i 14 måneder. Den abiotiske omdannelse (fx hydrolyse) af disse stoffer blev bestemt ved brug af mineralvand, og sorption til suspenderet faststof og biofilm blev endvidere bestemt. To sæt af eksperimenter blev udført og anvendt til model kalibrering of model validering.

Med henblik på at forudse skæbnen af narkotika-biomarkører i urenset spildevand blev der udført sideløbene evaluering og modellering af substratoptag og mikrobiel vækst. Hypotesen var, at aktive biomassedynamikker under batchforsøg (grundet høj tilgængelighed af substrat og betydelig mikrobiel vækst) kan påvirke forudsigelsen af biotransformationsrater signifikant. Med dette formål blev modellen "Wastewater Aerobic/anaerobic Transformations in Sewers" (WATS) kombineret med modellen "Activated Sludge Model for Xenobiotics" (ASM-X) til at bestemme skæbnen af narkotika-biomarkører samt de primære metaboliske processer. To nye processer blev taget i betragtning; nemlig sorption-desorption til reaktorens vægge samt abiotisk omdannelse. For biofilm blev den udvidede ASM-X model yderligere ændret til at tage højde for diffusive begrænsninger for massetransport af narkotika-biomarkører fra bulk fasen til biofilmen samt inde i biofilmen.

Udvalgte modelparametre blev estimeret ved hjælp af den Bayesianske optimeringsmetode $DREAM_{(ZS)}$. En kalibreringsmetode blev udviklet med fokus på spredningen af usikkerheder blandt modelparametre, f.eks. fra abiotiske til biotiske omdannelsesrater. Efterfølgende blev en usikkerhedsanalyse udført med henblik på at bestemme effekten af variabilitet i modelparametre på modellens output. Ydermere blev forskellige omdannelsesveje testet for udvalgte biomarkører, og nye veje blev identificeret baseret på massebalancer, usikkerhedsanalyse og vurdering af mulige omdannelsesveje (baseret på en eksisterende database).

Resultater fra de eksperimentelle - og modelbaserede evalueringer indikerer, at udeladelse af primære metabolske processer i urenset spildevand vil medføre en betydelig overestimering (op til 385%) af omdannelsesrater under aerobe forhold, mens ingen forskel blev funder for anaerobe forhold. Abiotiske transformationsprocesser var den dominerende mekanisme for fjernelse af mange af de udvalgte kemikalier (såsom kokain: 80-100%, batch forsøg med råt spildevand) under både aerobiske og anaerobiske forhold. Mange af de udvalgte kemikalier undergik betydelig biotransformation, f.eks.blev heroin og morfin-3-glucoronide næsten fuldstændig omsat i 12 timers batch forsøg med råt spildevand. Det blev yderligere observeret, at kloakkernes biofilm kan forstærke biotransformation af mange udvalgte kemikalier såsom benzoylecgonine og 6-monoacetylmorfin. Overordnet viste redoxforholdene sig at have stor indflydelse på biologiske omdannelsesrater (særligt for metadon) og i mindre grad på abiotiske omdannelsesrater. Kun for nogle få kemikalier, såsom 11-hydroxy-THC, blev der observeret væsentlig sorption til suspenderede stoffer og biofilm. Validering af de kalibrerede modeller med uafhængige data var succesfuld for de fleste stoffer, med metadon som den vigtigste undtagelse under aerobe forhold.

For at demonstrere effekten af processer i kloaksystemer på estimation af det daglige narkotikaforbrug på oplandsniveau blev en generisk scenarieanalyse udført, med fokus på at bestemme usikkerheden forbundet med processer i kloaksystem og prøvetagningen. Ved at se bort fra processer i kloakken for kokain og dets stofskifteprodukt benzylecgonine kan fejl opstå på op til 11% i estimater af det daglige kokain forbrug (median værdi for et *stort* opland). Denne fejl var på henholdvis 43% og 11% for estimater af det daglige kokain forbrug ved brug af 6-monoacetylmorfin og morfin som kandidat biomarkører. I modsætning hertil var effekten af fejl i prøvetagningen (flow proportional metode) størst for små oplande , helt op til 17% for kokain.

Efterfølgende blev målinger af kokain- og benzoylecgonine-belastningen gennem en to-ugers overvågningskampagne ved indløbet til Lynetten rensningsanlæg (København, DK) anvendt til at estimere kokainforbruget i to opstrøms oplande ved samtidig at tage højde for omdannelsesprocesserne i kloakken. Der blev observeret betydelige forskelle i udviklingen i forbruget mellem ugedage, weekender og helligdage Det gennemsnitlige forbrug af kokain i forbindelse med en gademusikfestival var dobbelt så stort som på normale arbejdsdage. Spildevandsbaseret epidemiologi er en interdisciplinær arbejdsmetode, hvor ingeniørmæssige beregninger, herunder modellerne udviklet og afprøvet i denne afhandling, kan forbedre præcisionen af narkotika forbruget i byområder.

Table of contents

1 Introduction

1.1 Background

With the increase of drug consumption in recent decades, in particular cocaine and cannabis, the social impact on society has also propagated (EMCDDA, 2015a). These consequences include rising healthcare costs, crime rates, and economic losses. Therefore, it is imperative that policy makers gain knowledge of the trends, usage levels, hot spots, and overall prevalence of illicit drug consumption, in order to develop proper prevention campaigns and effective intervention strategies.

Estimation of drug use at the population level is traditionally performed mainly via socio-epidemiological methods, such as population surveys and seizure data. These data are subject to significant uncertainties in measurement and selection, for example, self-reporting bias from false reports, unaware or misinformed consumers, and limited population coverage of the study (Banta-Green and Field, 2011; Castiglioni et al., 2014).

A new concept to assess collective drug consumption, based on measuring concentrations of illicit drugs and their excreted human metabolites in untreated sewage, was proposed by Daughton et al. (2001). The technique is based on the principle that excreted drug residuals in the sewage can be collected by the downstream end of urban drainage systems and subsequently analysed. Later, this approach, termed wastewater-based epidemiology (WBE), was applied by Zuccato et al. (2005) in several Italian cities. Levels of illicit drugs (parent compounds or metabolites) in wastewater can be used to back-calculate the total consumption of drug of abuse by the population served by a particular wastewater treatment plant (WWTP). Therefore, wastewater analysis was found to overcome some of the inherent limitation of survey-based methods, by providing evidence-based and objective estimates.

Sampling campaigns for the purpose of detection of excreted drug residuals (drug biomarkers) are usually performed at the inlet to WWTPs. There have been many studies to monitor temporal and spatial patterns of drug use in selected urban sewer catchments (Castiglioni et al., 2013; Gomes et al., 2009; Plósz et al., 2013); allowing, more recently, for the undertaking of international comparative studies (Ort et al., 2014; Thomas et al., 2012). These findings demonstrated that wastewater analysis techniques have the potential to complement conventional surveillance data. Estimation of drug use via wastewater analysis has been further expanded to include monitoring during

special events (e.g. music festivals) (Gerrity et al., 2011; Lai et al., 2013), as well as within smaller communities (e.g. fitness centers or prisons) (Postigo et al., 2011; Schröder et al., 2010). Despite the claim for protection of anonymity and non-intrusive approaches, various ethical concerns and debates have been raised with regards to the privacy of individuals and how far upstream one can perform monitoring in this way (Hall et al., 2012).

Estimation of drug use via wastewater analysis consists of several consecutive steps (Castiglioni et al., 2014) which allow researchers to back-calculate the parent drug consumptions, based on observations of the drug or its human metabolites at a sampling point:

1. As first step, representative sampling techniques are used to collect raw wastewater. Since the occurrence of drug biomarkers in the sewer exhibits high dynamics, a suitable sampling strategy should be able to capture short term variations of drug loads. Different sampling guidelines for trace organics have been suggested for daily composite sampling (Ort, 2014; Ort et al., 2010c).
2. Following sample preparation e.g. concentrating and isolating the analytes of interest via solid phase extraction (SPE), wastewater samples are qualitatively and quantitatively analyzed by a chromatographer coupled to a mass analyzer e.g. liquid chromatography-mass spectroscopy (LC-MS/MS). The challenges and the analysis techniques are reported in most WBE studies (Bijlsma et al., 2013; Gheorghe et al., 2008; Pinhancos et al., 2011). In this step, the stability of drug biomarkers during sample handling, known as *in-sample* stability should be further considered (e.g. Baker & Kasprzyk-Hordern 2011; Castiglioni et al. 2006).
3. The amount of drug residuals detected at the treatment plan (e.g. g d^{-1}) is then calculated from the quantified concentration, based on the average wastewater flowrate to the WWTP.
4. The total daily drug load is then estimated based on a candidate biomarker which fulfils several requirements, i.e. it is excreted in consistent amounts in urine, detectable and stable in wastewater, and is present in sewers only due to human excretion (Castiglioni et al., 2016). Estimation should involve a correction factor which includes the excretion ratio and molecular mass ratio of parent to candidate bi-

omarker (Gracia-lor et al., 2016; Lai et al., 2012; van Nuijs et al., 2011; Zuccato et al., 2008).

5. Total daily drug consumption is then normalized to the population size so the data can be compared with the results from other geographical areas. Estimation of the population size that actually contributed to the wastewater sample is very challenging. Although census data are most widely used, these estimations based on a single day may not have a high reliability. Other methods such as estimates based on the loads of specific chemicals e.g. cotinine (metabolite of nicotine) or pharmaceuticals are also proposed (Chen et al., 2014; Lai et al., 2015). Based on estimation on the average consumed dose, drug consumption estimates are also reported as dose per capita per day.

1.2 Motivation of the study

In the commonly applied methodological steps in WBE studies, it is assumed that a candidate biomarker does not undergo a significant transformation during its in-sewer transport time. This general assumption is however not accurate for many of chemicals. For example, benzoylecgonine, BE (a major human metabolite of cocaine, COC) that is widely used as a candidate biomarker to back-calculate COC consumption, is reported to actually form considerably from hydrolysis of cocaine in raw wastewater (Bisceglia et al., 2012). Other studies also found transformation of 6-monoacetylmorphine, 6MAM (a candidate biomarker for heroin, HER) in raw wastewater (van Nuijs et al., 2012) as well as in sewer biofilms (Thai et al., 2014). These studies demonstrated that many of commonly accepted candidate drug biomarkers undergo in-sewer transformation. The importance of accounting for in-sewer processes in back-calculation methods is demonstrated (Plósz et al., 2013). As opposed to *in-sample stability* only few studies assessed transformation of illicit drug biomarkers in the sewer (*in-sewer stability*) (Bisceglia et al., 2012; McCall et al., 2016; Plósz et al., 2013; Senta et al., 2014). In fact, the sewer network is not only a system of collection and conveyance of wastewater, but also a bioreactor for chemical and microbial transformations (Hvitved-Jacobsen et al., 2002). Wastewater composition at the WWTP influent is impacted by the design features and the operation conditions of the sewer system leading to alteration of organic matter during the sewer hydraulic residence time (Nielsen et al., 1992). Consequently, illicit drug biomarkers, as trace organic chemicals, can be influenced by physico-chemical and biological processes (fate processes) in the sewer. Ignoring the fate of

illicit drugs in the sewer may lead to significant under,- or over-estimation of drug consumption estimates in wastewater-based epidemiological studies. Therefore it is important to develop models that can be used to predict in-sewer transformation of drug biomarkers, which eventually will lead to more accurate estimation of drug consumption.

1.3 Research objectives

This thesis aims to provide a systematic procedure for model identification of in-sewer fate processes for illicit drug biomarkers. To this end, a variety of tools and methodologies have been used such as, experimental assessments, analytical procedures, modeling frameworks and statistical analyses. The research outcomes were then applied in the context of wastewater-based epidemiology where an objective assessment using uncertainty analysis was added to the conventional methods. Fig. 1.1 illustrates the research focus areas of this thesis. The main research objectives of this thesis are:

i. To experimentally assess in-sewer chemical and biological transformation and partitioning processes for selected illicit drug biomarkers under environmentally relevant conditions prevailing in sewer systems. Various experiments were performed in batch mode to investigate fate processes and biological activities in raw wastewater (suspended biomass) and sewer biofilms (**Paper I** and **III**).
ii. To develop mathematical models and evaluate different model structure to predict transformation of illicit drug biomarkers in raw wastewater and sewer biofilms (**Paper I** and **III**).
iii. Calibration of chemical pathway models combined with reaction kinetics using heroin drug biomarkers as an example. This includes the reliable propagation and prediction of uncertainty for drug transformation rates according to their transformation pathways. The methodology was assessed with parameter correlation analysis and compared with other common estimation methods (**Paper II**).
iv. To objectively assess the uncertainty associated with neglecting in-sewer processes as compared to uncertainty in sampling via a model-based approach at the catchment level. This includes generation of short-term variations of drug loads based on stochastic sewer inputs. (**Paper IV**).

v. To back-calculate selected drug biomarkers based on monitoring data from a sampling campaign in Copenhagen, Denmark.

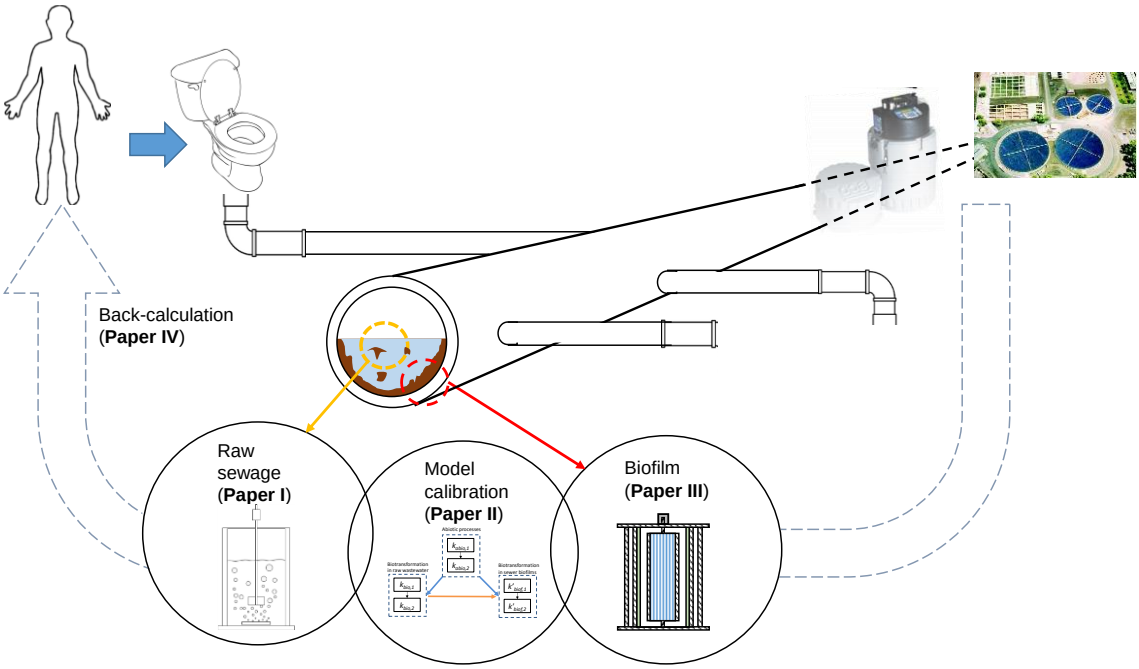


Figure 1.1 Schematic representations of 4 research focus areas in this thesis.

The research objectives are investigated for 5 chemical groups comprising 16 drug biomarkers: (i) mephedrone (MEPH); (ii) methadone (METD) and its human metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP); (iii) cocaine (COC) and its metabolites benzoylecgonine (BE), ecgonine methyl ester (EME), and cocaethylene (CE); (iv) heroin (HER) and its metabolites 6-monoacetylmorphine (6MAM), morphine (MOR), and morphine-3-β-D-glucuronide (MORG); codeine (COE) and its metabolite norcodeine (NCOE); (v) tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ9-THC (THCOH), and 11-nor-9-carboxy-Δ9-THC (THCCOOH).

1.4 Overview of methodology

Fig. 1.2 shows the systematic procedure applied to achieve the objectives in this thesis. This procedure involves model identification and model application. *Model identification* is a process that includes identification of a suitable model structure and unique estimates of model parameters.

First, based on the framework and objectives of the study, relevant experiments are designed and data are collected. Data quality must be assured either by repeating experiments or by replicating chemical analysis and data processing. Eventually, a suitable mathematical model structure is formulated based on experimental data and available modeling frameworks for model calibration. In prior analysis, model parameters are initially set as default values (in case of previous estimations by other studies). If the simulation results do not adequately predict the experimental data, e.g. the deviation (residuals) is significant, a selection of parameter subset – chosen based on sensitivity analysis or expert knowledge – is considered for estimation. If the parameters are not identifiable based on available criterion in literature, a new parameters subset is chosen. Subsequently, if a significant and systematic deviation is observed, the structure of the model is modified. Alternatively, new experiments should be conducted in case of identifiability problem (not part of this thesis).

Once both model structure identification and the subsequent calibration is successful, model validation should be assured by testing the model prediction against an independent dataset. The validated model is then reliably used for application.

In this PhD thesis, experimental assessments and model formulations were performed for laboratory scale studies, whilst model application was conducted at catchment level. Model application involves back-calculation of daily drug consumption based on monitoring data of WWTP influent.

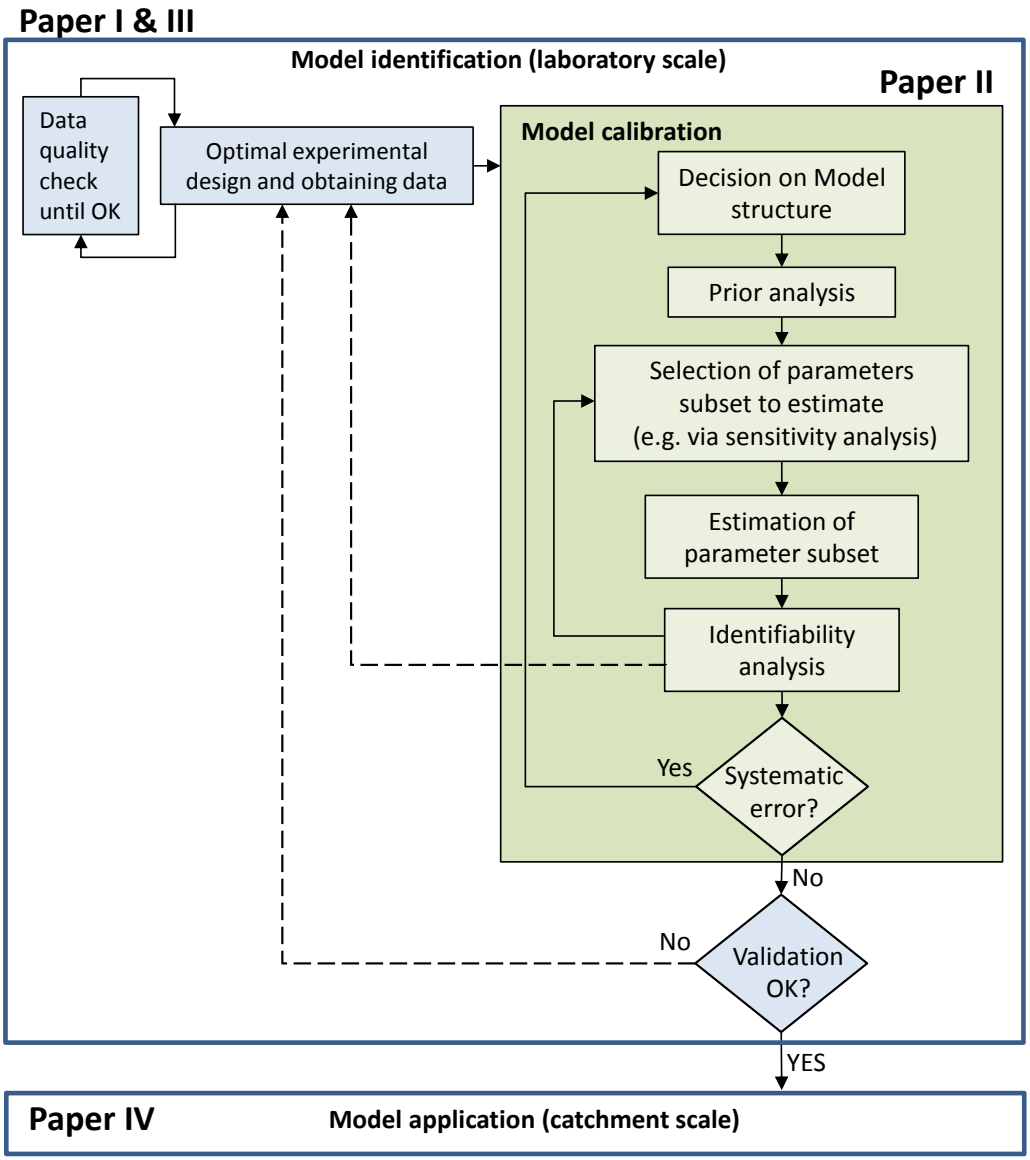


Figure 1.2 Overview of the methodology considered in this PhD thesis including model identification and model application. Dashed lines are not included in this thesis.

