Measuring and predicting the fate of contaminants of emerging concern during wastewater treatment

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ABSTRACT

The presence of contaminants of emerging concern (CECs) in the aquatic environment and the associated proven toxic impacts have increasingly alarmed researchers. The discharge of wastewater into surface water was identified as the major source for the release of CECs into the environment. Despite the available data on the removal of CECs in wastewater treatment plants (WWTPs) in the literature, previous studies had several shortcomings, resulting in an inaccurate prediction of the CEC fate. This PhD project aimed at monitoring the fate of CECs in different treatment steps with special consideration to the hydrodynamics of the treatment units and adsorption to sludge. The project also aimed at developing and calibrating a model to predict the fate of target CECs in the most widespread secondary treatment technology: the activated sludge process. Among the various classes of CECs, this thesis focused on the widely consumed pharmaceuticals, personal care products, drugs of abuse, hormones, stimulants and artificial sweeteners based on evidences of their presence in treated wastewater implying their inefficient removal during treatment.

Recently, the hydraulic characteristics of WWTPs were demonstrated to bias the calculations of CEC removal if not accounted for. To address this issue, the fractionated approach that integrates hydraulic modelling and improved sampling strategies was recently proposed. In order to verify the capability of the fractionated approach at capturing the hydraulic differences, the temperature and electric conductivity of wastewater were used as tracers to model the hydraulics of two full-scale WWTPs. Results demonstrated that a distinctive model was necessary to describe the hydraulics in each WWTP, requiring different number of days for sampling, as well as different CEC removal calculations.

In order to explore the contribution of the different fate pathways to the removal of CEC during wastewater treatment, a sampling campaign was performed in a WWTP using an optimized sampling strategy based on the fractionated approach to collect and chemically analyze both wastewater and sludge samples. This allowed performing a mass balance on the incoming load of CECs, which was carried out for the first time with consideration to the hydraulic characteristics. Results indicated that for 21 out of 24 investigated CECs, degradation was the major removal process, with sorption accounting for <10% of the input CEC load fate in the primary clarifier and <5% in the activated sludge process. Most target CECs (22 out of 25) were relatively persistent in rotating biological contactors and sand filtration compared to activated sludge treatment.

In order to predict the fate of CECs, a fate model based on the widespread Activated Sludge Model No. 2d (ASM2d) was further modified to better describe the CEC fate processes in aeration tanks. The state-of-the-art Bürger-Diehl secondary clarifier model was extended to include the CEC fate processes for the first time. The resulting secondary treatment model was calibrated to predict the fate of four target CECs that belong to different classes and undergo different fate processes. Results from global sensitivity analysis indicated that depending on the contaminant's properties, a different set of parameters deserved more attention. Further, dynamic sensitivity analysis should be taken into consideration in future sampling campaigns for model calibration.

RÉSUMÉ

La présence de contaminants d’intérêt émergent (CIE) dans l’environnement aquatique et les impacts toxiques associés à leur présence alarment de plus en plus les chercheurs. Le rejet d’eaux usées dans les eaux de surface a été identifié comme la source principale du relâchement de ces CIE dans l'environnement. Malgré les données disponibles dans la littérature relativement à l’enlèvement des CIE dans les stations d’épuration des eaux usées (STEP), plusieurs lacunes de ces études doivent être résolues. Ce projet de doctorat visait à évaluer le devenir des CIE dans les différentes étapes de traitement, en portant une attention particulière à l’hydrodynamique des unités de traitement. Le projet visait également à développer et calibrer un modèle de prédiction du devenir des CIE cibles en cours de traitement par boues activées, la technologie de traitement secondaire la plus répandue. Parmi les différentes classes de CIE, cette thèse se concentre sur les produits pharmaceutiques de grande consommation, les produits de soins personnels, les drogues, les hormones, les stimulants et les édulcorants artificiels. La sélection des CIE cibles s’est basée sur leur présence démontrée dans les eaux usées traitées impliquant leur élimination inefficace pendant le traitement.

Récemment, il a été démontré que les caractéristiques hydrauliques des STEP influencent les calculs d'enlèvement des CIE s’ils ne sont pas prises en compte. Pour résoudre ce problème, l'approche fractionnée qui intègre une modélisation hydraulique et des stratégies d'échantillonnage améliorées a été récemment proposée. La conductivité électrique et la température ont été utilisées comme traceurs pour modéliser le comportement hydraulique de deux stations d'épuration, permettant ainsi de vérifier l’efficacité de l'approche fractionnée à capter les différences hydrauliques. Les résultats ont démontré qu’un modèle distinct est nécessaire pour décrire l'hydraulique dans chaque station d'épuration, imposant donc un nombre de jours différent pour l’échantillonnage ainsi que des calculs différents d'élimination des CIE.