2 Processes in sewer systems

2.1 Sewer characteristics

Sewer systems are primarily designed to collect and convey wastewater from households and industries as well as runoff water from precipitation to storage tanks (e.g. retention basins), treatment and disposal facilities (e.g. WWTPs). The ultimate goal of this collection system is to maintain public hygiene and also prevent flooding. It consists of a network of pipes with a number of infrastructures such as manholes, valves, pumps and overflow weirs. In addition to transport mechanism in the sewer, sewer systems can also be considered as bioreactors where the organic matter can undergo significant alterations (Hvitved-Jacobsen et al., 2002; Warith et al., 1998). These alterations influence the interaction between the sewer and the subsequent treatment processes at the WWTP. Although urban drainage systems was not traditionally considered as "biologically active systems" when designing the sewer systems, there have been many studies on self-purification and the potential of sewers for biological wastewater treatment since the 1970s (Pomeroy and Parkhurst, 1972; Stoyer, 1970). A summary of sewer characteristics is depicted in Figure 2.1. In this perspective, sewer features are divided into design and operation aspects, i.e. the physical structure of the sewer and the operational mode, as well as in-sewer processes i.e. transport and fate processes (sewage including organic compounds). In the following section, sewer characteristics are briefly discussed from a water quality point of view.

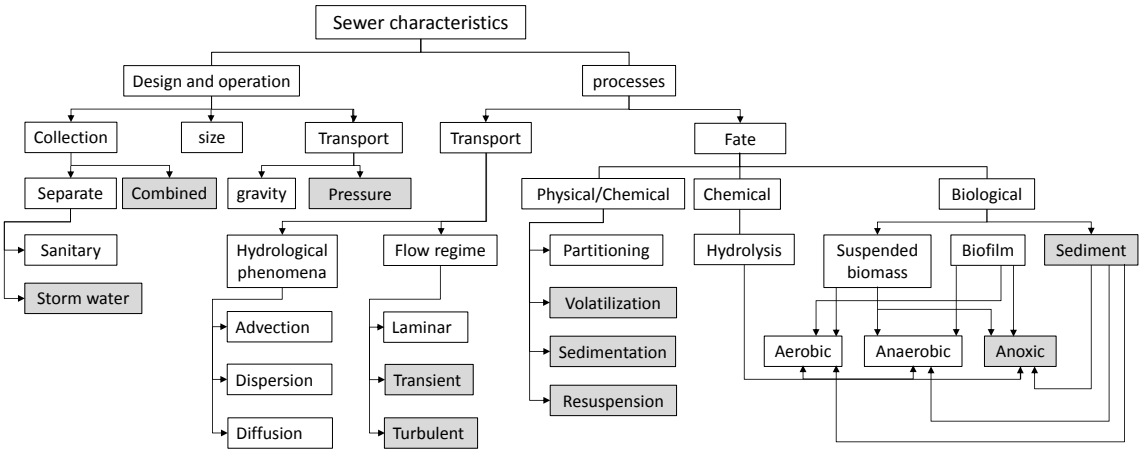


Figure 2.1 Diagram of sewer characteristics. Shaded boxes are not the focus of this thesis.

Water quality modelling in urban drainage systems involves two main aspects; physical transport processes and physicochemical and biological processes of the substances. To design an appropriate experiment (laboratory or full-scale) or simulate the sewer processes with reasonable approximation, it is essential to understand the principles behind these processes. This is essentially important in order to make assumptions and simplifications for experimental and modelling purposes knowing the fact that in-sewer processes have complex nature. The transport-related phenomena are not the central focus of this thesis, and they are only briefly evaluated in **Paper III** for a laboratory scale and in **Paper IV** for a catchment-scale study. Below is an attempt to discuss the relevant phenomena and present modelling approaches described in the literature, following the structure laid out in Figure 2.1.

2.2 Design and operation

The variability of sewer design and its operational mode creates different environmental conditions which influence the wastewater composition and its microbial community (Henze et al., 2000; Liu et al., 2015; Warith et al., 1998). These variabilities can be categorized according to (Hvitved-Jacobsen et al., 2013): (i) sewage collection, (ii) sewage transport, and (iii) sewer size:

Sewage collection: Sewers are designed to transport wastewater from households and industries (sanitary sewers) and also to transport runoff and infiltrated water after rain events (storm sewers). In this definition, sewers can be sanitary, storm or combined. In sanitary sewers, the wastewater contains high levels of biodegradable organic matter and it is considered a biologically active matrix, which contains excessive amounts of substrates for biomass. In contrary, storm sewers contain smaller amounts of organic matter but potentially more inorganic matter (e.g. street dust and sand) and toxic substances such as metals, plasticisers and pesticides (Erickson et al., 2013). The composition of wastewater is more dynamic and oscillatory in combined sewers as the dynamics and the loading of organic matter can vary significantly when the dry weather flow is combined with storm water. Moreover, other site-specific factors such as cross-connections, leakage in broken pipes, illicit connections etc. can be also influential (Panasiuk et al., 2015). Nevertheless, this thesis focuses on the sanitary and combined sewers under dry-weather flow conditions.

Sewage transport: Sewage is conveyed in the sewer pipes under gravitation (gravity sewers) or it is pumped in lift stations along the sewer (pressure sewers). In gravity sewers, the sewage is exposed to the sewer atmosphere,

allowing for reaeration and exchange of volatile compounds. Aerobic conditions in the bulk phase together with long residence time in gravity sewers allows for reduction of biodegradable substrates and production of biomass. In contrary, sewage in pressure sewers is transported with higher velocity and possibly under anaerobic conditions favoring the conditions for anaerobic activity of biomass.

Sewer size: Sewer systems consist of many interconnecting pipes covering an area (catchment) from the most upstream part to the downstream regions close to the WWTP. Pipes are designed with increasing diameter throughout the system as they collect more wastewater. Sewer biofilms (attached microbial communities on sewer walls) can potentially enhance microbial processes in the sewer (Huisman and Gujer, 2002).

2.3 Transport processes

Flow in sewer pipes can fill the whole cross-section (pipe flow) or partially fill the pipe (part-full pipe flow). The presence of these two flow types depends on sewer (i.e. gravity or pressure) and the type of flow (dry- vs. wet-weather flow). However the second flow type is the most common condition in sewers: gravity-driven flow with free surface (Butler and Davies, 2004). This implies that the redox conditions in the bulk liquid of the pipes are dominated by aerobic conditions (favoring the conditions for aerobic heterotrophic biomass), an assumption that was considered at catchment scale in **Paper IV**.

The flow regime in sewer pipes can be either laminar (e.g. in dry-weather flow), turbulent (e.g. storm flow) or an in-between regime known as transient. In laminar flow, the water flows in parallel layers with no cross-current among the layers. The velocity of water is at the minimum close to the pipe wall due to the flow resistance (viscous effect) and reaches a maximum after a specific length known as the boundary layer thickness. In a turbulent regime, flow and pressure change chaotically and intense mixing occurs at the microscopic scale. In this regime the flow velocity has a higher gradient close to the wall (high drag force) and the boundary layer is unsteady. The average flow velocity in laminar flow is defined at the average cross-sectional velocity profile. The impact of flow regime on the mass transfer of substances between the bulk liquid and the sewer biofilms is discussed in **Paper III**.

Transport of substances in the sewer can be explained by hydrodynamic phenomena, defined as advection, diffusion and dispersion.

Advection is the process of transport of water and the substances with the mean flow velocity. In this mode of flow, the flux vector for all substances in the liquid phase is equal. In contrary, diffusion refers to the random motion of compounds at the molecular scale and it is caused by concentration gradient due to unequal distribution of the compounds such as drug biomarkers. In sewer systems, diffusion is also enhanced by mixing in manholes. Diffusion depends on temperature, size of the compounds and viscosity of the fluid. In addition, dispersion is caused by combined action of variation of velocity over the cross section (e.g. pipe) and molecular diffusion causing the spreading of the compounds (Taylor, 1953). Dispersion along the pipe can be calculated based on a dispersion coefficient that can be calculated based on observed distribution of concentrations (Nordin and Troutman, 1980; Singh and Beck, 2003). Dispersion can also result from deviation of the real flow field from a one dimensional model. This deviation is known as *numerical dispersion* as simplified models cannot capture complex three dimensional fluid profiles. In simulation tools, numerical dispersion can substitute real dispersion (Gujer, 2008). This is briefly discussed and demonstrated in **Paper IV**.

2.4 Fate processes

The sewer is not a homogenous system and consists of different distinct phases or compartments; wastewater; biofilm/sediment and sewer atmosphere (in gravity sewers), Fig. 2.2. Wastewater components, such as soluble, colloidal and suspended matter, undergo different physical, chemicals and biological processes (fate processes) within each of the sewer phases. Although the primary goal of this thesis is the fate assessment of drug biomarkers in the sewer, their fate can be directly related to the primary metabolic processes which include the biological activities relevant for utilization of organic growth substrates and formation of biomass. Understanding the principles behind these processes – and also other physical and chemical processes that could indirectly impact the fate of drug biomarkers – would help to properly design experiments and construct appropriate mathematical models.

Physical, chemical and biological processes, under the environmental conditions prevailing in sewer systems, lead to alteration of organic compounds and trace organics (e.g. drug biomarkers) during their transport time in the sewer. Physical processes include volatilization, sedimentation/resuspension as well as partitioning i.e. sorption/desorption to suspended solids and biomass (a physico-chemical process); chemical processes can be in the form of hydrolysis; and biological processes are microbially-mediated transfor-

mations due to presence of biomass in suspended solids, sewer biofilms or sediments. In general, chemical and biological processes can occur under different redox conditions, i.e. aerobic, anaerobic and anoxic. Nevertheless, due to these physical and biological processes the distribution and composition of substances can alter in space and time via mass exchange across the boundaries of sewer compartments. Different sewer compartments are depicted for gravity sewers and pressure sewers in Figure 2.2.

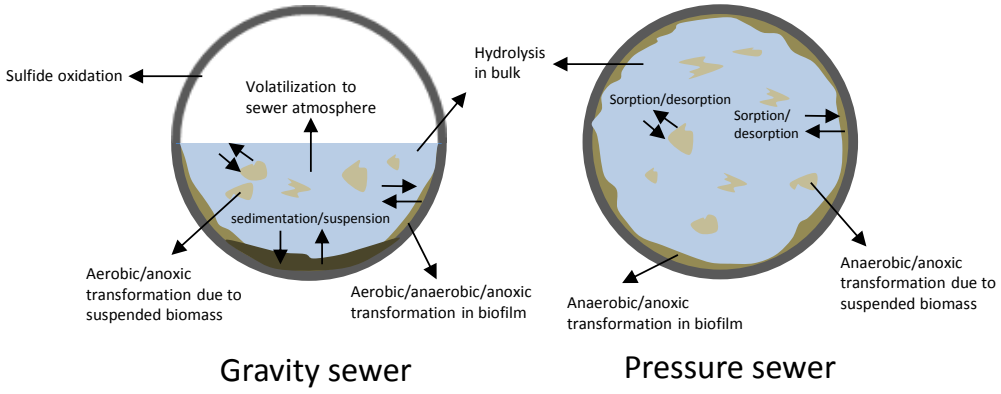


Figure 2.2 Sewer compartments and the main physical, chemical and biological processes for organic and trace organic chemicals.

Partitioning

If a drug biomarker has a high partitioning affinity to solids, it can diffuse and sorb onto biosolids in the sewer (sediment/suspended solids/biofilm). Since the bioavailability of the compounds is a prerequisite for microbial transformation, the rate of biotransformation can be significantly impacted by the sorption processes (Schwarzenbach et al., 2003). The interaction between sorbing chemical (*sorbate*) and solids (*sorbent*) can be governed by chemical properties and involve different mechanisms, including: (i) hydrophobicity of chemical or van der Waals interactions which are identified by an equilibrium partitioning between a hydrophobic phase and water. An octanol-water partition coefficient, K_{OW} or pH-dependent distribution coefficient (Ingram et al., 2011), are normally used as a predictor for such partitioning, (ii) electrostatic interaction between cations and anions with charged sites. Dissociation of an acid or a base highly depends on the pH value of the solution. The acid dissociation constant, pKa is normally used as predictor of non-dissociation or dissociation. An acid in a solution with a pH higher than its pKa would be more in dissociated form whereas for a base this implies non-dissociation. The opposite occurs when pH of the solution is lower than the pKa value of an acid or a base. Sorption process may involve other binding forces such as cation

exchange, surface complexation and hydrogen binding (Hyland et al., 2012; Trapp et al., 2010) and may also be associated with multiple interactions e.g. electrostatic interaction and surface complexation (Mackay and Vasudevan, 2012). Therefore, capturing a range of interactions between the chemicals and solids may be required to improve the prediction accuracy (Polesel et al., 2015; Sathyamoorthy and Ramsburg, 2013). Moreover, to simulate sorption processes in biofilm, diffusion of sorbate to the biofilm should also be considered (Wicke et al., 2007).

Chemical Hydrolysis

Chemical bounds of organic matters in water might cleave off due to a chemical reaction known as hydrolysis. By cleavage of a leaving group a new bond is formed as H+ or OH-. Transformation of drug biomarkers is often possible as these molecules usually have different functional groups and structural moieties such as ester, amide, lactone etc. that are susceptible to cleavage in water (Li, 2012). Beside the hydrolysis mechanism in neutral environments, hydrolysis can be catalyzed by acids or basis. Chemical hydrolysis is relevant for both primary substrates and drug biomarkers. It must be noted that chemical hydrolysis is different from enzymatic hydrolysis (breaking down large organics to smaller components by enzymes).

Biological processes

Biological processes in the sewers follow a basic principle: removal of substrates for anabolic (cellular growth) and catabolic (as energy source) reactions. These processes are referred to as *primary metabolic processes* which involve biomass growth and substrate utilization. The underlying concepts to simulate these processes are similar to the ones applied for activated sludge systems (Henze et al., 2000). Together with other organic chemicals trace organics also undergo biological transformations leading to conversion to other chemical structures. These processes are referred to as *secondary metabolic processes*. These microbial processes are one of the focal points of this thesis which are further discussed in this chapter.

2.5 Modelling approaches

Sewer systems have very dynamic characters from both water quantity and water quality perspectives. Modeling urban catchments involves generation of dry weather flow and run off water as well as description of transport processes for flowrate and pollutant load. Different modelling approaches are

extensively described in literature (Achleitner et al., 2007; Butler and Graham, 1995; Rauch et al., 2002).

Sewer flow is commonly simulated either by a physically-based description of flow using partial differential equations such as the Saint-Venant equation e.g. in MOUSE–HD (Garsdal et al., 1995) or conceptual-based models formulated as ordinary differential equations such as the tank-in-series approach e.g. in CITYDRAIN (Achleitner et al., 2007). Besides such deterministic models, the stochastic phenomena of sewer flow are also modelled by stochastic differential equations in the stochastic grey-box modelling approach (Breinholt et al., 2011). Moreover, mechanistic models based on advection-dispersion principles with stochastic model inputs is also suggested (Ort et al., 2005) to simulate stochastic load variations of trace chemicals such as illicit drugs (Mathieu et al., 2011). This model was used in **Paper IV**.

Compared to sewer flow modelling, fewer studies have focused on water quality modelling aspects. Water quality models received a major attention after sewer modelers attempted to develop an integrated urban water system model to combine sewer system and wastewater treatment into one model for better management and control strategies (Bach et al., 2014; Meirlaen et al., 2002; Olsson and Jeppsson, 2006; Rauch et al., 2002). Due to limited understanding of in-sewer chemical and biological processes, water quality models have been applied to a limited extent (e.g. Benedetti et al., 2013). Different models have been proposed to describe biological processes similar to existing models for treatment plants (Huisman and Gujer, 2002; Mourato et al., 2003; Tanaka and Hvitved-Jacobsen, 1998). Although these models are developed to describe primary metabolic processes, there are also models that include prediction of fate of trace organic chemicals and micropollutants (Benedetti et al., 2013; Keyser et al., 2010; Lindblom et al., 2006; Snip et al., 2014; Vezzaro et al., 2014). However, fate processes for trace organic chemicals are often compound-specific and requires understanding the principles behind sorption and transformation processes. A model identification procedure is discussed in detail in chapter 4, for illicit drug biomarkers.

Primary metabolic processes:

As discussed earlier, wastewater in sewers consists of soluble and particulate fractions. A biological modeling concept should include these two groups of components and their relevant kinetic characteristics. In this perspective, the traditional reporting on removal of biological oxygen demand (BOD) and chemical oxygen demand (COD) in the bulk phase of raw sewage (Pomeroy

and Parkhurst, 1972; Stoyer, 1970) is not a reliable method to characterize the in-sewer biological processes. Microbial organisms in raw wastewater, to some extent, are similar to activated sludge biomass in WWTPs (Henze and Comeau, 2008). Hence, the existing models to predict microbial activity in activated sludge could be relevant in sewers as well. Process models developed by the International Water Association (IWA) Task Group on design and operation of biological wastewater treatment are the most widespread models which primarily focus on modelling of activated sludge systems (ASMs) (Henze et al., 2000). These models are widely described and discussed in literature (Decostere et al., 2016; Kappeler and Gujer, 1992; G Sin et al., 2009; Vanrolleghem et al., 1999).

Despite similarities between microbial processes in a sewer and in a wastewater treatment plant, these two systems are conceptually different mainly due to two reasons (Hvitved-Jacobsen et al., 2013): (i) The principle of biological processes are not the same, i.e. a WWTP is a controlled and optimized system to remove organic carbon, nitrogen and phosphorous, whereas a sewer is a highly dynamic environment in which the microbial transformation can be utilized for other purposes, e.g. preventing the emission of hydrogen sulfide or preserving biodegradable substrates removal for following denitrification and biological phosphorous removal in a WWTP. (ii) The process conditions are different. In WWTPs, active heterotrophic biomass is present in abundance in activated sludge systems and the biomass is often under the conditions of limited readily biodegradable substrates. Due to high solids retention time (SRT) in WWTPs, the biomass contains high microbial diversity. In contrary, due to comparably shorter SRT, or in-sewer hydraulic residence time (HRT), the biodiversity is expected to be lower in the bulk phase of the sewers (suspended biomass). Readily biodegradable substrates are in abundance in the sewer, although oxygen can be limited. Biomass in sewers exhibit less microbial diversity partially due to short SRT of biomass e.g. autotrophic biomass can be expected to be close to zero (Jiang et al., 2009). These differences are summarized in Table 2.1.

Based on these differences, the microbial community underlying processes in the sewer system should be characterized differently than in WWTPs in terms of availability of growth substrates and terminal electron acceptors and fraction of active biomass.

Table 2.1 The difference between sewers and a typical WWTP (sludge system) in the view of process conditions for biological processes

	Sewer	**WWTP** (Activated sludge system)
Biologically active biomass		
• Autotrophic biomass	Negligible	High
• Heterotrophic biomass	Low	Very high
Oxygen	Possibly limiting (gravity) Low (pressure)	High (aerobic tank) Negligible (anaerobic tank)
Readily degradable substrate	Non-limiting	limiting
Substrate-to-biomass ratio	High (> 5 gCOD/gCOD)	Low (< 1 gCOD/gCOD)
Particulate hydrolysable factions	High	Medium
SRT/HRT	In range of hours	In range of days
Form of biomass	Suspended/biofilm/sediment	Suspended (activated sludge) Biofilm (e.g. moving bed biofilm reactors) Suspended + biofilm (hybrid systems)

According to these concepts, the Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) model framework was introduced by Hvitved-Jacobsen et al. (1998). In its first version, the model focused primarily on microbiologically-mediated aerobic transformation of organic carbon. Since then, the model has been extended to include anoxic and anaerobic heterotrophic growth processes as well as biochemical processes related to the sulfur cycle (Hvitved-Jacobsen et al., 2013).

In the aerobic WATS model, the maintenance energy requirement is used to account for any non-growth related substrate removal as proposed by Hvitved-Jacobsen et al. (1998), who found that biomass decay is less relevant for untreated wastewater in sewers due to unlimited growth in the presence of excess organic carbon. Furthermore, hydrolysis of particulate organic matter to substrate used for bacterial growth and maintenance is divided into two fractions: slow, X_{S2}, and rapid X_{S1} hydrolysable substrates. The inert soluble and inert particulate fractions used in the ASM1 framework are neglected, due to the low residence time in sewers, and are being lumped into the slow hydrolysable COD fraction, X_{S2} (Vollertsen and Hvitved-Jacobsen, 2002).

In the anaerobic WATS model, readily biodegradable substrate is divided into two fractions: fermentable substrates, S_F, and fermentation products, i.e. volatile fatty acids (VFAs). S_F and S_A are both electron donors and are utilized for cell growth. Low-molecular weight organic chemicals such as VFA are pre-

sent in raw wastewater and also produced via fermentation of S_F. Only heterotrophic biomass is considered for enzymatic hydrolysis since polymeric organic compounds are not typically direct substrate for sulfate reducing bacteria (SRB) (Muyzer and Stams, 2008). A decay rate and no growth rate are considered for heterotrophic biomass (X_H). The decay of biomass results in the formation of X_{S2}. However, growth of SRB is considered as detached biofilm from the pressure sewer that could be present in the sample. Rudelle et al. (2012) concluded that for wastewater that has already undergone anaerobic process it is important to consider sulfate respiration even in the bulk wastewater. Methanogenesis is assumed to be negligible as it is typically not observed in sewers and only in the permanent layer of deposits. Acetogenic bacteria are also considered to be outcompeted by SRB species, especially when acetate is present. WATS model calibration and simulation is presented in chapter 4 (**Paper I**).

Secondary metabolic processes:

Microorganisms use different strategies to catalyze transformation of trace organics such as hydrolysis, oxidation, reduction and photolysis (not relevant for sewers). The rate of biotransformation may be determined by the processes that limit the delivery of substrates to microorganisms such as mass transport limitation of substrates across intervening media e.g. in biofilm (Rittmann, 1995) or sorption of hydrophobic organic compounds (Zhang et al., 1998). Since most trace organic chemicals (such as drug biomarkers), also called *xenobiotics*, are foreign to the microorganism, the cells may not have extracellular systems to actively pick up these chemicals and carry them to the interior part of the cell. Although the uptake is facilitated for nonpolar species, the uptake rate of trace organics may dictate the overall transformation (Schwarzenbach et al., 2003). Moreover, the *bioavailability* of trace organics can be affected by other phenomena, such as sequestration to solids matrix (Alexander, 2000) or cometabolism processes (Criddle, 1993).

Once trace organics and enzymes coexist, if the metabolism of the chemicals results in enough energy yield, the microorganisms may develop new cells. In such case, the dynamic of microbial population can be expressed based on Monod-kinetics and transformation of trace organic chemicals can follow this principle (Lindblom et al., 2006; Rittmann, 1995; Siegrist et al., 1989), eq. 2.1, Table 2.2. In this formulation, C_{LI} (g L^{-1}) is the concentration in the bulk, μ_C (d^{-1}) and Y_C ($gX_B\ gC_{LI}^{-1}$) are the maximum specific growth rate and yield

coefficients and K_C ($gC_{LI}\ L^{-1}$) is the affinity constant. The biomass is indicated by X_B which could be heterotrophic and also autotrophic biomass.

However, trace organics are primarily different from primary substrates because: (i) they present at very low concentration (in the range of nanograms to micrograms), significantly lower than their affinity constant and unlike primary substrates they are usually considered as non-growth substrates, (ii) their transformation can be catalyzed by non-specific enzymes widely present in wastewater and biofilm. From these stand points, biotransformation can be described based on a pseudo-first-order rate equation (Schwarzenbach et al., 2003) in which the removal rate is described by the concentration of the trace organic and a constant microbial population, X (eq. 2.2). In this formulation, k_{bio} ($L\ gX^{-1}\ d^{-1}$) is the biotransformation rate constant and it is assumed that biomass growth can be considered negligible during the time of investigation.

Following the assumption of negligible biomass growth, the extent of transformation can be expressed by a transformation rate, k'_{bio} (d^{-1}) in a first-order rate equation i.e. $k'_{bio}=k_{bio} \times X$, eq 2.3 (Bisceglia and Lippa, 2014; Senta et al., 2014; Vezzaro et al., 2014). It is argued that a transformation rate based on X would reflect an empirical character i.e. a system-specific behavior in a tested environment, and may not be applicable to other experimental conditions (Jacobsen et al., 1996). In this respect, more appropriate biotransformation can be expressed for a specific active biomass concentration, X_B, rather than total biomass (eq. 2.4). Moreover, sorption of trace organics can be considered as a lumped parameter within the expression for biotransformation (Joss et al., 2006; Plósz et al., 2013), assuming that sorption is fast compared to biotransformation (Wang and Grady, 1995), eq 2.5. In this sorption-biotransformation expression the bioavailability of the chemical due to sorption is inherently considered assuming that the trace chemical is bioavailable in aqueous phase and it is not bioavailable once it is sorbed (Schwarzenbach et al., 2003).

Many of trace organic chemicals such as illicit drugs (Kasprzyk-Hordern and Baker, 2012) have two or more enantiomers (a pair of molecules with a mirror image of each other). This implies that biotransformation of such compounds may need to address enantiomer-specific processes i.e. different transformation kinetics for each of the compounds (Polesel, 2016) eq. 2.6.

In the view of co-metabolism (co-digestion of primary and secondary substrates by enzymes), primary substrates may play a significant role in biotransformation of trace organic chemicals, suggesting that a biotransfor-

mation process that only includes the concentration of trace organics (even with including biomass, X_B in eq. 2.4) may not reflect a cometabolic mechanism. Presence of growth substrates has been observed to effectively enhance the biotransformation of trace chemicals (Delgadillo-Mirquez et al., 2011; Plósz et al., 2012a; Tan et al., 2013; Torresi et al., 2016). Consequently, the impact of the presence and absence of primary substrates on biotransformation of trace organics can be modeled using a switch function by including a half saturation coefficient, K_S for S_S, eq. 2.7 (Plósz et al., 2012b; Torresi et al., 2016). In this formulation the removal rate of C_{LI}, is slowed down by complete removal S_S. Conversely, inhibition of transformation by primary substrates was also reported due to e.g. competition for non-specific enzymes (Mazioti et al., 2015; Plósz et al., 2010; Su et al., 2015). The inhibition impact is formulated using a correction factor, η_{bio} for inhibition (eq. 2.8) according to (Plósz et al., 2010). Nevertheless, co-metabolism may also depend on the level of organic chemicals (Alexander, 1985) and this should also be considered when applying the suggested equations.

Besides biotransformation, trace organic chemicals may also undergo a formation process in which they are formed as a transformation product. In case of drug biomarkers (also Pharmaceuticals and Personal Care Products, PCPs) these formation processes follow a human metabolism before their release in the sewer. During the in-sewer transport time drug biomarkers may form again from their parent drug or other metabolites. A well-known example is net formation of benzoylecgonine from cocaine in sewers (Bisceglia et al., 2012; Plósz et al., 2013; van Nuijs et al., 2012). Formation processes can be expressed similar to transformation (with opposite sign) and also considering the molecular weight ratio e.g. a transformation product to a parent (Plósz et al., 2013). In this thesis, process rates to describe sorption to suspended solids, biotransformation and formation processes, were adopted according to activated sludge model for xenobiotics (ASM-X) framework (Plósz et al., 2013, 2012a).

Table 2.2 Secondary metabolic process rate equations according to literature studies to describe transformation of trace organic chemicals, with typical references and an indication of what papers in this thesis they have been used.

eq.		Biotransformation process for C_{LI}	Reference	
(2.1)	Second-order (Monod-based kinetics)	$\frac{dC_{LI}}{dt} = -\frac{\mu_C}{Y_C}\frac{C_{LI}}{(C_{LI}+K_C)}X_B$	Siegrist et al. 1989 Rittmann 1995 Lindblom et al. 2006	
(2.2)	Pseudo-first order	$\frac{dC_{LI}}{dt} = -k_{bio}C_{LI}X$	Schwarzenbach et al. 2003	Paper **I, II, III**
(2.3)	first order	$\frac{dC_{LI}}{dt} = -k'_{bio}C_{LI}$	Senta et al. 2014 Bisceglia & Lippa 2014 Vezzaro et al. 2014	Paper **I, II, III**
(2.4)	Second order	$\frac{dC_{LI}}{dt} = -k_{bio}C_{LI}X_B$	Jacobsen et al. 1996	Paper **I**
(2.5)	Pseudo-first order (instantaneous sorption)	$\frac{\mathrm{dC_{LI}}}{\mathrm{dt}} = -\frac{k_{bio}}{(1+K_dX)}\mathrm{C_{LI}}X$	Joss et al. 2006 Plósz et al. 2013	Paper **III, IV**
(2.6)	Two Pseudo-first order (Enantioselective)	$\frac{\mathrm{dC_{LI}}}{\mathrm{dt}} = -\frac{k_{bio,1}}{(1+K_dX)}\mathrm{C_{LI,1}}X - \frac{k_{bio,2}}{(1+K_dX)}\mathrm{C_{LI,2}}X$	Polesel 2016	
(2.7)	Pseudo-first order (co-metabolism; enhancement)	$\frac{dC_{LI}}{dt} = -\left(q_C\frac{S_S}{K_S+S_S}+k_{bio}\right)C_{LI}X$	Plósz et al. 2012 Torresi et al. 2016	
(2.8)	Pseudo-first order (co-metabolism; inhibition)	$\frac{dC_{LI}}{dt} = -\frac{k_{bio}}{1+\frac{S_S}{\eta_{bio}K_S}}C_{LI}X$	Plósz et al. 2010	

3 Sampling and quantification of drug biomarkers in wastewater

3.1 Representative sampling

As mentioned in the introduction, sampling campaigns are usually performed at the WWTP inlet. Collection of representative samples is a prerequisite for further methodological steps. A non-representative sample can be a major source of inaccuracy in consumption estimates which cannot be compensated with a highly accurate analytical procedure or very sophisticated backcalculation method. It is well-known that substances in the sewer are generally subject to systematic variations (i.e. seasonal, weekly, diurnal). However, du to infrequent release of many trace organic chemicals including drug biomarkers, these variations can occur in significantly shorter time frames, e.g. minutes or even seconds. Such short-term variations can result in a non-representative sample in case of inappropriate sampling. There have been different sampling guidelines for decades (Clesceri et al., 1998; U.S. EPA, 1982). However many studies have not considered these guidelines or they lack a transparent explanation of the sampling procedure used (Ort et al., 2010b). Performing sampling according to best-practice sampling protocols can be challenging, since these guidelines are provided to be of general validity and it might be difficult to adapt them for specific cases. Depending on the characteristics of the sewer (e.g. design and operational condition) the occurrence of substances might significantly differ from one site to the other. These variations are also substance-specific. For instance it has been shown that the variations of benzotriazole – contained in dishwasher detergents – as an example of a house hold chemical can be very different from human related PPCPs that are excreted with urine (Rieckermann et al., 2011). Even among excreted compounds (e.g. pharmaceuticals and drug biomarkers), high variability can be expected in one sampling site (Ort et al., 2010b). Regardless of sewer characteristics and catchment layout, the variability of excreted compounds is intrinsically dependent on the frequency of usage and excretion ratios (how much of parent is excreted as metabolites) which can be different from one person to another (Khan and Nicell, 2011). Moreover, the requirements for representative sampling might be not in access e.g. lack of access to flow sensor for flow-proportional sampling. Decision on the sampling location is also important. If the sampling is performed after the primary clarifier (or simply in a tank) the short term variations can be attenuated (Ort et

al., 2010b). However, this sampling location is not proper for particulate matters as they are substantially removed in primary clarifiers (Ort, 2014).

A "true" representative sampling is only possible with online automatic instrumentation or continuous sampling by diversion of a side stream of the inflow to the WWTP proportional to the flow (Ort and Gujer, 2006). For drug biomarkers no such online sensors exist and most available commercial samplers do not support continuous flow-proportional sampling. Instead, discrete sampling is usually applied following three sampling modes: (i) Time-proportional sampling where a fixed sample volume is taken at fixed predetermined time intervals. (ii) Volume-proportional sampling where a fixed sampling volume is collected at variable time intervals, which are triggered by a predefined wastewater volume arriving at the measurement point. (iii) Flow-proportional sampling where a variable sampling volume is collected at fixed time intervals and the sampling volume is proportional to the flow at the time of sampling.

A decision on sampling interval is highly dependent on the dynamic nature of the substance load with relation to the flow (i.e. concentration variations). The substances are released in the sewer with intermittent wastewater pulses. Due to dispersion and mixing effects in the sewer, the pulses overlay each other throughout the transport and form series of larger superimposed pulses. In case of scarce release of a substance or short transport time, the pulses can appear distinctly at the sampling site. Capturing such pulses requires very short sampling interval (Ort et al., 2010a) that could be as short as 2 min to correctly capture short-term fluctuations (Ort, 2014). As mentioned earlier, the geometry and structure of the sewers can have huge impact on flow variations. Since the occurrence of the drug biomarkers (as mainly dissolved substance in wastewater) depends on the flow, the fluctuation of the flow should be considered during sampling. In time proportional sampling mode, the samples are collected irrespective of the flow while in flow-proportional sampling the samples are collected with a volume weighted according to the flow. Although representativeness of the sample is improved with this sampling mode, the drawback is when wastewater flow is repeatedly stagnant (no flow). In such case volume-proportional sampling is preferred.

Choice of the right sampling mode and sampling interval is only possible with detailed preliminary knowledge which is often site and compound specific. By using a model-based approach and generating realistic load patterns, different sampling scenarios can be investigated a priori. A sewage Pattern

Generator (SPG) was introduced (Ort et al., 2005) to simulate stochastic load variation of the substances in the sewer, which can also be used to compare different sampling modes on drug biomarkers (Ort, 2016). This software computes superposition of wastewater pulses emitted from users distributed over a catchment. The transport of solutes in gravity sewer networks is simulated using a 1-D advection dispersion model. The SPG package requires prior knowledge such as number of pulses, drug mass per pulse and pulse duration. The drawback of this software is that it does not assess the impact of sorption and biotransformation on generation of drug load patterns.

As part of this thesis number of sampling campaigns were performed using flow,- and volume-proportional sampling modes (chapter 5). An objective assessment was also performed on the uncertainty in daily drug estimates that is due to inaccurate sampling. Moreover, different sampling modes were tested through scenario analysis (**Paper IV**).

3.2 Quantitative chemical analysis

Quantifying the concentration of drug pharmaceuticals and drug biomarkers in wastewater requires robust and reliable analytical methods. A number of challenges can be addressed: (i) target compounds are present in trace amounts, which necessitates extensive pre-concentration during sample preparation; (ii) Wastewater is a complex matrix, accuracy and reproducibility of quantitative analysis can be severely impacted by the presence of interfering compounds present in the matrix. This requires sample purification prior to mass detection and using isotope-labelled internal standards (ILIS) (Bijlsma et al., 2013); (iii) it is likely that there are other compounds present in a wastewater sample similar to the target compounds i.e. having similar mass. Presence of possible enantiomers for the same compound should be also considered in analysis (Kasprzyk-Hordern and Baker, 2012). The analytical method should be able to distinguish and evaluate these contributions through confirmation steps. Measurement (quantification) is normally performed via spectrometric techniques comprising of sample preparation, analysis using a mass spectrometer, and data processing. In this study these steps were performed for the samples collected during batch experiments (**Paper I & III**).

Sample preparation:

This step includes the preparation of samples after sample collection and before injection to a mass spectrometer. Sample collection might be from a monitoring study or from a laboratory-scale reactor. To prevent possible al-

teration of compound concentrations due to biological activity or partitioning to solids, samples in monitoring studies are preferably kept refrigerated during sample collection and transport to a lab. Depending on the stability of the compound in wastewater, the sample might need to be adjusted to acidic pH (van Nuijs et al., 2011). It is recommended that the samples are spiked with target analytes soon after sample collection. After transport, if the analysis is to be done later on, samples are stored in the dark at -20°C. After defrosting, samples are vacuum filtered through glass fiber filters to remove particulate matters from the matrix and clean the sample. For further sample clean-up, pre-concentration as well as isolation of the analytes of interest, filtered samples are passed through solid phase extraction (SPE) cartridges. The analytes are retained by the sorbent inside the cartridges with different mechanisms including hydrophobic interaction (nonpolar-nonpolar), hydrophilic interaction (polar-polar), ion exchange (electrostatic attraction) or mixed mode (two or more retention mechanisms). The choice of SPE sorbent highly depends on the analyte functional groups and polarity. SPE can be done manually or automatic (Trenholm et al., 2009) following this procedure: (i) sample pretreatment which includes pH adjustment, (ii) conditioning of the cartridge e.g. with MeOH or acetonitrile, (iii) loading of the sample, (iv) washing the cartridges to remove undesired contaminants, and (v) disrupting the retentive interaction between the analytes and the sorbent so the analytes are eluted from the cartridge.

The stability of the target compounds should be well assessed during sample collection, storage and preparation. It has been demonstrated that the in-sample stability of many drug biomarkers can be significantly affected by the choice and the length of the procedures before analysis (Baker and Kasprzyk-Hordern, 2011). A study on the impact of short-term (7 days) and long-term (60 and days) storage of wastewater samples on the analysis of PPCPs revealed that for short-term storage, keeping the sample in the fridge is better than freezing while for long-term storage freezing is preferred (Fedorova et al., 2014). Optimizing a method for sample handling can be very rigorous if a sample should be prepared for multi-analyte analysis in which analytes have very different physicochemical properties, e.g. a wide range of polarity (Borova et al., 2014).

Analysis using a mass spectrometer:

Following sample extraction, the sample is injected by a mobile phase at high pressure into a liquid chromatographic (LC) column that is packed with a stationary phase. Based on the specific physical interaction between the analytes

and the stationary phase, analytes move in the column with different velocity resulting in separate elution of analytes. The mass of separated analytes are then measured using mass spectrometry (MS) technique via ionizing the analyte molecules and sort the ions – including any fragment ion – based on their mass-to-charge ratio. Ionization, ion analysis and detection can be performed using different techniques, resulting in many different types of mass spectrometers. These three are the main parts of a mass analyzer: (i) an ion source to create atomic and molecular ions from sample molecules, (ii) mass analyzer to separate ions based on mass-to-charge ratio, (iii) following the ejection of ions from mass analyzer, they are collected by a detector such as electron multiplier to multiply incident charges.

Electrospray ionization (ESI) is a common method for ionization of organic compounds in which a beam of high energetic electrons knock off an electron from analytes to form an ion. During ionization different fragment ions – acquiring positive or negative charges – are produced which can then be used to identify target compounds.

Among the most common mass analyzers (Fitzgerald et al., 1997) are time of flight (TOFMS), quadrupole (QMS), ion trap (ITMS) and orbitrap. In TOFMS method, the mass-to-charge ratio is determined by the velocity (time) of the accelerated ion in electric field. In QMS, ions are filtered depending on their trajectories in an oscillating electric field. Only ions with a certain mass-to-charge will reach the detector. In ITMS, the ions – formed before or inside the trap – are trapped between two endcap electrodes and a ring electrode where they are oscillated by acceleration and deceleration. The ions leave the cap at specific mass-to-charge ratios. In Orbitrap ions cycle around a central electrode and simultaneously oscillate along a horizontal axis. The mass analysis is then performed in Fourier transform mode (by measuring oscillations) or mass selective instability mode (ejection and collection by detector).

Employed method in this thesis:

In this thesis, the selected drug biomarkers have different physicochemical characteristics, e.g. low (logKow=7.61 for THC) to high hydrophilicity (logKow<0 for MORG). Most of them are weak bases (except THC, THCOH, and THCCOOH, which are weak acids, and BE and MORG, zwitterions) and undergo cationic-neutral speciation (except THC which undergoes neutral-anionic; THCCOOH is always anionic; BE and MORG are zwitterionic-anionic) at typical wastewater pH=7-8. Acid dissociation constants range

from pKa = 10.6 for THC to pKa = 3.6 for BE. Based on the variation of properties, an all-purpose cartridge (Oasis HLB) was selected. This cartridge has strongly hydrophilic filter with water-wettable polymer and has a unique hydrophilic-lipophilic balance. SPE was performed manually using MeOH and MiliQ for conditioning and MiliQ for washing. The extraction was performed using MeOH followed by nitrogen evaporation. Samples were analyzed with high performance liquid chromatography coupled with linear ion trap-orbitrap mass spectrometry. Analytical methods were developed based on the accurate mass of the protonated ion with one nominal mass product ion and retention time.

4 Fate model identification

4.1 Experimental design

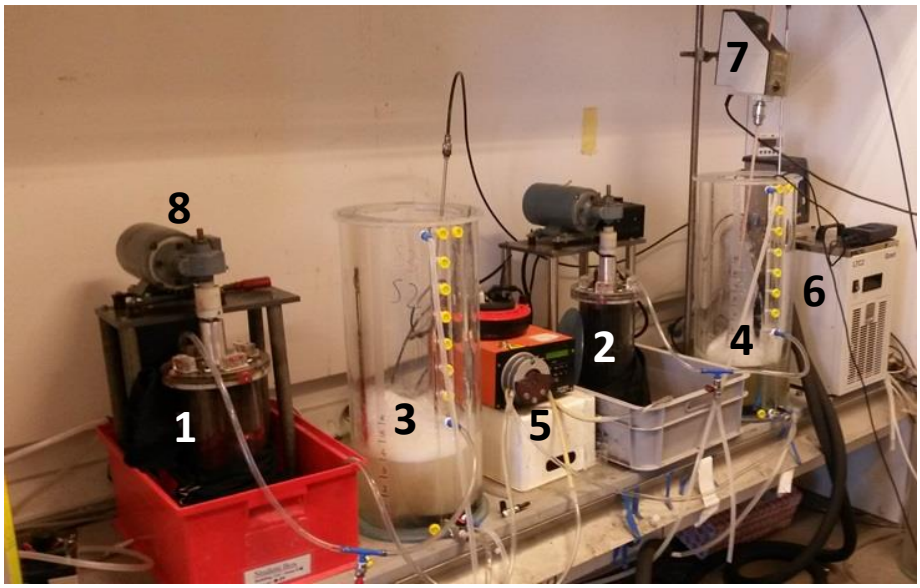
In-sewer fate processes were experimentally investigated by assessing transformation of illicit drug biomarkers in the sewer as well as abiotic transformations such as chemical hydrolysis. Moreover, partitioning of these chemicals to sewer solids was examined. All experimental assessments were performed in laboratory scale systems.