Afin d'explorer la contribution des différentes voies d’élimination des CIE au cours du traitement des eaux usées, une campagne d'échantillonnage a été effectuée dans une station d'épuration en utilisant une stratégie d'échantillonnage optimisée basée sur l'approche fractionnée. Les échantillons d’eau et de boue recueillis et analysés au cours de cette campagne ont permis d'effectuer un bilan de matière sur la charge entrante de CIE, ceci étant réalisé pour la première fois en tenant compte des caractéristiques hydrauliques. Les résultats indiquent que pour 21 des 24 CIE, la dégradation a été le processus principal d'élimination, avec une sorption représentant <10% de l’élimination des charges entrantes de CIE au clarificateur primaire et <5% au procédé de boues activées. La plupart des CIE cibles (22 sur 25) étaient relativement persistants dans les contacteurs biologiques rotatifs et la filtration sur sable par rapport au traitement par boues activées.

Afin de prédire le devenir de CIE au cours du traitement des eaux usées, un modèle basé sur le modèle connu de boues activées No.2d (ASM2d) a été développé afin de mieux décrire les processus qui affectent les CIE dans les bassins d'aération. Les processus d’élimination des CIE ont été ajoutés au modèle Bürger-Diehl de décantation pour la première fois. Les mesures de concentration des CIE ont été utilisées pour calibrer le modèle de traitement secondaire afin de prédire le devenir de quatre CIE cibles qui appartiennent à des classes différentes de CIE et subissent différents processus d’élimination. Les résultats de l'analyse globale de sensibilité ont indiqué qu'un ensemble de paramètres différents méritent une attention particulière selon les propriétés des CIE. L’analyse de sensibilité dynamique pourrait être mis à profit dans des campagnes futures de mesure visant la calibration de modèles.

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ABBREVIATIONS AND UNITS

AE	Aeration effluent
APHA	American Public Health Association
ASE	Accelerated solvent extraction
ASM2d	Activated Sludge Model No. 2d
AWWA	American Water Works Association
CEC	Contaminant of emerging concern
CEPT	Chemically-enhanced primary treatment
CMZ-DiOH	rac trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine
CMZ-EP	Carbamazepine 10,11-Epoxide
COD	Chemical oxygen demand
d	days
DF	Detection frequency
DO	Dissolved oxygen
DR	Detection ratio
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
F	Fahrenheit
g/d	grams per day
gSS	grams of suspended solids
h	hours
HESI	Heated electrospray ionization source
HLB	Hydrophilic-lipophilic-balanced
HRT	Hydraulic retention time
k_{bio}	Biodegradation rate constant
K_d	Solid-liquid distribution coefficient
k_{des}	Desorption rate constant
kg	kilograms
k_{ow}	Octanol-water partition coefficient
k_{sor}	Sorption rate constant
L	liters
LC-MS/MS	Liquid chromatography with tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
LS-HRMS	Liquid chromatography with high resolution mass spectrometry
m^3	cubic meter
MAX	Mixed-mode anion exchange
MCX	Mixed-mode cation exchange
MDA	methylene dioxyamphetamine
mg	milligrams
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
MP	Micropollutant
MRM	Multiple reaction monitoring
MW	Molecular weight
ng	nanograms
PE	Primary effluent
pk_a	Logarithmic acid dissociation constant
PLE	Pressurized liquid extraction

PPCPs	Pharmaceuticals and personal care products
RBC	Rotating biological contactor
RMSE	Root mean square error
RTD	Residence time distribution
SE	Secondary effluent
S_{MP}	Micropollutant concentration in soluble phase
SPE	Solid phase extraction
SRT	Solids retention time
STD	Standard deviation
STOWA	Dutch Foundation for Applied Water Research
TKN	Total Kejeldahl Nitrogen
TSS	Total suspended solids
UNODC	United Nations Office of Drugs and Crime
VODE	Variable-coefficient ODE solver
WAS	Secondary waste sludge
WWTP	Wastewater treatment plant
X_{MP}	Micropollutant concentration in particulate phase
µS/cm	microSiemens per centimeter
χ^2	Chi-square error
°C	Degrees Celsius
k_La	Oxygen transfer rate