Biotransformation experiments

Biotransformation in presence of sewer suspended solids was tested by performing laboratory scale experiments using raw wastewater (**Paper I**). Two-jacketed reactors, filled with fresh raw influent wastewater were operated at 15°C under aerobic and anaerobic conditions by sparging air and nitrogen in the reactors respectively. Throughout a 2-day experiment, 9-12 samples were withdrawn from each reactor. Experiments were initially performed without spiking target drug biomarkers to test the biotransformation of drug biomarkers already present in the background. In this procedure (P0) the hypothesis was to test biotransformation under representative environmental conditions as previously suggested (Alexander, 1985). Following chemical analysis, the concentrations of drug biomarkers in many of the samples were below quantification levels. Subsequently, the experimental procedure was revaluated and further refined by spiking internal standards at a high level (10 $\mu g\ L^{-1}$), such that presence of background concentrations would not cause any interference. In this procedure (P1), almost all target chemicals were detected in samples, and the quality of data were assured with replicate analysis and confirmation steps. An additional set of experiments was also performed with an alternative procedure (P2) by spiking and targeting labelled internal standards (2 $\mu g\ L^{-1}$) with the hypothesis that transformation of these chemicals can be traced without any influence from back-ground concentrations.

To test the biotransformation in sewer biofilms (**Paper III**), two annular rotating biofilm reactors were used to simulate the shear conditions as in the sewer. Each reactor consisted of a stationary inner cylinder (diameter=9 cm) and an outer rotating drum (diameter=11.4 cm). The advantage of the reactors were high area to volume ratio (A/V=175 $m^2\ m^{-3}$) which allowed a high surface area for grow of biofilm. Each of the reactors were fed continuously (4 L d^{-1}) via an external tank (T=4°C), filled with pre-clarified wastewater. One external tank was sparged with air, and the other one with nitrogen to main-

tain aerobic and anaerobic conditions for the biofilms. Two batch experiments were performed for aerobic and anaerobic biofilms by spiking internal standards (P1) after 14 months of continuous operation. For these experiments, reactors were connected in a closed loop – flow rate of 4 L h^{-1} – with jacketed tanks in which they were sparged with air and nitrogen. These tanks were connected to aerobic and anaerobic biofilms respectively. Fresh preclarified wastewater samples were centrifuged (20 min, 4700 rpm) and then vacuum filtered (Advantec MFS, Inc., GA-55 grade) to further remove the suspended solids content and then wastewater was introduced to the tanks. Batch experiments (t=17°C) were performed for two days and 9 samples were collected from the outlet of the biofilm reactors. Similar experiments were also conducted with spiking labelled internal standards (P2) after 7 months of continuous operation. During all operational time, rotation of reactors was set at 20 rpm. Biofilm thickness (aerobic biofilms, 0.75 mm; anaerobic biofilms, 1 mm), was approximated after P1 experiments by the difference between the empty reactors and completely drained reactors with biofilm. It was then assumed that during the P2 experiments biofilm had the same thickness. Fig. 4.1 shows the experimental setup during batch experiments.



1. Aerobic rotating biofilm reactor
2. Anaerobic rotating biofilm reactor
3. Wastewater tank sparged with air
4. Wastewater tank sparged with nitrogen
5. Pump
6. Recirculating cooling bath
7. Mixer
8. Rotating motor

Figure 4.1 Experimental setup for sewer biofilm studies using annular rotating biofilm reactors under aerobic and anaerobic conditions (**Paper III**).

During biotransformation experiments using raw wastewater and in biofilm reactors (P1), biological activity of biomass was monitored by taking additional samples to measure total and soluble chemical oxygen demand (COD), total suspended solids (TSS), nitrate, ammonium and sulfate. Moreover, the activity of heterotrophic biomass was concomitantly investigated during aerobic biotransformation experiments in raw wastewater by measuring oxygen uptake rate in fresh wastewater samples.

Abiotic experiments

Transformation of selected drug biomarkers due to abiotic processes, i.e. chemical hydrolysis in absence of biomass, was tested using mineral water. Experiments were conducted in jacketed reactors (t=15°C) under aerobic and anaerobic conditions by following the procedure of spiking internal standards (P1) and taking 5 samples over 2-day experiments. To assess possible attachments of drug biomarkers to the reactor wall (plexiglass), a sample was taken at 15 min, from each reactor, by hypothesizing that any partitioning to the reactor wall, especially for hydrophobic compounds, would occur rapidly.

Sorption experiments

Sorption to suspended solids was assessed using primary sludge in batch reactors. Biomass was deactivated using 0.05% (v/v) sodium azide. Experiments were performing by spiking internal standards (P1) and samples were collected over 4 h. Two experiments were conducted at pH~8.4 and 7.7 to replicate the pH conditions during biotransformation experiments with raw wastewater (P1) (**Paper I**).

To investigate sorption to sewer biofilms, sewer biofilms on two strips, from each reactor separately, were suspended in tap water and biomass was deactivated as previous. The hypothesis was to test instantaneous sorption without interference of diffusion limitation (**Paper III**). Two experiments were performed in total for 4 h, for aerobic biofilms and anaerobic biofilm (pH~8.8). For all sorption experiments, control experiments were also performed with mineral water with same amount of sodium azide and approximately same pH.

4.2 Model structure

4.2.1 Physico-chemical processes

Partitioning to solids

Sorption and desorption to suspended solids was modeled using two first order differential equations in opposite directions; one to describe the sorption (partitioning) of compounds from the aqueous phase, C_{LI} (g L^{-1}), onto solids phase C_{SL} (g L^{-1}) and one to describe the desorption of already sorbed compounds from the solid phase back to the aqueous phase (Joss et al., 2006). In this formulations the extent of sorption was described with partition coefficients of biomarkers, K_d (L gTSS d^{-1}) and the sorption rate was defined as $K_d \times K_{des}$ in which K_{des} (d^{-1}) is the desorption rate from suspended solids (**Paper I**). Sorption to sewer biofilms however defined together with biotransformation processes, similar to eq. 2.5 in Table 2.2. It was assumed that desorption rate from suspended biofilm was rather fast, e.g. k_{desf}=100 (d^{-1})(Plósz et al., 2013) and equilibrium between sorbed and dissolved chemicals were reached instantaneously. In this formulations the stress is on the impact of sorption on bioavailability of drug biomarkers (Schwarzenbach et al., 2003), rather than predicting the dynamics of sorption and desorption. Nevertheless, the sorption coefficient for sewer biofilms k_{df} (L gTSS d^{-1}) was described and subsequently estimated as linear equilibrium distribution between sorbate and sorbent. Since drug biomarkers can undergo abiotic processes even at the presence of sodium azide, these processes were also included, from control experiments, in the estimation of k_d. The dynamics of sorption and desorption processes can be simulated during sorption experiments with both suspended solids and biofilm. Fig. 4.2 presents a concentration profile of ecgonine methyl ester (EME), as an example, during a sorption experiment with anaerobic biofilms and during a control experiment. Estimated sorption coefficient, (k_{df}=1.59 L gTSS d^{-1}), could simulate the removal of this compound due to sorption.

To simulate sorption and desorption processes onto the reactor wall, two first-order kinetics were formulated. Similar to partitioning to suspended solids, the extent of sorption was correlated with the partitioning coefficient to the reactor wall, k_{dw} (m^3 m^{-2}). Furthermore abiotic processes were described with first-order kinetics, in which removal or formation of a drug biomarker is proportional only to its concentration.

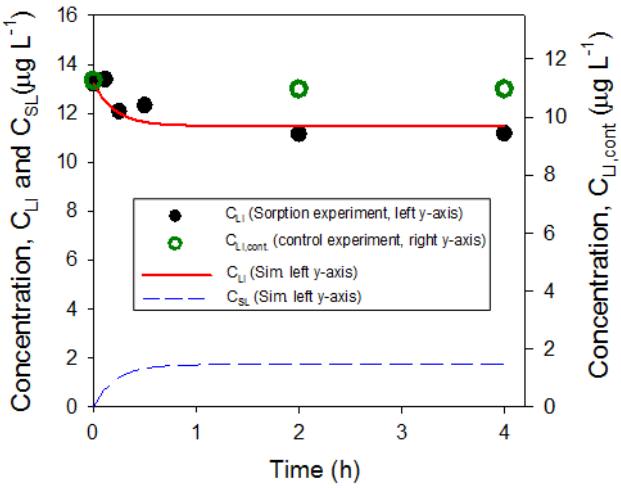


Figure 4.2 Ecgonine methyl ester (EME) concentration in bulk liquid (C_{LI} µg L^{-1}) during a sorption experiment with suspended anaerobic biofilms (left Y-axis) and EME data in the bulk liquid phase during a control experiment with mineral water ($C_{LI,cont}$) (right Y-axis). C_{LI} and concentration in solids (C_{SL}) are simulated with two reverse first-order kinetics.

In Fig. 4.3 it is shown that simulation of abiotic processes for mephedrone (MEPH) without accounting for partitioning to the reactor wall, even with best estimates k_{abio}=0.17 d^{-1}, could not well predict experimental data (green line). By including partitioning (K_{df}=1.6 L gTSS d^{-1}), model could capture data pints (k_{abio}=0.1 d^{-1}) more precisely.

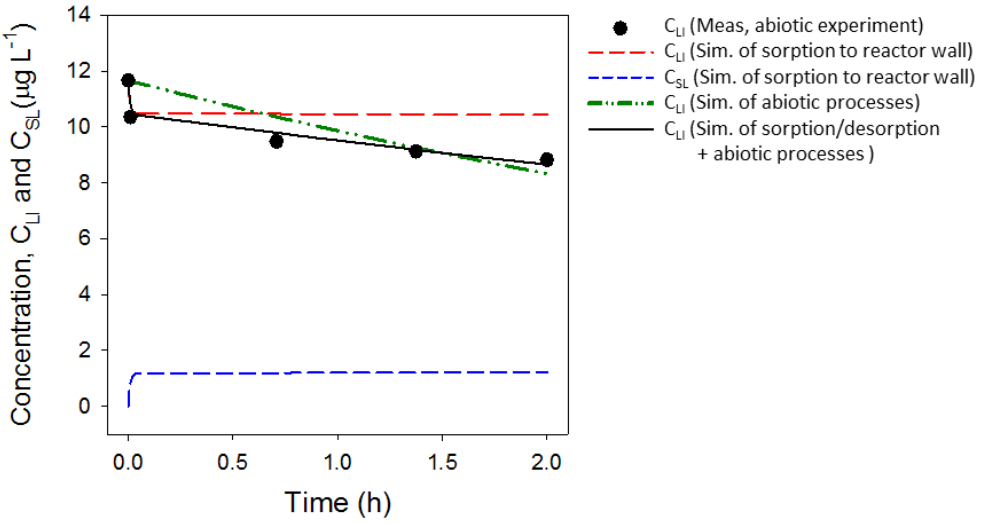


Figure 4.3 Mephedrone (MEPH) concentration in bulk liquid during an abiotic experiment under aerobic conditions. Simulation results reflect on the variation of the simulated bulk concentration, C_{LI} and the attached concentration to the reactor wall, C_{SL}, during the abiotic processes and sorption/ desorption onto and from reactor wall.

4.2.2 Biological processes

Simulating biological processes, i.e. microbially-mediated transformations or shortly biotransformations, is one of the main modeling focuses of this thesis. As presented in Table 2.2, these processes are defined with different formulations to correlate removal or formation processes to one or two constants, or time-dependent variables, and biotransformation rate(s).

In this thesis, to model biological processes in raw wastewater, two model structures were tested. (i) A model based on the activated sludge model for xenobiotics (ASM-X) framework which was extended with abiotic processes and sorption and desorption processes onto the reactor wall. (ii) The Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) model combined with extended ASM-X (WATS—ASM-X). With respect to the first model, biotransformation processes were described – in the same way as for both aerobic and anaerobic conditions – as pseudo-first order kinetic equations. In this model, the dynamics of drug concentrations were correlated with TSS-normalized biotransformation rates, k^*_{bio} (L $gTSS^{-1}$ d^{-1}), assuming that suspended solids are fixed during simulation time (same length as experiments, 2 day). In the WAST—ASM-X model, biotransformations were described in second order kinetic equations, in which the active fraction of heterotrophic biomass considered as time-dependent state variable. Biotransformation rates were described as COD-based rate constants, k_{bio} (L $gCOD^{-1}$ d^{-1}). One of the research questions was to compare the biotransformation rates, k^*_{bio} and k_{bio} estimated for each model structure. The WATS—ASM-X stoichiometric and process rate matrix is presented in Table 4.1. All model parameters and their values are reported in **Paper I**. An additional state variable as S_{Me} (gCOD m^{-3}), was considered to account for methanol evaporation during experiment.

It was found that methanol – presented in reactors from spiked standard solutions –is not subject to utilization by the bacterial community in raw wastewater, at least during the duration of experiments. This conclusion was made following comparison of oxygen uptake rate in raw wastewater samples with and without methanol (**Paper I**). In both the ASM-X and the WATS-ASM-X models, partitioning and abiotic processes were also included.

Table 4.1 Stoichiometric matrix and process rates in the WAST—ASM-X model (**Paper I**).

	State variables →	X_{Hw}	X_{SRB}	S_F	S_A	S_S	S_{Me}	X_{S1}	X_{S2}	S_O	S_{SO4}	C_{LI}	C_{SL}	C_{CJ}	C_{SW}	
	Definition →	Heterotrophic biomass	Sulfate reducing bacteria	Fermentable substrate	Fermentation product	Readily degradable COD	Methanol	Rapid hydrolysable substrate	Slow hydrolysable substrate	Dissolved oxygen	Sulfate	Biomarker in aqueous phase	Biomarker in suspended solids	Biomarker transforming to C_{LI}	Biomarker onto reactor wall	**Process rates ↓**
	Processes ↓ **Unit →**	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gCOD m^{-3}	gO_2 m^{-3}	gS m^{-3}	g L^{-1}	g L^{-1}	g L^{-1}	g L^{-1}	
WATS - aerobic	Growth of X_{Hw}	1				$-\frac{1}{Y_{Hw}}$				$-\frac{(1-Y_{Hw})}{Y_{Hw}}$						$\mu_H S_S/(S_S+K_{Sw})X_{Hw}\alpha_w^{(T-20)}$
	Maintenance	-1				-1				-1						$q_m X_{Hw}\alpha_w^{(T-20)}$
	Hydrolysis, rapid					1		-1								$k_{h1}(X_{S1}/X_{Hw})/(X_{S1}/X_{Hw}+K_{X1})X_{Hw}\alpha_w^{(T-20)}$
	Hydrolysis, slow					1			-1							$k_{h2}(X_{S2}/X_{Hw})/(X_{S2}/X_{Hw}+K_{X2})X_{Hw}\alpha_w^{(T-20)}$
	Methanol evaporation						-1									$k_{eva,ae}S_{Me}$
WATS - anaerobic	Decay of X_{Hw}	-1							1							$d_H X_{Hw}\alpha_S^{(T-20)}$
	Growth of X_{SRB} for S_F		1	$-\frac{1}{Y_{SRB}}$							$-0.5\frac{1-Y_{SRB}}{Y_{SRB}}$					$\mu_{SRB}\frac{S_F}{S_F+K_{SRB,S}}\frac{S_F}{S_F+S_A}\frac{S_{SO4}}{(S_{SO4}+K_{SRB,SO4})}X_{SRB}\alpha_S^{(T-20)}$
	Growth of X_{SRB} for S_A		1		$-\frac{1}{Y_{SRB}}$						$-0.5\frac{1-Y_{SRB}}{Y_{SRB}}$					$\mu_{SRB}\frac{S_A}{S_A+K_{SRB,S}}\frac{S_A}{S_F+S_A}\frac{S_{SO4}}{(S_{SO4}+K_{SRB,SO4})}X_{SRB}\alpha_S^{(T-20)}$
	Hydrolysis, rapid			1				-1								$\eta_h k_{h1}(X_{S1}/X_{Hw})/(X_{S1}/X_{Hw}+K_{X1})X_{Hw}\alpha_S^{(T-20)}$
	Hydrolysis, slow			1					-1							$\eta_h k_{h2}(X_{S2}/X_{Hw})/(X_{S2}/X_{Hw}+K_{X2})X_{Hw}\alpha_S^{(T-20)}$
	Fermentation			-1	1											$q_{fe}\frac{S_F}{S_F+K_{fe}}X_{Hw}\alpha_S^{(T-20)}$
	Methanol evaporation						-1									$k_{eva,an}S_{Me}$
ASM-X (aerobic / anaerobic)	Desorption from wall											1		1	-1	$k_{des,W}C_{SW}$
	Sorption to wall											-1		-1	1	$\sigma_W k_{des,W}K_{d,W}C_{LI}$ (or C_{CJ})
	Desorption from suspended solids											1	-1	1		$k_{des}C_{SL}$
	Sorption to suspended solids											-1	1	-1		$k_{des}K_d(X_{Hw}+X_{SRB}+X_{S1}+X_{S2})f_{SS}10^{-3}C_{LI}$ (or C_{CJ})
	Abiotic transformation											-1				$k_{abio,LI}C_{LI}$
	Abiotic formation											$\frac{M_{LI}}{M_{CJ}}$		-1		$k_{abio,CJ}C_{CJ}$
	Biotransformation											-1				$k_{bio,LI}C_{LI}(X_{Hw}+X_{SRB})10^{-3}$
	Biotic formation											$\frac{M_{LI}}{M_{CJ}}$		-1		$k_{bio,CJ}C_{CJ}(X_{Hw}+X_{SRB})10^{-3}$

WATS - aerobic: μ_H: maximum specific growth rate of X_{Hw}; Y_{Hw}: heterotrophic growth yield; K_{Sw}: affinity constant of X_{Hw} for S_S; q_m: maintenance rate; k_{h1}: rapid hydrolysis rate; k_{h2}: slow hydrolysis rate; K_{X1}: affinity constant for rapid hydrolysis; K_{X2}: affinity constant for slow hydrolysis; α_w: aerobic Arrhenius temperature coefficient; $k_{eva,ae}$: aerobic methanol evaporation rate; T: Temperature

WATS - anaerobic: μ_{SRB}: maximum specific growth rate of X_{SRB}; Y_{SRB}: growth yield of X_{SRB}; $K_{SRB,S}$: affinity constant of X_{SRB} for S_S; $K_{SRB,SO4}$: affinity constant of X_{SRB} for S_{SO4}; d_H: decay rate of X_{Hw}; η_h: anaerobic reduction factor for hydrolysis; q_{fe}: maximum fermentation rate; K_{fe}: affinity constant for S_F; α_S: anaerobic Arrhenius temperature coefficient; $k_{eva,an}$: anaerobic methanol evaporation rate

ASM-X: $k_{des,w}$: desorption rate from reactor wall; K_{dw}: wall-liquid partition coefficient; k_{des}: desorption rate from suspended solids; K_d: solid-liquid partition coefficient; σ_W: wet-surface-to-volume ratio; $k_{abio,LI}$: abiotic transformation rate; $k_{abio,CJ}$: abiotic formation rate; $k_{bio,LI}$: biotransformation rate constant; $k_{bio,CJ}$: biotic formation rate constant; f_{SS}: TSS-to-particulate-COD ratio; M: biomarker molecular weight

To simulate biotransformations in biofilm reactors, an additional pseudo-first order kinetic equation was added to the extended ASM-X model. Biotransformation rates were expressed either based on the biofilm density, k_{biof} (L gTSS d^{-1}) or normalized to the area-to-volume ratio, k'_{biof} (m^3 m^{-2} d^{-1}). Due to presence of some fractions of suspended solids during biofilm experiments, despite filtration of the medium, a TSS-normalized biotransformation rate, k^*_{bio} (d^{-1}) was also included in the model. The kinetic model also comprised of abiotic processes. The assumption of the modelling approach was that the bulk phase in the reactors was in complete mixed conditions i.e. uniform distribution of drugs mass in the aqueous phase. Moreover, due to samples withdrawn during biofilm experiments, the external tanks connected to the biofilm reactors were modeled by accounting for the dynamics of the volume during batch experiment. This was especially important to simulate the increasing contact time for drug biomarkers in the aqueous phase and at the biofilm surface.

4.2.3 Mass transfer by diffusion

In comparison to no expected mass transfer limitation in the bulk phase of jacketed reactors during biotransformation experiments with raw wastewater (due to excessive mixing by a mixer and aeration), the mass transfer from the aqueous phase of the biofilm reactors into the biofilms can be limited. Biofilms in reactors had high density (aerobic biofilms=56 gTSS L^{-1}, anaerobic biofilms=83 gTSS L^{-1}). Under these conditions, the only mass transfer mechanism that would substantially contribute to delivery of drug biomarkers (in soluble form) from the bulk liquid phase into sewer biofilms is diffusion. In this thesis, a 1-D dynamic diffusion-reaction model was considered to simulate the concentration profiles of drug biomarkers in the depth of the biofilms. An example of such a profile is presented in Figure 4.4 for mephedrone (MEPH) concentrations in aerobic and anaerobic biofilm reactors throughout a 2-day experiment. 80 discretization layers were used for this simulation (**Paper III**).

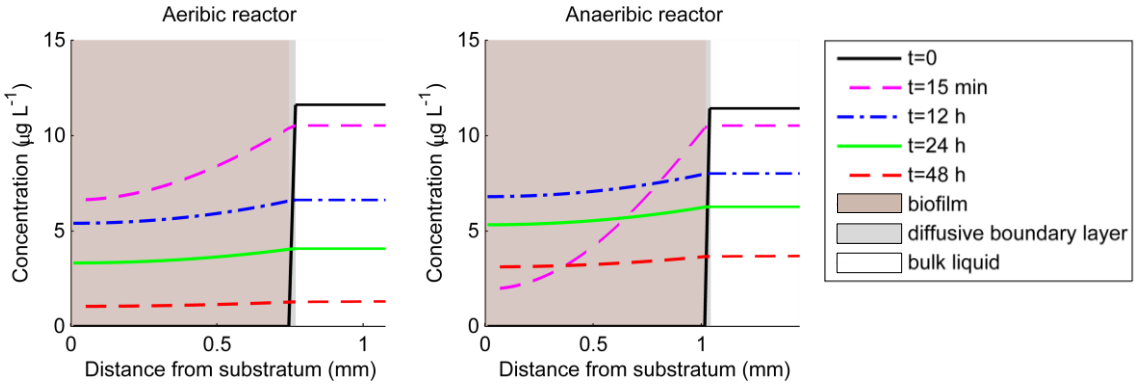


Figure 4.4 Mephedrone (MEPH) concentration profiles insides the aerobic and anaerobic biofilm reactors for different time (t) throughout 2-day biofilm experiments in batch mode.

4.2.4 Transformation pathways

As previously mentioned, trace organic chemicals can transform and also form. Since these chemicals often coexist in the sewer, they can transform from one to another according to their in-sewer transformation pathways. These pathways do not necessarily follow those of human metabolism as the enzymes responsible for most transformations in the sewer are probably different and much more diverse than enzymes in humans. Moreover, in-sewer transformation and formation due to abiotic processes, biotransformation in suspended biomass or biotransformation in sewer biofilms might also be different. Therefore, a suitable transformation pathway for each chemical should be considered in the model structure. In this thesis the pathways were initially assumed to be similar to human metabolism. From this perspective, the selected illicit drugs were sub-divided into five groups (**Paper I and III**). Any deviations from the initial pathways were identified based on mass balances, uncertainty analysis and assessment of the feasibility of transformations – which is only available for raw wastewater ("EAWAG-BBD," 2016). Fig. 4.5 illustrates the identified in-sewer transformation pathway of HER, COE and their human metabolites (**Paper I and III**). Although the transformation pathways for these chemicals in the sewer were found to be similar to human metabolism, new (undetected) transformation pathways were identified for HER and MORG in raw wastewater (suspended solids). The simulation of HER and 6MAM before identifying the pathway and after identifying the pathway for HER is shown in Fig. 4.6. Prediction of 6MAM was improved substantially after considering an additional transformation product for HER.

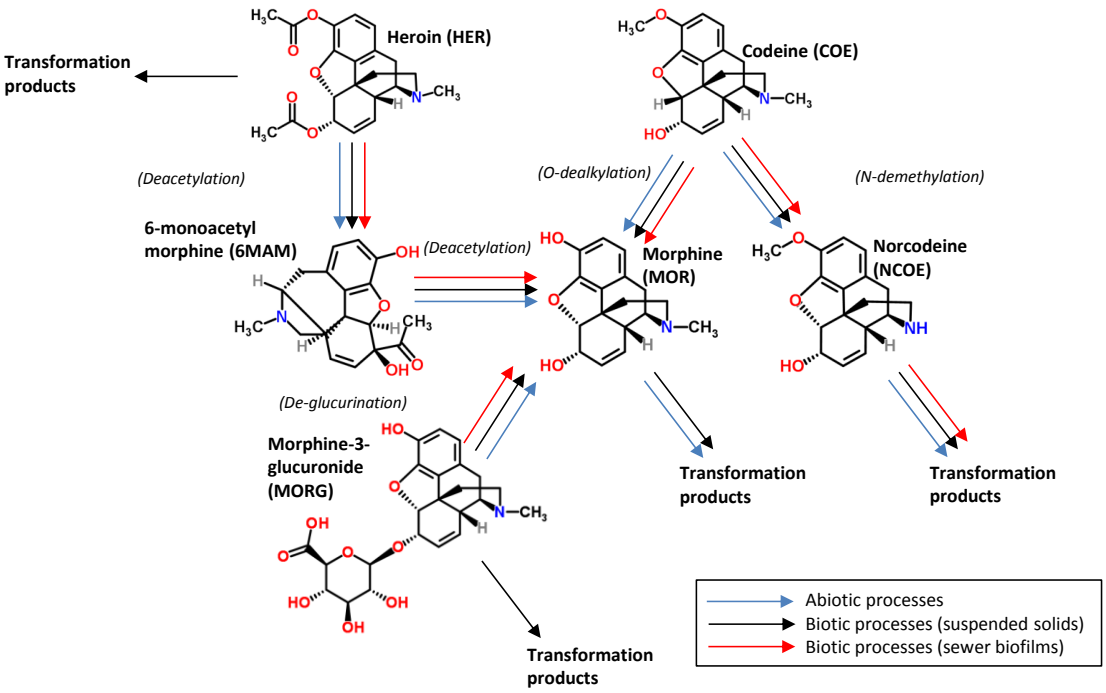


Figure 4.5 in sewer transformation of HER and its human metabolites, 6MAM, MOR and MORG as well as CE and its human metabolite NCOE (**Paper I, II & III**).

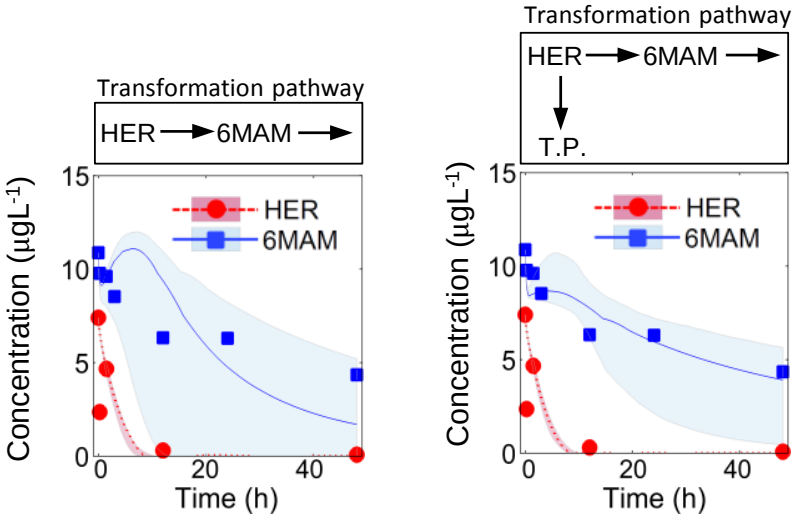


Figure 4.6 Identifying a new major transformation pathway for heroin (HER) in raw wastewater under aerobic conditions. Without such pathway, 6-monoacetylmorphine concentration profile cannot be predicted well. However, addition of an (undetected) transformation product for HER improves the predictions. Marks are the measured data and lines are simulation results with the WATS—ASM-X model. The shaded area is a 95% credibility interval (**Paper I**).

4.3 Model calibration

General procedure

Model calibration in this thesis consisted of number of steps in succession. The procedure includes 4 main steps: i) generation of a prior knowledge on range and uncertainty of parameters. This was mainly done by literature review and manual trial and error of parameter values and evaluating the response of the model system. A uniform distribution was considered for parameters with unknown distributions; ii) selection of parameter subsets for estimation. For WATS model parameters, a global sensitivity analysis (GSA) was performed with linear regression of Monte Carlo analysis (Saltelli et al., 2008) – also known as standard regression coefficients (SRC). For other model parameters, i.e. transformation rates, selection was made based on limited available information. For instance transformation of COC to EME in raw wastewater was assumed negligible (Bisceglia et al., 2012; Plósz et al., 2013). However, this was rarely the case, and most transformation rates, in transformation pathway of drug biomarkers, were estimated; iii) estimation of model parameters either directly from measurements or in a formal procedure with the Bayesian optimization method DREAM(ZS) (Vrugt et al., 2003). The optimization method runs multiple chains for effective posterior exploration by employing the Markov Chain Monte Carlo (MCMC) method. The method uses the sampling from an archive of prior states in order to generate candidates in each chain; iv) testing if parameters can be uniquely identified which was mainly done based on parameter correlation analysis and uncertainty range (Frutiger et al., 2016).

Parameter estimation for the WATS model

Fig. 4.7 presents results from a GSA study on the aerobic WAST model according to the SRC method after simulation time of 2.5 hours. The model was linear with linear model determination coefficients (R^2) higher than 0.9. A sensitivity measure (defined as scaled coefficients of linear models), β, was used for parameter significance ranking for different model outputs (in this case state variables). The parameter ranges were defined according to parameter uncertainty ranges reported elsewhere (Gurkan Sin et al., 2009). In this GSA method, higher β indicates higher effect of the corresponding parameters on the output. Moreover, a positive sign indicates a positive effect on the output and vice versa. These results indicate that maximum specific growth rate, μ_H (d^{-1}) and rapid hydrolysis rate, k_{h1} (d^{-1}) and slow hydrolysis rate, k_{h2} (d^{-1}) are in general the most influential parameters.

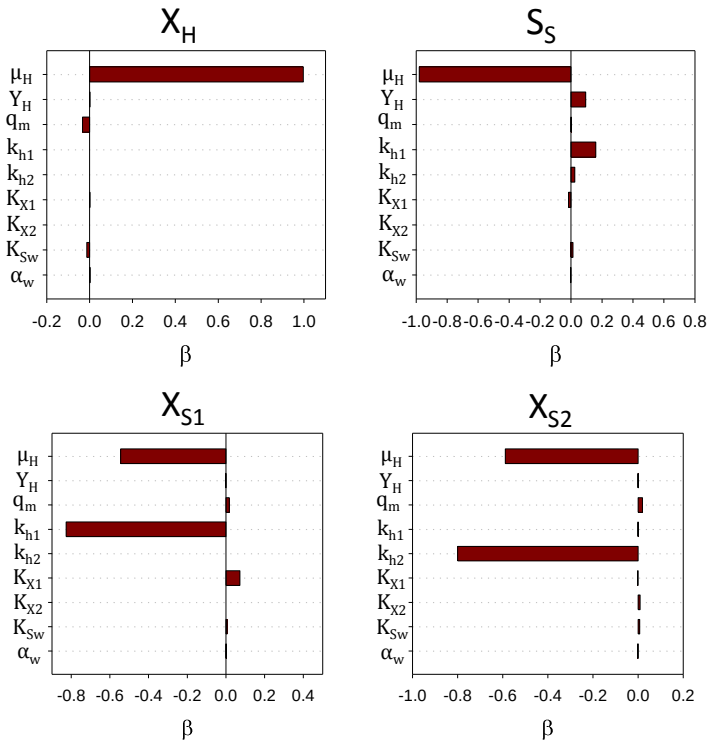


Figure 4.7 Parameters significant ranking for aerobic WAST model outputs at 2.5 hours through simulation. β is sensitivity measures.

μ_H (d^{-1}) could be estimated directly from oxygen uptake rate (OUR) measurements during the exponential growth phase of biomass (Vollertsen and Hvitved-Jacobsen, 1999). k_{h1} was considered for parameter estimation using $DREAM_{(ZS)}$ and k_{h2} (d^{-1}) was set at 0.5 (d^{-1}) based on a reported value (Hvitved-Jacobsen et al., 2013). Simulation of OUR after parameter estimation (k_{h1}= 3.42 d^{-1}) is presented in Fig. 4.8 It should be noted that OUR highly depends on the biomass yield, Y_H (g COD $gCOD^{-1}$), and can be easily formulated based on utilization of readily biodegradable substrate, S_S (gCOD d^{-1}) (Vollertsen and Hvitved-Jacobsen, 1999). For the anaerobic WATS model parameter values were taken from other studies (Rudelle et al., 2012).

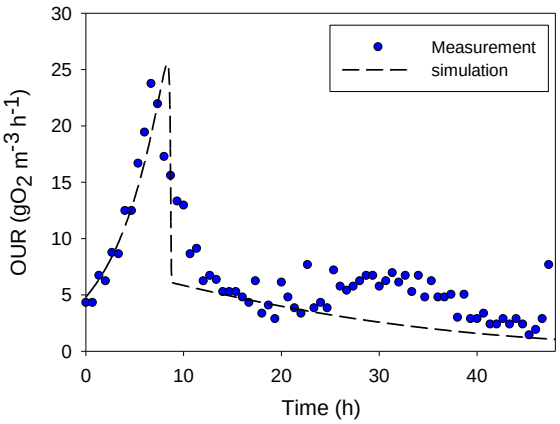


Figure 4.8 Measured oxygen uptake rate (OUR) in raw wastewater and simulation of aerobic WAST model (i.e. OUR, based on S_S) after estimation of rapid hydrolysis rate, k_{h1} (d^{-1}).

Estimation of transformation rates

Abiotic transformation rates and biotransformation rates were estimated by the $DREAM_{(ZS)}$ algorithm. The estimations also included formation rates according to transformation pathways as discussed previously. To estimate reliable parameter values and associated uncertainties, a method was developed by propagation of uncertainty among illicit drug transformation or formation rates that are present in the same pathway. This method employs parameter ranges and their probability distributions at any transformation level in the pathway as prior knowledge for the subsequent parameters in the pathway. As compared to other model calibration methods e.g. estimation of all parameters at the same time or fixing parameters for the subsequent estimations, this method could improve both the transformation pathway identification efficiency and the parameter identifiability (**Paper II**). A simple representation of error propagation for drug biomarker is illustrated in Fig. 4.9. drug biomarker **A** transforming to drug biomarker **B**. This method includes estimation of three types of in-sewer transformation rates, namely abiotic transformation rates, k_{abio} (d^{-1}), biotransformation rate in raw wastewater, k_{bio} (d^{-1}), and biotransformation in sewer biofilms, k'_{biof} (m^3 m^{-2} d^{-1}). All arrows indicate the flow of information i.e. uncertainty range and parameter distribution among different in-sewer transformation rates. These rates were described by the ASM-X or WAST—ASM-X model (for abiotic and biotransformation in raw wastewater) and biofilm model (for biotransformation in sewer biofilms) (**Paper II** and **III**). It should be noted that in the biofilm model (**Paper III**), TSS-normalized biotransformation rate, k^*_{bio} (L gTSS d^{-1}) was propagated for estimation of k'_{biof} (m^3 m^{-2} d^{-1}).

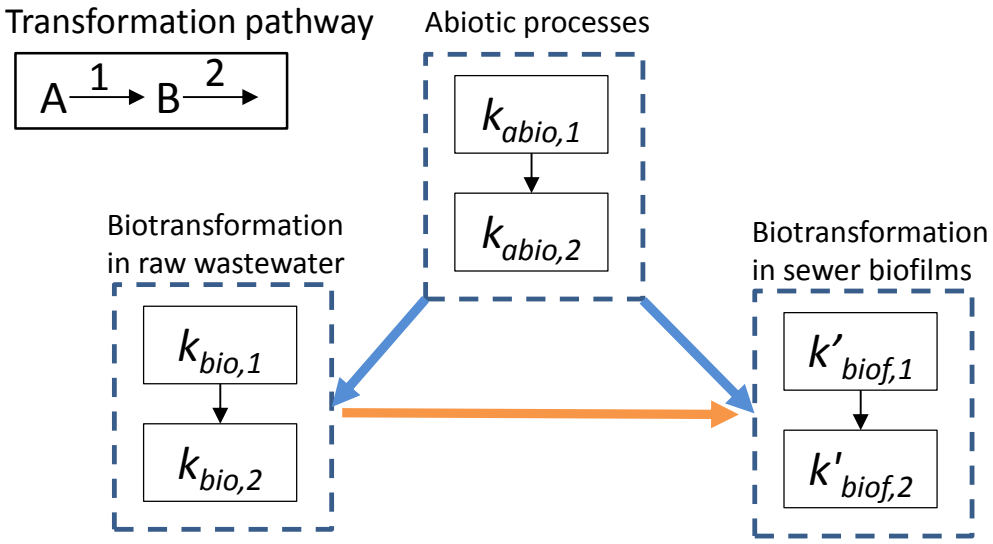


Figure 4.9 Propagation of error among different model parameters for reliable parameter estimation and their associated uncertainties. The method is demonstrated for a simple transformation pathway including two illicit drugs A and B.

All estimated transformation rates are presented in Fig. 4.10. The value from each bar corresponds to the median of the posterior distribution of model parameters including the upper 95% credibility bound. Despite apparent correlation among aerobic and anaerobic transformation rates, for many drug biomarkers a significant difference was observed between transformation rates under the two redox conditions. For instance, aerobic biotransformation of MEPH and METD in raw wastewater was found to be zero while their anaerobic biotransformation was substantial. The opposite was found for COC and BE. Although MORG significantly transformed in raw wastewater under both redox conditions, it did not transform at all in sewer biofilms. Nevertheless since transformation rates have different units comparison of estimated values is not straight forward. Chapter 6 attempts to assess the contribution of different transformation processes in overall drug biomarker in-sewer transformations.

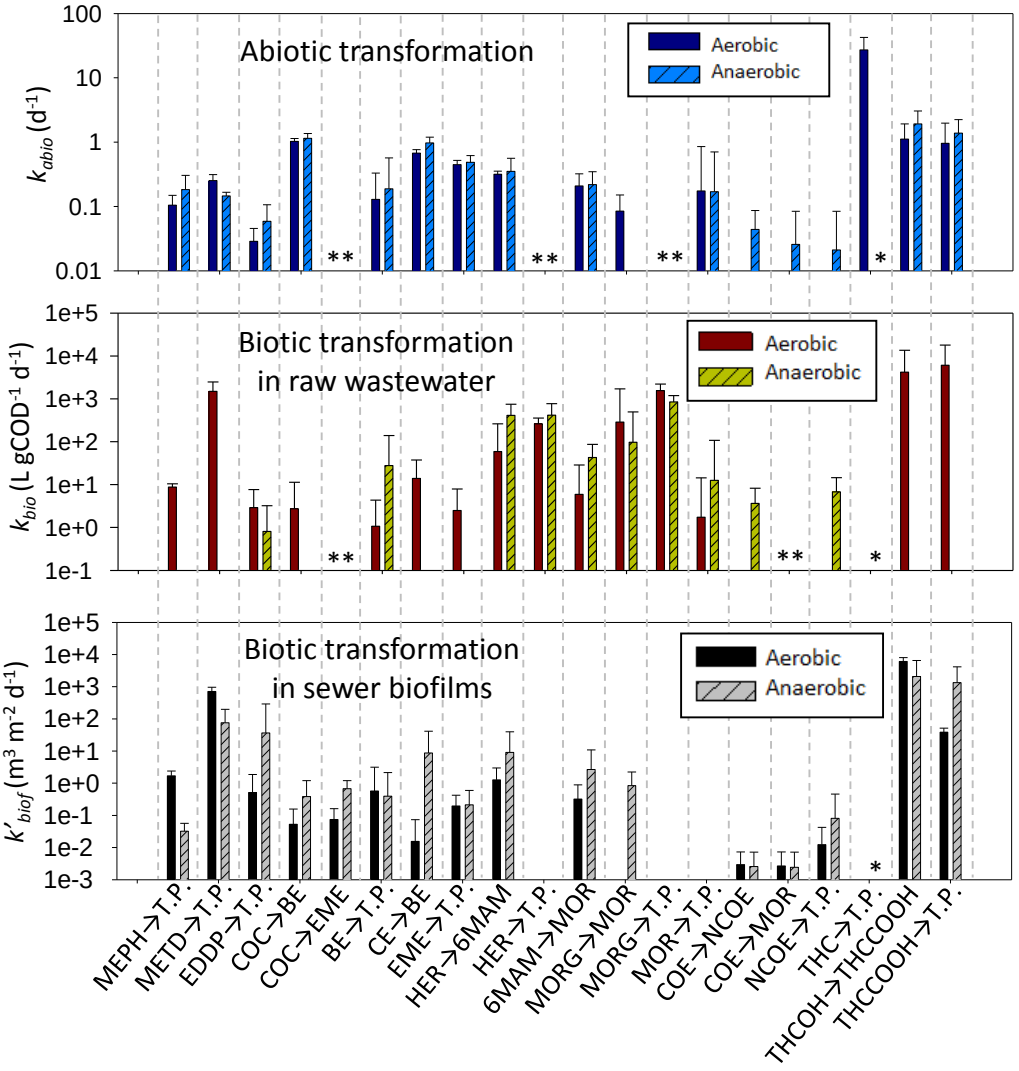


Figure 4.10 Estimated abiotic transformation rate, k_{abio} (d^{-1}) according to the extended ASM-X model, biotransformation rate in raw wastewater (T=15°C), k_{bio} (L gCOD^{-1} d^{-1}) in the WAST—ASM-X model, and biotransformation rate in sewer biofilms (T=17°C), k'_{biof} (m^3 m^{-2} d^{-1}) in the biofilm model. Undetermined transformations rates are indicated with asterisk (*).

Impact of temperature on biotransformation rates

In this thesis estimated transformation rates are valid for investigated temperatures. However, wastewater temperature in sewers can be very different based on geographical or climate conditions. Bisceglia et al. (2014) tested transformation of COC drug biomarkers in raw wastewater at three different temperatures (9, 21 and 31°C). He observed that increasing temperature can effectively enhance the biotransformation rates. Other studies also reported on biotransformation rates at different temperatures e.g. biotransformation of

COC in raw wastewater at 10 and 20°C (Senta et al., 2014), and biotransformation of COC and CE in raw wastewater at 21±1°C (McCall et al., 2016). Fig. 4.11 shows reported k'_{bio}(d^{-1}) values in these studies including the values estimated in this thesis in raw wastewater at 15°C. The impact of temperature on process rates is calculated using a modified Arrhenius equation similar to the WATS model (Table 4.1): $k'_{bio,T} = k'_{bio,T0} \times \alpha_b^{(T-T0)}$, in which α_b is the temperature coefficient and $k'_{bio,T0}$ is the biotransformation rate at a reference temperature, T_0. Although T_0 is normally the standard temperature (20°C), due to insufficient number of data points $k'_{bio,20}$ could not be accurately estimated, hence T_0 was considered at 15°C and α_b was estimated based on $k'_{bio,15}$. Estimation was performed with regression using reported mean or median values of k'_{bio}; $\alpha_{b,COC}$=1.14, $\alpha_{b,EME}$=1.13, $\alpha_{b,CE}$=1.14.

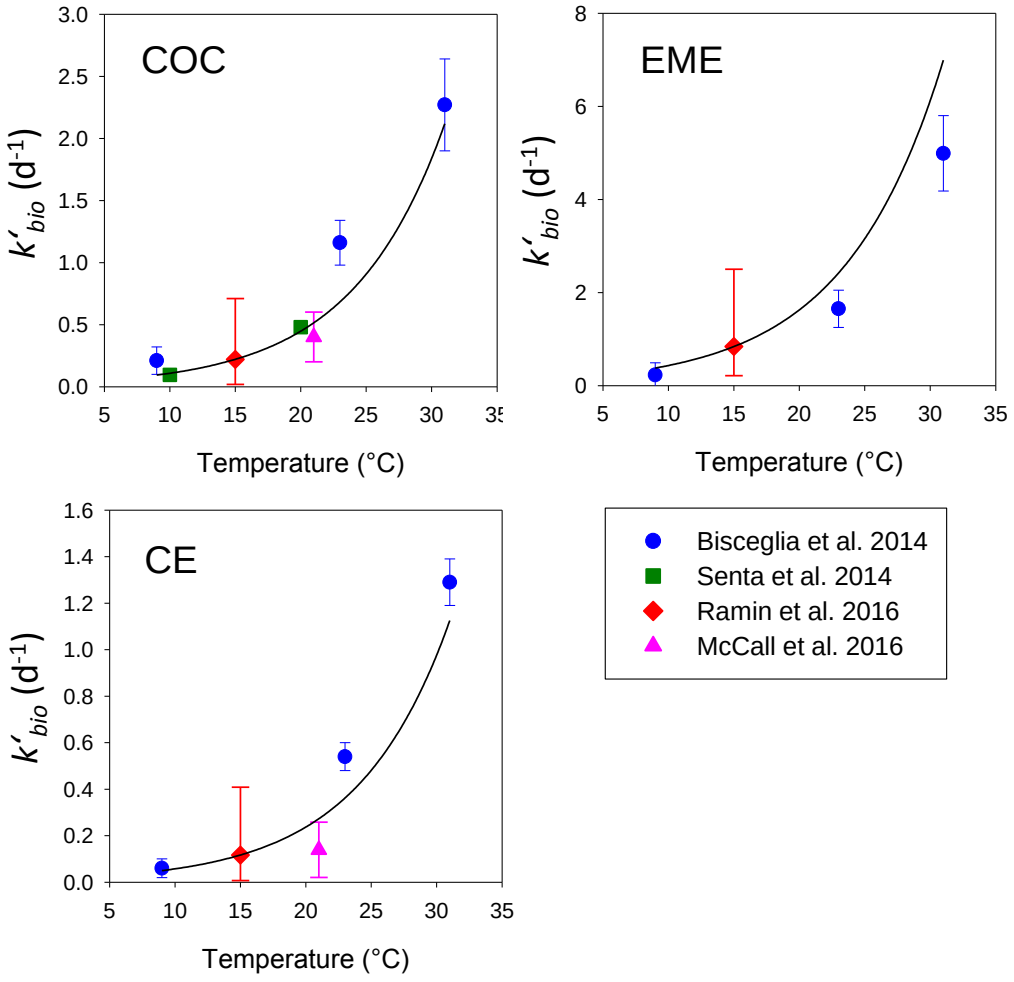


Figure 4.11 Biotransformation rate, k'_{bio} (d^{-1}) for COC, EME and CE at different temperatures reported by different studies including the values from this thesis (**Paper I**). Regression was used to estimate temperature coefficient, α_b according to a modified Arrhenius equation.

4.4 Model validation

Experimental data obtained via spiking deuterated standards were used as an independent dataset for model validation. Estimated partitioning coefficients and transformation rates were used in the WATS—ASM-X model and the biofilm model and concentration profiles were simulated. For many of the drug biomarkers, e.g. COC and its human metabolites, good agreement between simulations and an independent dataset was achieved. However, for a number of drug biomarkers such as METD, model simulations could not adequately approximate measured data. Fig. 4.12 presents measured concentrations from biotransformation experiments with raw wastewater under aerobic conditions for COC, METD and their human metabolites. Although model validation was successful for COC and its metabolites as well as EDDP, the simulation model for METD during validation could not capture the measured data.

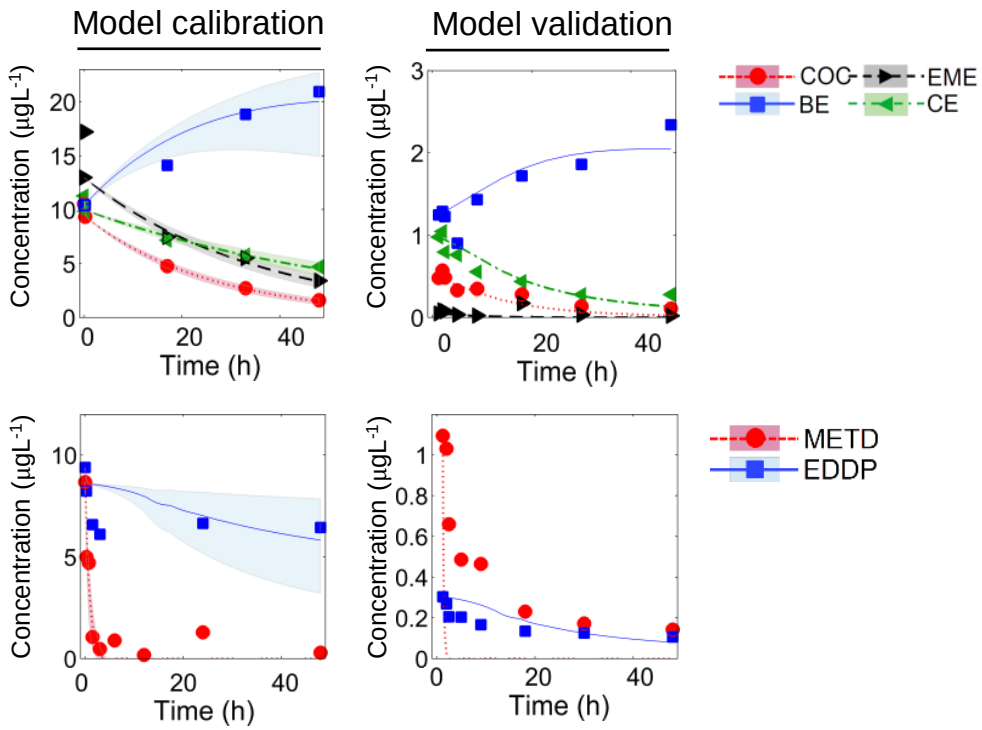


Figure 4.12 Concentration profiles for COC and its human metabolites, EME, BE and CE as well as METD and its human metabolite EDDP during biotransformation experiments with raw wastewater under aerobic conditions. The experiments were performed following two procedures; spiking internal standards (P1) and spiking deuterated internal standards (P2). Data from P1 was used for model calibration and data from P2 was used for model validation (**Paper I**).

5 Monitoring campaigns

Sampling from Lynetten WWTP

As part of this thesis, several sampling campaigns were performed at the inlet of Lynetten WWTP located in the city center of Copenhagen (Denmark). The catchment covers Copenhagen and seven other municipalities with total reduced area of 7600 ha and an estimated population of 531000 inhabitants (last census: 2009). A major part of the drainage system is built as a combined system where domestic and industrial wastewater as well as storm water is conveyed to the treatment plant. The plant has a design capacity of 750000 PE with an average daily wastewater inflow of 175000 m^3 d^{-1}. The treated effluent is discharged in the Øresund strait. At the inlet of the plant there are three pressurized collectors: the northern pipe stretching from Strandvænget pump station which receives flow also from the Skovshoved pump station, and two southern pipes from Kløvermarken pump station (Breinholt and Sharma, 2010).

Participation in Europe-wide monitoring campaign

The first Europe-wide monitoring campaign was performed in 2011 by the Sewage Analysis CORe group Europe (SCORE) network and is still annually performed. This group aims at providing patterns of drug use at geographical and temporal resolution across European cities. The network has established a robust protocol concerning representative sample collection, sample preparation, analysis and data reporting. A minor part of this PhD thesis work was devoted to contributing to SCORE group activities by performing 1-week sampling campaigns during March in 2013, 2014 and 2015. Samplings were performed after the primary clarifier with a volume-proportional automatic sampler installed at this location (sampling interval of every 2000 m^3 sewage). Samples were collected in refrigerated plastic containers. At the end of each sampling day, collected samples were acidified (1% v/v, acetic acid) and transported to a freezer. Following defrosting and sample preparation (i.e. SPE), samples were sent to project partners for analysis. Briefly, analysis was carried out using the Waters Acquity UPLC system, with a Xevo G2-S QTOF detector according to developed methods (Baz-Lomba et al., 2016). Fig. 5.1, reports the illicit drug biomarkers that were detected in the samples for each year. This figure includes additional illicit drugs, Amphetamine (AMPH), Methamphetamine (METH) and 3,4-Methylenedioxymethamphetamine (MDMA) that are not discussed in this thesis. The average concentrations of biomarkers during weekday and weekends in Copenhagen (CPH) are com-

pared with the average values reported from European cities. In 2013 in total 47 WWTPs in 21 countries (42 cities) (Ort et al., 2014), and in 2014 in total 46 WWTPs in 18 countries (44 cities) (EMCDDA, 2015b) were included in the sampling campaign. Data from 2015 is still not fully available from other participants, however some of the results are published (Baz-Lomba et al., 2016). Based on a report by European Monitoring Centre for Drug and Drug Addiction (EMCDDA, 2015a), after UK and Spain, Denmark has the third place in cocaine use with slightly decreasing prevalence. This is also apparent from COC and BE data in Fig. 5.1, showing a decreasing trend from 2013 to 2015. Drug consumption during the weekend as compared to weekdays seems to be higher for most drugs.

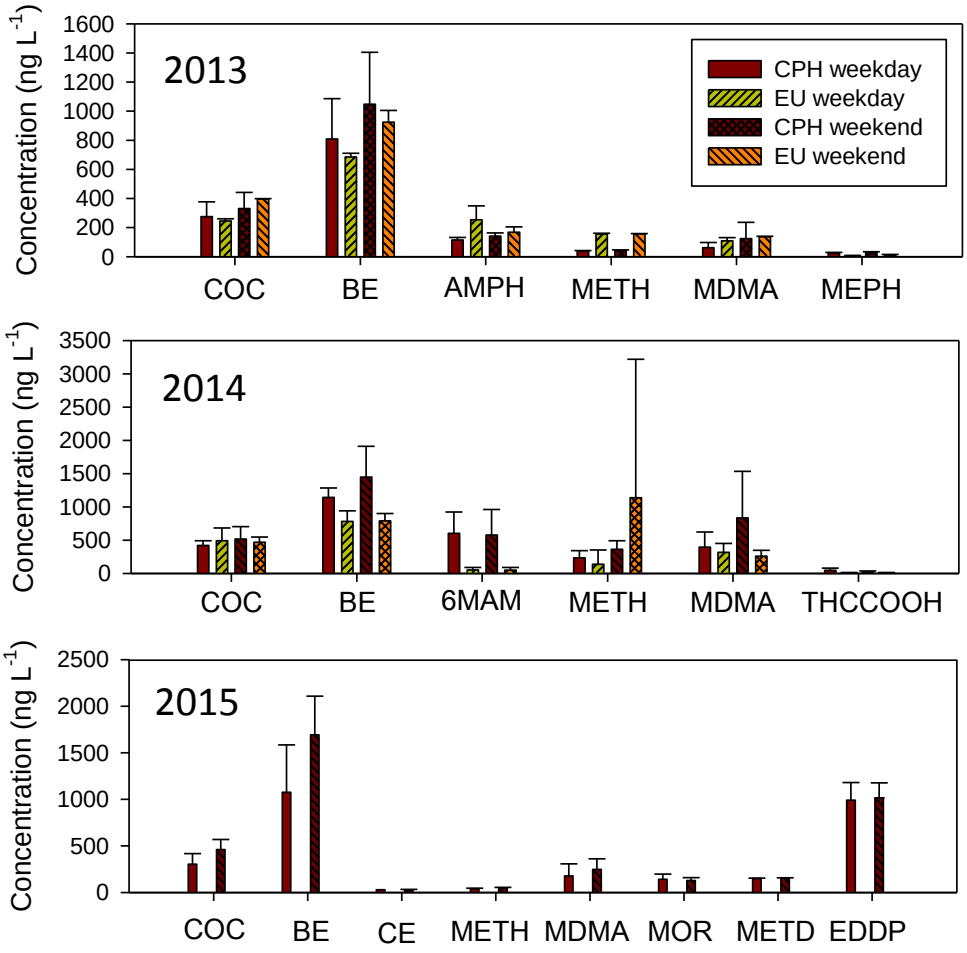


Figure 5.1 Average concentrations of different illicit drugs monitored during the annular European sampling campaign (SCORE). The results are presented as average values of 7-day monitoring for Copenhagen (CPH) and average concentration for European countries (EU) during weekend and weekdays. Bars are average values and error bars are standard deviations. (Baz-Lomba et al., 2016; EMCDDA, 2015b; Ort et al., 2014).

Organized sampling campaign

A 2-week sampling campaign was performed at the inlet to Lynetten WWTP from 28 May until 11 June 2014. This period included a street music festival period as well as holidays and weekends. Two sampling sites were considered: one at the arrival of wastewater from Kløvermarken pump station (southern part of catchment, mainly city center) and one connected to Strandvaenget pressurized pipe from north of catchment. Portable automatic samplers were used to collect 24 composite samples per day starting from 7 AM. Sampling hoses were placed right after the mechanical bar screens (Fig. 5.2). The samples were collected time-proportionally with sampling interval of 6 min. At the end of each sampling day, composite samples were mixed flow-proportionally to prepare 4 composite samples per day. To preserve the samples from biological activities, a glass vial containing acetic acid was placed in each bottle prior to sampling to reach final concentration of 0.1% and drop the pH to 4.7±0.1 during sampling. The samples were then transported to a freezer and kept at -20°C until time of analysis. Analysis was performed by other partners of the SEWPROF project as described previously. During the sampling period, flow data was measured from each of the two influent pipes, using already installed flowmeters with a resolution of 2 min. Moreover, two TSS sensors were installed for the period of sampling campaign at each of the two sampling locations and data were recorded every 1 min. Fig. 5.3 presents the concentrations of COC and BE measured during the sampling campaign for the North and South catchments.

Figure 5.2 An automatic sampler in time-proportional mode during 2-week sampling campaign from raw influent after screening in Lynetten WWTP. The sampler included 24 bottle (left) allowing collection of hourly composite samples. At the end of each sampling day, hourly samples were mixed flow-propotionally to make 6-hourly samples. TSS was also measured during sampling using sensor (right).

From the Fig. 5.3, weekly variations of the concentrations of COC and BE can be clearly seen. Lowest concentrations were found during normal working days, especially Monday night, and highest concentrations were observed for the festival period, especially the last day of the festival. Increase of COC and BE concentration is also evident during the public holiday in the first week of the campaign.

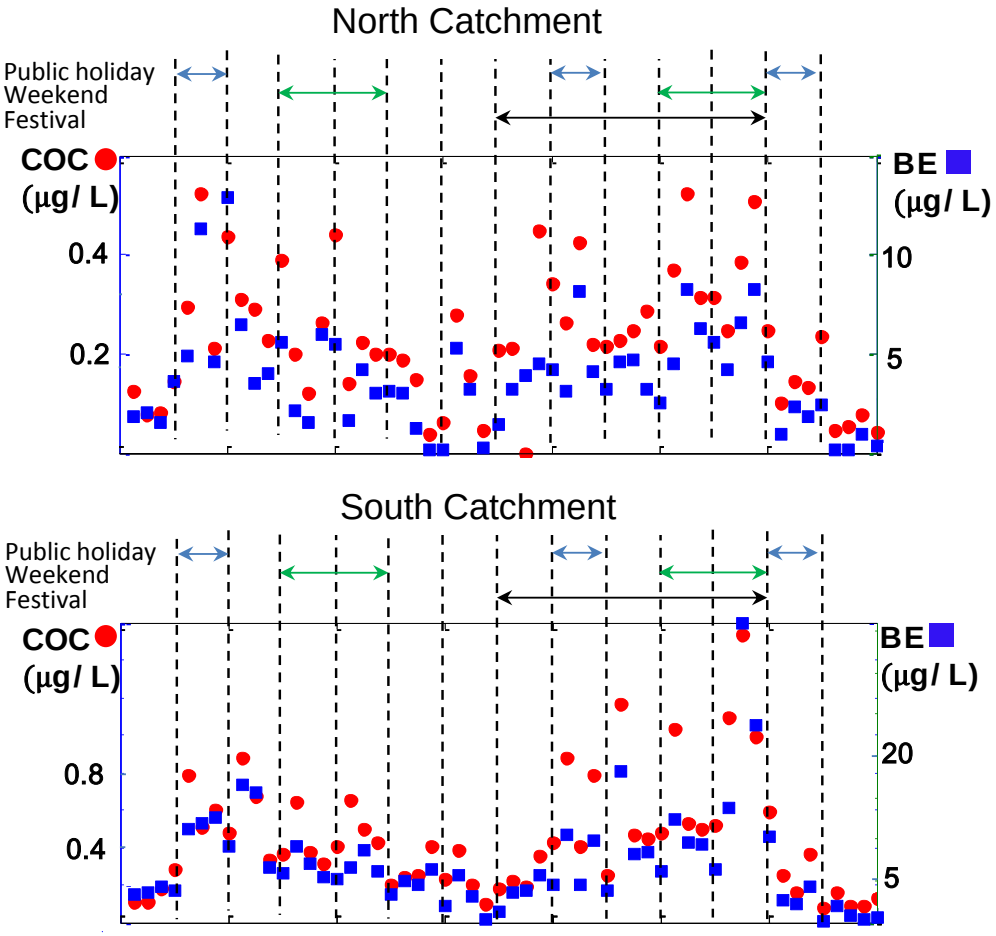


Figure 5.3 Measured concentrations of COC and BE at two inlets of Lynetten WWTP, the North inlet (B) and South inlet (A). Monitoring was performed between 28 May and 11 June 2014 at 4 different periods including: weekday, weekend, public holiday and a street music festival.

6 Estimation of drug use in catchments

Generic assessment by scenario analysis

One of the concluding goals of this thesis is to refine the existing drug use estimation methods by accounting for in-sewer processes. These processes should be included in back-calculation schemes at the catchment level. The extent of in-sewer drug biomarker transformations significantly depends on the hydraulic residence time in the sewer. Drug biomarkers enter the sewer system mostly after toilet flushes across the catchment. Depending on the distance of drug release to the sampling point, the flow velocity (and other sewer design factors such as pump sump volume) and the hydraulic residence time can be very variable. Moreover, beside diurnal variations (resolution of hours), the loads of drug biomarkers exhibit short term fluctuations (resolution of seconds to minutes) as previously reported (Ort et al., 2010c). Employing in-sewer processes in such a highly dynamic systems – across spatio-temporal scales – is very challenging. However, since estimations in WBE studies are normally based on hourly to daily drug consumption, the challenge of short-term variations of drug loads remains mainly for representative sampling (**Paper IV**) and not the estimation of transformation of drug biomarkers in the sewer. Nevertheless, prediction of spatial variability of drug release – and hence hydraulic residence time – seems rather crucial.

In this thesis, a simple approach was considered by assuming a theoretical discharge point in the catchment. This discharge point has no physical meaning but is rather conceptually defined based on the average hydraulics residence time in the catchment. The error introduced by neglecting in-sewer processes was calculated by comparing the daily drug load at the theoretical discharge point estimated with and without in-sewer processes. The contribution of uncertainties from different transformations i.e. abiotic transformation and biotransformation in raw wastewater and sewer biofilms was also quantified by neglecting each of these processes individually and comparison of back-calculation with the reference scenario. This assessment was performed for COC, BE, MOR, MORG and 6MAM with three different catchment sizes: small, 10000 PE; medium, 50000 PE and large, 200000 PE.

The sewage pattern generator (SPG) software (Ort, 2016) was used to generate drug load profiles with short term variations at the WWTPs inlet. Selected drug pharmacokinetics, i.e. excretion profiles, together with other assumptions e.g. flow distance distribution, were used as input to the model. Subse-

quently, through Monte Carlo simulations, the error introduced by neglecting in-sewer processes (with known transformation rate distributions) was calculated for 10000 simulation days. Fig. 6.1 presents the median values of the absolute error (defined as absolute relative difference between simulation scenarios and reference scenarios for each day) for drug biomarkers in each catchment (**Paper IV**) as well as the average for all catchments. The absolute error increases in larger catchments with higher residence time. Notably, for COC and BE most uncertainties were introduced by neglecting abiotic processes, whereas for MORG, biotransformation in raw wastewater was the dominant source of uncertainty. Sewer biofilms played the most important role for 6MAM.

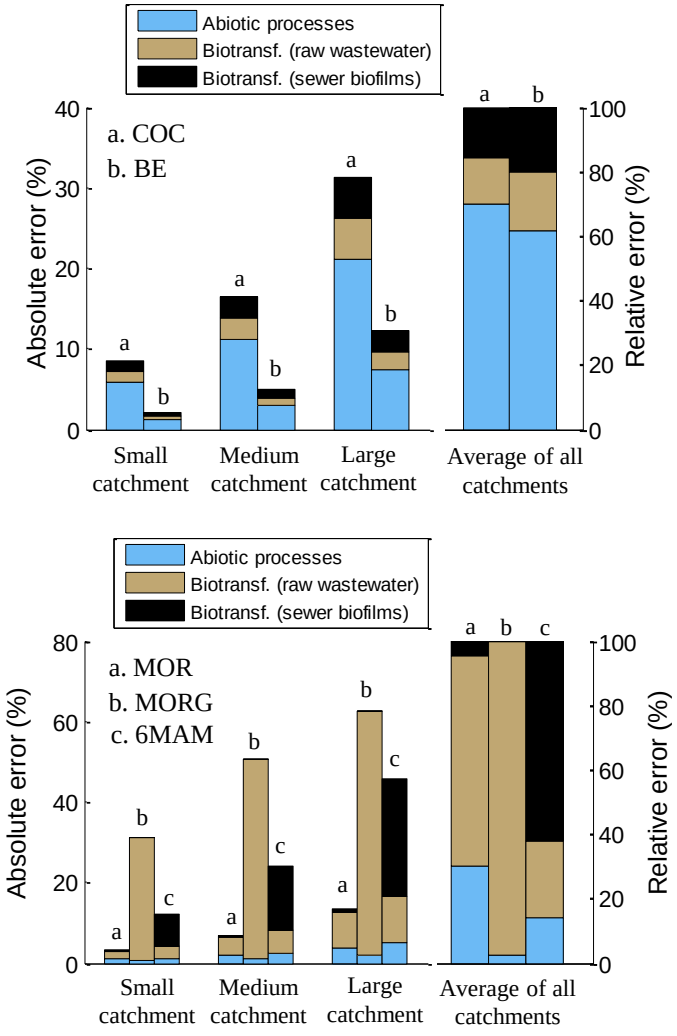


Figure 6.1 Absolute and relative error associated with ignoring the effect of in-sewer transformations, for COC and BE (top) and MOR, MORG, 6MAM (bottom), on estimation of daily load at a theoretical discharge point (**Paper IV**).

Estimation of daily cocaine use in Lynetten catchment

Based on measured concentrations of COC and BE (Fig. 5.3), the COC consumption based on the candidate biomarker, BE was estimated for different periods. Fig. 6.2 reports on population-normalized estimated values (gCOC $6h^{-1}$ $1000PE^{-1}$) with a time interval of 6 hours. For these estimations 10000 Monte Carlo simulations were performed with 6 hour simulation by randomly selecting transformation rates from known distributions (**Paper I** and **III**). Several strong assumptions were considered. i) The population of each catchment (north=205000 PE, south=354000) was estimated based on available online statistics ("statistikbanken 2014, Second quarter") and an assumption of uniform distribution of the population in each catchment area reported by Breinholt and Sharma (2010), ii) the average hydraulic residence time in each catchment (τ=6 h) was approximating based on an average distance with a flow-velocity of 0.5 m s^{-1} (Breinholt and Sharma, 2010). The average residence time for every 6 h was then estimated based on τ (h^{-1}) and variation of flow according to a reported correlation (Plósz et al., 2013), iii) the sewer is primarily operating with no limitation of oxygen in the bulk or in the biofilm, and iv) an in-sewer area to volume ratio of 4.7 m^2 m^{-3} was assumed to estimate transformation rates in sewer biofilms. These results indicate that the COC consumption was increased during weekends and holiday periods as compared to weekday data. This increase is noticeable during the festival period (on average more than twice of normal weekdays). Estimation of COC use in the South catchment was on average 2 times higher than the COC use estimates from the North catchment. This difference was especially noticeable during holidays and festival periods. In this example, estimation of COC and BE was based on monitoring data and in-sewer transformation rates and shows an application of the developed model and estimated parameters, namely partitioning coefficients to suspended solids and sewer biofilms, abiotic transformation rates, biotransformation rate in raw wastewater, and biotransformation in sewer biofilms, in estimation of drug use at catchment level.

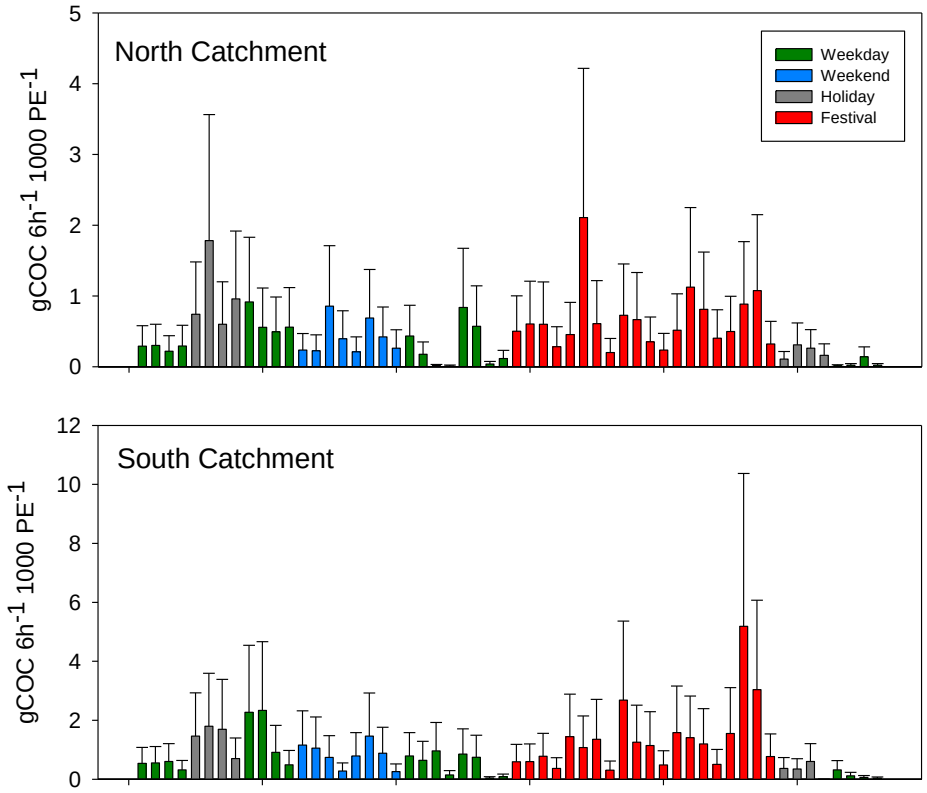


Figure 6.2 COC consumption with 6 h time interval (X axis) during a 2-week sampling campaign at the influent of Lynetten WWTP in Copenhagen. Estimated consumptions are reported as gCOC $6h^{-1}$ $1000PE^{-1}$ for the North catchment (top) and South catchments (bottom).

7 Conclusion

The study presented in this thesis is the first example of developing a detailed mathematical description of transformation and partitioning of illicit drug biomarkers in sewer systems. To reach the major conclusion of this thesis, prediction of in-sewer fate processes were addressed from five different perspectives - namely experimental assessments, model development, model calibration, uncertainty analysis for daily drug use estimates and model application.

- Through different experimental assessments, it was found that drug biomarkers can potentially undergo significant sorption and transformation in the sewers. Among the selected drug biomarkers in this thesis such as COC and CE, chemical hydrolysis and to a lesser extent biotransformation was responsible for most transformations in the sewer bulk. However, significant removal of HER and MORG was also observed in raw wastewater with much less removal in mineral water. Overall, aerobic and anaerobic transformations in mineral water showed higher similarity than aerobic and anaerobic biotransformation in raw wastewater and sewer biofilms. Redox conditions played an important role in transformation of a number of chemicals. For instance, substantial removal of METD was observed in raw wastewater under aerobic conditions, whereas no removal was shown under anaerobic conditions. This may suggest that the activity of the bacterial community, responsible for biotransformation of drug biomarkers, can be very different under different redox conditions. Sewer biofilms can significantly enhance the overall transformation of drug biomarkers. This was especially observed for EDDP, COC, EME and 6MAM. However, the extent of biotransformation was different in aerobic and anaerobic biofilms. Following sorption experiments, the highest sorption capacity to suspended solids and biofilms (in suspended form) was shown for THCOH and THCCOOH due to very high hydrophobicity of these chemicals. For other drug biomarkers, rather than 6MAM, no significant partitioning to suspended solids was observed. EME was only sorbed to anaerobic biofilms and not the aerobic one. These evidences provided new insights on the fate of drug biomarkers in the presence of different sewer biosolids and supported the requirements for prediction the fate of these chemicals in sewers.

- Sorption and transformation of drug biomarkers in mineral water and raw wastewater (presence of suspended solids) could be adequately predicted by the extended Activated Sludge Modelling framework for Xenobiotics (ASM-X). The model was then further extended with prediction of primary metabolic processes using the Wastewater Aerobic/anaerobic Transformations in Sewers (WATS) model. Following estimation of transformation rates, it was found that neglecting the dynamics of biomass during batch experiments could result in significant over estimation of aerobic biotransformation rates in raw wastewater. However, no significant difference was observed for the biotransformation rates under anaerobic conditions. These results suggest that considering the variation of biomass is rather crucial for accurate estimation of aerobic biotransformation rates in raw wastewater. This is especially relevant for sewers which are often operated under the conditions of significance biomass growth e.g. in gravity sewers, due to high substrate-to-biomass ratio. Moreover, the 1-D biofilm model was successful in simulating concentrations of drug biomarkers in the aqueous phase of biofilm reactors. It was shown that an inaccurate boundary layer thickness and number of discretization for spatial integration in the model can deteriorate the accuracy of simulation. All models could be validated for most tested drug biomarkers, except a few such as METD. The validation was performed by using an independent dataset.
- It was demonstrated that transformation pathways in sewers may be different from human metabolism. A calibration method was developed based on propagating uncertainty associated with model parameters. The method developed was compared with estimation methods commonly used in literature (i.e. estimation of all parameters at the same time or fixing parameters at earlier levels). Proposed method could outperform other methods in terms of prediction accuracy, transformation pathway identification efficiency and parameter identifiability
- At the catchment level, uncertainties in estimation of daily drug consumption, that are associated with neglecting, or imprecisely predicting, in-sewer transformation, were assessed through scenario analysis for different catchment sizes. Moreover the error imposed by inaccurate sampling was also assessed. Under the tested conditions, the results indicated that ignoring in-sewer transformation of drug bi-

omarkers can introduce significant error as compared to inaccurate sampling, especially for larger catchments. It was found that abiotic processes play an important role in prediction of daily COC consumption based on BE. However, in estimation of HER consumption based on 6MAM, it was the sewer biofilms that dominated the total transformations.

- Eventually, the developed model was successfully applied to estimate COC consumption in Lynetten catchment, in Copenhagen, Denmark, based on a 2-week monitoring campaign performed at Lynetten WWTP inlet. The full-scale evaluation for Lynetten catchment presented in this thesis represents one of the first examples of estimation of drug use by including a detailed fate processes.

8 Future perspectives

Experimental assessments carried out in this thesis demonstrated that many of selected drug biomarkers undergo significant transformations. Different factors can impact the transformation of drug biomarkers that were not assessed in this thesis including i) transformation under different pH levels. This can be an influential factor as the activity of bacterial community can be impacted at different pH levels; ii) transformation tests with representative concentration of drug biomarkers. High concentration of drug biomarkers in batch experiments might have strong impact on cometabolism of drug biomarkers. Possible inhibition of microorganisms that capable of biotransformation of selected drug biomarkers should be considered (Alexander, 1985); iii) monitoring formation of drug biomarkers with deuterated standards. This would allow direct observation of illicit drugs that are formed such as benzoylecgonine (McCall et al., 2016; Thai et al., 2014); iv) biotransformation experiments with sewer sediments; v) transformation tests in sewer sections similar to the experiment performed by Jelic et al. (2014) for pharmaceuticals. The tests should be conducted in both aerobic and anaerobic sewer sections.

The mathematical models presented in this thesis could adequately predict most transformation processes. However, model simulation and calibration were validated for transformations in laboratory scale systems. The prediction potential of these models should be validated in sewer systems. At catchment level, the fate of illicit drugs should be formulated together with prediction of flowrates, such as the modeling tool for dynamic transport and fate of micropollutants (Vezzaro et al., 2014). The model should ideally include prediction of drug biomarkers at temporal and spatial scales.

The biofilm model in this thesis is rather simplified by assuming the density and thickness of biofilm as fixed parameters. This is while the concentration of all electron donors and acceptors and biomass composition exhibit a gradient inside the biofilm. In this respect, the biofilm is not a homogeneous system. Different multispecies model has been developed for sewer biofilms including the interactions among different microbial species (Huisman et al., 2003; Jiang et al., 2009). Incorporating the active biomass fraction in prediction of illicit drug transformation processes can be beneficial for more accurate estimation of transformation rates, similar to the approach in WATS—ASM-X model.

9 References

Achleitner, S., Möderl, M., Rauch, W., 2007. CITY DRAIN © – An open source approach for simulation of integrated urban drainage systems. Environ. Model. Softw. 22, 1184–1195.

Alexander, M., 2000. Aging, bioavailability, and overestimation of risk from environmental pollutants. Environ. Sci. Technol. 34, 4259–4265.

Alexander, M., 1985. Biodegradation of organic chemicals. Environ. Sci. Technol. 19, 106–111.

Bach, P.M., Rauch, W., Mikkelsen, P.S., Mccarthy, D.T., Deletic, A., 2014. A critical review of integrated urban water modelling - Urban drainage and beyond. Environ. Model. Softw. 54, 88–107.

Baker, D.R., Kasprzyk-Hordern, B., 2011. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography-mass spectrometry. J. Chromatogr. A 1218, 8036–59.

Banta-Green, C., Field, J., 2011. City-wide drug testing using municipal wastewater. Significance 8, 70–74.

Baz-Lomba, J.A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignanò, E., Causanilles, A., Hernandez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.-K., van Nuijs, A., Ort, C., Plósz, B.G., Ramin, P., Reid, M., Rousis, N.I., Ryu, Y., de Voogt, P., Bramness, J., Thomas, K., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16.

Benedetti, L., Langeveld, J., Comeau, A., Corominas, L., Daigger, G., Martin, C., Mikkelsen, P.S., Vezzaro, L., Weijers, S., Vanrolleghem, P.A., 2013. Modelling and monitoring of integrated urban wastewater systems: review on status and perspectives. Water Sci. Technol. 68, 1203–1215.

Bijlsma, L., Emke, E., Hernández, F., de Voogt, P., 2013. Performance of the linear ion trap Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant metabolites in sewage water. Anal. Chim. Acta 768, 102–110.

Bisceglia, K.J., Roberts, A.L., Lippa, K.A., 2012. A hydrolysis procedure for the analysis of total cocaine residues in wastewater. Anal. Bioanal. Chem. 402, 1277–1287.

Bisceglia, K.J.K., Lippa, K. a, 2014. Stability of cocaine and its metabolites in municipal wastewater - the case for using metabolite consolidation to monitor cocaine utilization. Environ. Sci. Pollut. Res. 21, 4453–4460.

Borova, V.L., Maragou, N.C., Gago-Ferrero, P., Pistos, C., Thomaidis, N.S., 2014. Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 406, 4273–4285.

Breinholt, A., Sharma, A.K., 2010. Case Area Baseline Report Copenhagen Energy & Lynettefællesskabet.

Breinholt, A., Thordarson, F.Ö., Møller, J.K., Grum, M., Mikkelsen, P.S., Madsen, H., 2011. Grey-box modelling of flow in sewer systems with state-dependent diffusion. Environmetrics 22, 946–961.

Butler, D., Davies, J.W., 2004. Urban Drainage, Third. ed. CRC Press.

Butler, D., Graham, N.J.D., 1995. Modelling Dry Weather Wastewater Flow In Sewer Networks. J. Environ. Eng. 121, 161–173.

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Harman, C., Hernández, F., Kasprzyk-Hordern, B., Ort, C., van Nuijs, A.L.N., de Voogt, P., Zuccato, E., 2016. Estimating community drug use through wastewater-based epidemiology, Assessing illicit drugs in wastewater: advances in wastewater-based drug epidemiology, EMCDDA Insights 22.

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F., Reid, M., Ort, C., Thomas, K. V., Van Nuijs, A.L.N., De Voogt, P., Zuccato, E., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ. Sci. Technol. 47, 1452–1460.

Castiglioni, S., Thomas, K. V., Kasprzyk-Hordern, B., Vandam, L., Griffiths, P., 2014. Testing wastewater to detect illicit drugs: State of the art, potential and research needs. Sci. Total Environ. 487, 613–620.

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. Anal. Chem. 78, 8421–8429.

Chen, C., Kostakis, C., Gerber, J.P., Tscharke, B.J., Irvine, R.J., White, J.M., 2014. Towards finding a population biomarker for wastewater epidemiology studies. Sci. Total Environ. 487, 621–628.

Clesceri, L.S., Greenberg, A.E., Eaton, A.D., 1998. Standard Methods for the Examination of Water and Wastewater. Am. Public Heal. Assoc. 20, 1998.

Criddle, C.S., 1993. The kinetics of cometabolism. Biotechnol. Bioeng. 41, 1048–1056.

Daughton CG, 2001. Illicit drugs in municipal sewage: proposed new non-intrusive tool to heighten public awareness of societal use of illicit/abused drugs and their potential for ecological consequences, in: American Chemical Society, Symposium Series. American Chemical Society, Symposium Series, Washington, DC, p. 348–364.

Decostere, B., De Craene, J., Van Hoey, S., Vervaeren, H., Nopens, I., Van Hulle, S.W.H., 2016. Validation of a microalgal growth model accounting with inorganic carbon and nutrient kinetics for wastewater treatment. Chem. Eng. J. 285, 189–197.

Delgadillo-Mirquez, L., Lardon, L., Steyer, J.P., Patureau, D., 2011. A new dynamic model for bioavailability and cometabolism of micropollutants during anaerobic digestion. Water Res. 45, 4511–4521.

EAWAG-BBD [WWW Document], 2016. . Pathw. Predict. Syst. URL http://eawag-bbd.ethz.ch/predict/index.html (accessed 3.15.16).

EMCDDA, 2015a. European Drug Report.

EMCDDA, 2015b. Wastewater analysis and drugs: a European multi-city study, European Monitoring Centre for Drugs and Drug report.

Erickson, A.J., Weiss, P.T., Gulliver, J.S., 2013. Optimizing Stormwater Treatment Practices, Springer. ed.

Fedorova, G., Golovko, O., Randak, T., Grabic, R., 2014. Storage effect on the analysis of pharmaceuticals and personal care products in wastewater. Chemosphere 111, 55–60.

Fitzgerald, R.L., O'Neal, C.L., Hart, B.J., Poklis, a, Herold, D. a, 1997. Comparison of an ion-trap and a quadrupole mass spectrometer using diazepam as a model compound. J. Anal. Toxicol. 21, 445–50.

Frutiger, J., Marcarie, C., Abildskov, J., Sin, G., 2016. A Comprehensive Methodology for Development, Parameter Estimation, and Uncertainty Analysis of Group Contribution Based Property Models-An Application to the Heat of Combustion. J. Chem. Eng. Data 61, 602–613.

Garsdal, H., Mark, O., Dorge, J., Jepsen, S.E., 1995. MOUSETRAP: Modelling of water quality processes and the interaction of sediments and pollutants in sewers. Water Sci. Technol. 31, 33–41.

Gerrity, D., Trenholm, R.A., Snyder, S.A., 2011. Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. Water Res. 45, 5399–5411.

Gheorghe, A., van Nuijs, A., Pecceu, B., Bervoets, L., Jorens, P.G., Blust, R., Neels, H., Covaci, A., 2008. Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry. Anal. Bioanal. Chem. 391, 1309–1319.

Gomes, R.L., Scrimshaw, M.D., Lester, J.N., 2009. Fate of conjugated natural and synthetic steroid estrogens in crude sewage and activated sludge batch studies. Environ. Sci. Technol. 43, 3612–3618.

Gracia-lor, E., Zuccato, E., Castiglioni, S., 2016. Refining correction factors for back-calculation of illicit drug use. Sci. Total Environ.

Gujer, W., 2008. System analysis for water technology. Springer.

Hall, W., Prichard, J., Kirkbride, P., Bruno, R., Thai, P.K., Gartner, C., Lai, F.Y., Ort, C., Mueller, J.F., 2012. An analysis of ethical issues in using wastewater. Addiction 107, 1767–1773.

Henze, M., Comeau, Y., 2008. Wastewater Characterization. Biol. Wastewater Treat. Princ. Model. Des. 33–52.

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

Huisman, I.L., Krebs, P., Gujer, W., 2003. Integral and unified model for the sewer and wastewater treatment plant focusing on transformations. Water Sci. Technol. 47, 65–71.

Huisman, J.L., Gujer, W., 2002. Modelling wastewater transformation in sewers based on ASM3. Water Sci. Technol. 45, 51–60.

Hvitved-Jacobsen, T., Vollertsen, J., Matos, J.S., 2002. The sewer as a bioreactor - A dry weather approach. Water Sci. Technol. 45, 11–24.

Hvitved-Jacobsen, T., Vollertsen, J., Nielsen, A.H., 2013. Sewer Processes: Microbial and Chemical Process Engineering of Sewer Networks, second. ed. CRC Press.

Hvitved-Jacobsen, T., Vollertsen, J., Tanaka, N., 1998. Wastewater quality changes during transport in sewers - an integrated aerobic and anaerobic model concept for carbon and sulfur microbial transformations. Wat. Sci. Tech. 38, 257–264.

Hyland, K.C., Dickenson, E.R. V, Drewes, E., Higgins, C.P., 2012. Sorption of ionized and neutral emerging trace organic compounds onto activated sludge from different wastewater treatment configurations 6, 1958–1968.

Ingram, T., Richter, U., Mehling, T., Smirnova, I., 2011. Fluid Phase Equilibria Modelling of pH dependent n-octanol / water partition coefficients of ionizable pharmaceuticals. Fluid Phase Equilib. 305, 197–203.

Jacobsen, B.N., Becher, G., Jensen, B.K., Monarca, S., Heidrun Scholz-Muramatsu, J.S., 1996. Fate prediction of specific organic compounds in bioreactors. Environ. Sci. Technol. 33, 289–296.

Jelic, A., Rodriguez-Mozaz, S., Barceló, D., Gutierrez, O., 2014. Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res. 68, 98–108.

Jiang, F., Leung, D.H.-W.W., Li, S., Chen, G.-H.H., Okabe, S., van Loosdrecht, M.C.M., 2009. A biofilm model for prediction of pollutant transformation in sewers. Water Res. 43, 3187–98.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T. a., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Res. 40, 1686–1696.

Kappeler, J., Gujer, W., 1992. Estimation of kinetic parameters of heterotrophic biomass under aerobic conditions and characterization of wastewater for activated sludge modelling. Water Sci. Technol. 25, 125–139.

Kasprzyk-Hordern, B., Baker, D.R., 2012. Enantiomeric profiling of chiral drugs in wastewater and receiving waters. Environ. Sci. Technol. 46, 1681–1691.

Keyser, W. De, Gevaert, V., Verdonck, F., Nopens, I., Baets, B. De, Vanrolleghem, P.A., Mikkelsen, P.S., Benedetti, L., 2010. Combining multimedia models with integrated urban water system models for micropollutants. Water Sci. Technol. 62, 1614–1622.

Khan, U., Nicell, J.A., 2011. Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. Environ. Int. 37, 1236–1252.

Lai, F.Y., Anuj, S., Bruno, R., Carter, S., Gartner, C., Hall, W., Kirkbride, K.P., Mueller, J.F., O'Brien, J.W., Prichard, J., Thai, P.K., Ort, C., 2015. Systematic and day-to-day effects of chemical-derived population estimates on wastewater-based drug epidemiology. Environ. Sci. Technol. 49, 999–1008.

Lai, F.Y., Bruno, R., Hall, W., Gartner, C., Ort, C., Kirkbride, P., Prichard, J., Thai, P.K., Carter, S., Mueller, J.F., 2012. Profiles of illicit drug use during annual key holiday and control periods in Australia: Wastewater analysis in an urban, a semi-rural and a vacation area. Addiction 108, 556–565.

Lai, F.Y.I.N., Thai, P.K., Brien, J.O., Gartner, C., Bruno, R., Kele, B., Ort, C., Prichard, J., Kirkbride, P., Hall, W., Carter, S., Mueller, J.F., 2013. Using quantitative wastewater

analysis to measure daily usage of conventional and emerging illicit drugs at an annual music festival. Drug Alcohol Rev. 32, 594–602.

Li, M., 2012. Organic Chemistry of Drug Degradation. Royal Society of Chemistry.

Lindblom, E., Gernaey, K. V., Henze, M., Mikkelsen, P.S., 2006. Integrated modelling of two xenobiotic organic compounds. Water Sci. Technol. 54, 213–221.

Liu, Y., Dong, Q., Shi, H., 2015. Distribution and population structure characteristics of microorganisms in urban sewage system. Appl. Microbiol. Biotechnol. 99, 7723–7734.

Mackay, A.A., Vasudevan, D., 2012. Polyfunctional Ionogenic Compound Sorption: Challenges and New Approaches To Advance Predictive Models. Environ. Sci. Technol. 46, 9209–9223.

Mathieu, C., Rieckermann, J., Berset, J.D., Schürch, S., Brenneisen, R., 2011. Assessment of total uncertainty in cocaine and benzoylecgonine wastewater load measurements. Water Res. 45, 6650–6660.

Mazioti, A.A., Stasinakis, A.S., Gatidou, G., Thomaidis, N.S., Andersen, H.R., 2015. Sorption and biodegradation of selected benzotriazoles and hydroxybenzothiazole in activated sludge and estimation of their fate during wastewater treatment. Chemosphere 131, 117–123.

McCall, A.-K., Scheidegger, A., Madry, M.M., Steuer, A.E., Weissbrodt, D.G., Vanrolleghem, P.A., Kraemer, T., Morgenroth, E., Ort, C., 2016. Influence of Different Sewer Biofilms on Transformation Rates of Drugs. Environ. Sci. Technol.

Meirlaen, J., Assel, J. Van, Vanrolleghem, P.A., 2002. Real time control of the integrated urban wastewater system using simultaneously simulating surrogate models. water Sci. 45, 109–116.

Mourato, S., Matos, J., Almeida, M., Hvitved-Jacobsen, T., 2003. Modelling in-sewer pollutant degradation processes in the Costa do Estoril sewer system. Water Sci. Technol. 47, 93–100.

Muyzer, G., Stams, A.J.M., 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nat. Rev. Microbiol. 6, 441–454.

Nielsen, P.H., Raunkjrer, K., Norsker, N.H., Jensen, N.A., Hvitved-Jacobsen, T., 1992. Transformation of wastewater in sewer systems - a review. Wat. Sci. Tech. 25, 17–31.

Nordin, C.F., Troutman, B.M., 1980. Longitudinal Dispersion in Rivers: The Persistence of Skewness in Observed Data. Water Resour. Res. 16, 123–128.

Olsson, G., Jeppsson, U., 2006. Plant-wide control: dream, necessity or reality? Water Sci. Technol. 53, 121–129.

Ort, C., 2016. Sewage Pattern Generator (SPG) [WWW Document]. URL www.eawag.ch/spg (accessed 7.18.16).

Ort, C., 2014. Quality assurance/quality control in wastewater sampling, Future Science Ltd.

Ort, C., Gujer, W., 2006. Sampling for representative micropollutant loads in sewer systems. Water Sci. Technol. 54, 169–176.

Ort, C., Lawrence, M.G., Reungoat, J., Eaglesham, G., Carter, S., Keller, J., 2010a.

Determining the fraction of pharmaceutical residues in wastewater originating from a hospital. Water Res. 44, 605–615.

Ort, C., Lawrence, M.G., Reungoat, J., Mueller, J.F., 2010b. Sampling for PPCPs in Wastewater Systems: Comparison of Different Sampling Modes and Optimization Strategies. Environ. Sci. Technol. 44, 6289–6296.

Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010c. Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems : Are Your Conclusions Valid? A Critical Review. Environ. Sci. Technol. 44, 6024–6035.

Ort, C., Schaffner, C., Giger, W., Gujer, W., 2005. Modelling stochastic load variations in sewer systems. Water Sci. Technol. 52, 113–122.

Ort, C., van Nuijs, A.L.N., Berset, J.D., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., Fatta-Kassinos, D., Griffiths, P., Hernández, F., González-Mariño, I., Grabic, R., Kasprzyk-Hordern, B., Mastroianni, N., Meierjohann, A., Nefau, T., Östman, M., Pico, Y., Racamonde, I., Reid, M., Slobodnik, J., Terzic, S., Thomaidis, N., Thomas, K. V., 2014. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction 109, 1338–1352.

Panasiuk, O., Hedström, A., Marsalek, J., Ashley, R.M., Viklander, M., 2015. Contamination of stormwater by wastewater: A review of detection methods. J. Environ. Manage. 152, 241–250.

Pinhancos, R., Maass, S., Ramanathan, D.M., 2011. High-resolution mass spectrometry method for the detection, characterization and quantitation of pharmaceuticals in water. J. Mass Spectrom. 46, 1175–1181.

Plósz, B.G., Langford, K.H., Thomas, K. V., 2012a. An activated sludge modeling framework for xenobiotic trace chemicals (ASM-X): Assessment of diclofenac and carbamazepine. Biotechnol. Bioeng. 109, 2757–2769.

Plósz, B.G., Langford, K.H., Thomas, K. V., 2012b. An activated sludge modeling framework for xenobiotic trace chemicals (ASM-X): Assessment of diclofenac and carbamazepine. Biotechnol. Bioeng. 109, 2757–2769.

Plósz, B.G., Leknes, H., Thomas, K. V., 2010. Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. Environ. Sci. Technol. 44, 734–742.

Plósz, B.G., Reid, M.J., Borup, M., Langford, K.H., Thomas, K. V., 2013. Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. Water Res. 47, 2129–2140.

Polesel, F., 2016. Modelling the Fate of Xenobiotic Trace Chemicals via Wastewater Treatment and Agricultural Resource Reuse, PhD thesis.

Polesel, F., Lehnberg, K., Dott, W., Trapp, S., Thomas, K. V, Gy, B., 2015. Factors influencing sorption of ciprofloxacin onto activated sludge: Experimental assessment and modelling implications. Chemosphere 119, 105–111.

Pomeroy, R.D., Parkhurst, J.D., 1972. Self-purification in sewers, in: Advances in Water Pollution Research, 6th International Conference. pp. 291–308.

Postigo, C., Alda, M.L. de, Barceló, D., 2011. Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. Environ. Int. 37, 49–55.

Rauch, W., Bertrand-Krajewski, J.-L., Krebs, P., Mark, O., Schilling, W., Schütze, M., Vanrolleghem, P.A., 2002. Deterministic modelling of integrated urban drainage systems. Environ. Sci. Technol. 45, 81–94.

Rieckermann, J., Anta, J., Scheidegger, A., Ort, C., 2011. Assessing wastewater micropollutant loads with approximate bayesian computations. Environ. Sci. Technol. 45, 4399–4406.

Rittmann, B.E., 1995. Transformation of Organic Micropollutants by Biological Processes, in: Handbook of Environmental Chemistry. pp. 31–60.

Rudelle, E., Vollertsen, J., Hvitved-Jacobsen, T., Nielsen, A.H., 2012. Modeling anaerobic organic matter transformations in the wastewater phase of sewer networks. Water Sci. Technol. 66, 1728–1734.

Saltelli, A., Ratto, M., Andres, T., Francesca Campolongo, J.C., Gatelli, D., Saisana, M., Tarantola, S., 2008. Global sensitivity analysis: the primer. John Wiley & Sons, West Sussex.

Sathyamoorthy, S., Ramsburg, C.A., 2013. Assessment of quantitative structural property relationships for prediction of pharmaceutical sorption during biological wastewater treatment 92, 639–646.

Schröder, H.F., Gebhardt, W., Thevis, M., 2010. Anabolic, doping, and lifestyle drugs, and selected metabolites in wastewater—detection, quantification, and behaviour monitored by high-resolution MS and MSn before and after sewage treatment. Anal Bioanal Chem 398, 1207–1229.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 2003. Environmental Organic Chemistry. Wiley Interscience, Hoboken, NJ.

Senta, I., Krizman, I., Ahel, M., Terzic, S., 2014. Assessment of stability of drug biomarkers in municipal wastewater as a factor influencing the estimation of drug consumption using sewage epidemiology. Sci. Total Environ. 487, 659–665.

Siegrist, H., Alder, A., Gujer, W., Giger, W., 1989. Behaviour and modelling of NTA degradation in activated sludge systems. Water Sci. Technol. 21, 315–324.

Sin, G., Gernaey, K. V., Neumann, M.B., van Loosdrecht, M.C.M., Gujer, W., 2009. Uncertainty analysis in WWTP model applications: A critical discussion using an example from design. Water Res. 43, 2894–2906.

Sin, G., Gernaey, K. V., Neumannc, M.B., van Loosdrecht, M.C.M., Gujerc, W., 2009. Uncertainty analysis in WWTP model applications: A critical discussion using an example from design. Water Res. 43, 2894–2906.

Singh, S.K., Beck, M.B., 2003. Dispersion Coefficient of Streams from Tracer Experiment Data. J. Environ. Eng. 129, 539–546.

Snip, L.J.P., Flores-Alsina, X., Plósz, B.G., Jeppsson, U., Gernaey, K. V., 2014. Modelling the occurrence, transport and fate of pharmaceuticals in wastewater systems. Environ. Model. Softw. 62, 112–127.

statistikbanken [WWW Document], 2014. URL http://www.statistikbanken.dk/ (accessed 7.15.16).

Stoyer, R.L., 1970. The Pressure Pipe Wastewater Treatment System. Presents at 2nd annual sanitary engineering research laboratory workshop on wastewater and reuse.

Tahoe City, CA.

Su, L., Aga, D., Chandran, K., Khunjar, W.O., 2015. Factors impacting biotransformation kinetics of trace organic compounds in lab-scale activated sludge systems performing nitrification and denitrification. J. Hazard. Mater. 282, 116–124.

Tan, D.T., Arnold, W.A., Novak, P.J., 2013. Impact of organic carbon on the biodegradation of estrone in mixed culture systems. Environ. Sci. Technol. 47, 12359–65.

Tanaka, N., Hvitved-Jacobsen, T., 1998. Transformations of wastewater organic matter in sewers under changing aerobic/anaerobic conditions. Wat. Sci. Tech. 37, 105–113.

Taylor, G., 1953. Dispersion of Soluble Matter in Solvent Flowing Slowly through a Tube. Math. Phys. Sci. 219, 186–203.

Thai, P.K., Jiang, G., Gernjak, W., Yuan, Z., Lai, F.Y., Mueller, J.F., 2014. Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. Water Res. 48, 538–547.

Thomas, K. V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F., Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., Lopez de Alda, M., Meierjohann, A., Ort, C., Pico, Y., Quintana, J.B., Reid, M., Rieckermann, J., Terzic, S., van Nuijs, A.L.N., de Voogt, P., 2012. Comparing illicit drug use in 19 European cities through sewage analysis. Sci. Total Environ. 432, 432–439.

Torresi, E., Fowler, J.S., Polesel, F., Bester, K., Andersen, H.R., Smets, B.F., Plósz, B.G., Christensson, M., 2016. Biofilm thickness influences biodiversity in nitrifying MBBRs – Implications on micropollutant removal. Environ. Sci. Technol. 50, 9279–9288.

Trapp, S., Franco, A., Mackay, D., 2010. Activity-Based Concept for Transport and Partitioning of Ionizing Organics. Environ. Sci. Technol. 44, 6123–6129.

Trenholm, R.A., Vanderford, B.J., Snyder, S.A., 2009. On-line solid phase extraction LC-MS/MS analysis of pharmaceutical indicators in water: A green alternative to conventional methods. Talanta 79, 1425–1432.

U.S. EPA, 1982. Handbook for Sampling and Sample Preservation of Water and Wastewater; 600/4-82-029; Environmental Monitoring and Support Laboratory.

van Nuijs, A.L.N., Abdellati, K., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2012. The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology? J. Hazard. Mater. 239–240, 19–23.

van Nuijs, A.L.N., Castiglioni, S., Tarcomnicu, I., Postigo, C., de Alda, M.L., Neels, H., Zuccato, E., Barcelo, D., Covaci, A., 2011. Illicit drug consumption estimations derived from wastewater analysis: A critical review. Sci. Total Environ. 409, 3564–3577.

Vanrolleghem, P.A., Spanjers, H., Petersen, B., Ginestet, P., Takács, I., 1999. Estimating (combinations of) activated sludge model no. 1 parameters and components by respirometry. Wat. Sci. Tech 39, 195–214.

Vezzaro, L., Benedetti, L., Gevaert, V., De Keyser, W., Verdonck, F., De Baets, B., Nopens, I., Cloutier, F., Vanrolleghem, P. a., Mikkelsen, P.S., 2014. A model library for dynamic transport and fate of micropollutants in integrated urban wastewater and stormwater systems. Environ. Model. Softw. 53, 98–111.

Vollertsen, J., Hvitved-Jacobsen, T., 2002. Biodegradability of wastewater - A method for COD-fractionation. Water Sci. Technol. 45, 25–34.

Vollertsen, J., Hvitved-Jacobsen, T., 1999. Stoichiometric and kinetic model parameters for microbial transformation of suspended solid in combined sewer systems. Wat. Res. 33, 3127–3141.

Vrugt, J.A., Gupta, H. V., Bouten, W., Sorooshian, S., 2003. A Shuffled Complex Evolution Metropolis algorithm for optimization and uncertainty assessment of hydrologic model parameters. Water Resour. Res. 39, 1201.

Wang, X., Grady, C.P.L., 1995. Effects of biosorption and dissolution on the biodegradation of di-n-butyl phthalate 67, 863–871.

Warith, M.A., Kennedy, K., Reitsma, R., 1998. Use of sanitary sewers as wastewater pre-treatment systems. Waste Manag. 18, 235–247.

Wicke, D., Böckelmann, U., Reemtsma, T., 2007. Experimental and modeling approach to study sorption of dissolved hydrophobic organic contaminants to microbial biofilms. Water Res. 41, 2202–2210.

Zhang, W., Bouwer, E.J., Ball, W.P., 1998. Bioavailability of Hydrophobic Organic Contaminants: Effects and Implications of Sorption-Related Mass Transfer on Bioremediation. Groundw. Monit. Remediat. 18, 126–138.

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., Fanelli, R., 2008. Estimating Community Drug Abuse by Wastewater Analysis. Environ. Health Perspect. 116, 1027–1032.

Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., Fanelli, R., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ. Heal. A Glob. Access Sci. Source 4, 1–7.

10 Papers

I **Ramin, P.**, Brock, A.L., Polesel, F., Causanilles, A., Emke, E., de Voogt, P., Plósz, B.G. (2016). Transformation and sorption of illicit drug biomarkers in sewer systems : understanding the role of suspended solids in raw wastewater. Environmental Science and Technology. doi:10.1021 /acs.est.6b03049

II **Ramin, P.**, Valverde-Pérez, B., Polesel, F., Locatelli, L., Plósz, B.G. (2016). A systematic model calibration method for chemical and biochemical transformation pathway models – The case of transformation of heroin biomarkers in wastewater. *Submitted manuscript*

III **Ramin, P.**, Brock, A.L., Causanilles, A., Valverde-Pérez, B., Emke, E., de Voogt, P., Polesel, F., Plósz, B.G. (2016). Transformation and sorption of illicit drug biomarkers in sewer biofilms. *Submitted manuscript*

IV **Ramin, P.**, Vezzaro, L., Mikkelsen, P.S., Plósz, B.G. (2016). Back-calculating illicit drug abuse rates in urban areas – Quantifying uncertainties associated with in-sewer transformation and samplin. *Submitted manuscript.*

The Department of Environmental Engineering (DTU Environment) conducts science based engineering research within six sections: Water Resources Engineering, Water Technology, Urban Water Systems, Residual Resource Engineering, Environmental Chemistry and Atmospheric Environment.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

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