CONTRIBUTION OF AUTHORS

Manuscript 1- Estimating removals of contaminants of emerging concern from wastewater treatment plants: The critical role of wastewater hydrodynamics

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Chris Metcalfe	Contributed to the chemical analysis for a subset of the target contaminants and the manuscript revision
Viviane Yargeau	Contributed to the design of experiments and the field work and contributed to the manuscript revision

Manuscript 2- Fate and mass balance of contaminants of emerging concern during wastewater treatment determined using the fractionated approach

Author	**Contribution**
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Thomas Maere	Contributed to the hydraulic calibration and manuscript revision
Chris Metcalfe	Contributed to the chemical analysis for a subset of the target contaminants and manuscript revision
Peter Vanrolleghem	Contributed to the hydraulic modelling and manuscript revision
Viviane Yargeau	Contributed to the design of experiments and fieldwork and the manuscript revision

Manuscript 3- Dynamic modelling of solids in a full-scale activated sludge WWTP preceded by CEPT as a basic step for micropollutant modelling

Author	**Contribution**
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Thomas Maere	Contributed to the influent characterization, hydraulic calibration and manuscript revision
Viviane Yargeau	Contributed to the field work and manuscript revision
Peter Vanrolleghem	Contributed to the development of the model, the design of the simulations and manuscript revision

Manuscript 4 - Predicting the fate of micropollutants during wastewater treatment: Calibration and sensitivity analysis

Author	**Contribution**
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Peter Vanrolleghem	Contributed to the development of the model, the design of the simulations and manuscript revision
Viviane Yargeau	Contributed to the fieldwork and the manuscript revision

1. INTRODUCTION

With the increased development and use of pharmaceuticals, drugs of abuse and personal care products, these chemicals have been observed over the past two decades to end up in surface waters at trace concentrations of ng/L-μg/L (Buser et al., 1998; Daneshvar et al., 2010; García-Galán et al., 2011; Kasprzyk-Hordern et al., 2007; Lindström et al., 2002). It was proven that these contaminants in environmental waters could disrupt aquatic life (Burkhardt-Holm et al., 2008; Gay et al., 2016; Kidd et al., 2007; Purdom et al., 1994) and can make their way to drinking water resources (Heberer, 2002a; Rodayan et al., 2015). The presence of these contaminants, also called micropollutants (MPs) or contaminants of emerging concern (CECs), including PPCPs, drugs of abuse and hormones in the environment was shown to be mainly caused by discharges of wastewater treatment plants (WWTPs) into surface waters (Heberer, 2002a). In fact, WWTPs receive many of these micropollutants; however, considering that these are not regulated (i.e. unlike bulk conventional pollutants, there is no legislation specifying a discharge limit for micropollutants), WWTPs are not designed to remove CECs. In 2012, the Swiss Federal Office for the Environment established and enforced legislation regarding the discharge of micropollutants in Switzerland, which implied the upgrade of approximately 100 WWTPs, requiring a total investment of 1.2 billions CHF (Office Fédérale de l'Environnement, 2012). Even in countries with no current legislation concerning the discharge of CECs, global awareness is growing with regards to the adverse effects of the presence of low concentration of these CECs in water bodies, and, as a result, environmental protection agencies in some countries monitor the amount of discharge of some of the common CECs and their levels in surface waters (Ort et al., 2009).

In this context, obtaining reliable data on the efficacy of conventional treatment at removing these CECs, as well as tools to predict the removal when designing treatment units is becoming increasingly valuable. Among the different studies reporting data on CEC removal, large variations are observed in the data of CEC removal in various types of treatment (Luo et al., 2014; Verlicchi & Zambello, 2015). Also, in some cases, negative and fluctuating removals of CECs were observed (Behera et al., 2011; Blair et al., 2015; Rodayan et al., 2014a). Despite the numerous studies investigating the removal of CECs in WWTPs, a number of critical aspects remain understudied until today. Firstly, the effect of hydraulics on the transport of CECs within treatment units is often overlooked when determining removal levels (Majewsky et al., 2011; Ort et al., 2010). Secondly, most studies investigating the removal of CECs in WWTPs based their calculations on the concentrations measured in the aqueous phase only, without quantifying the partitioning of CECs onto sludge. It was previously shown that the main processes governing the removal of CECs are biodegradation (i.e. the transformation of organic compounds by biomass into by-products) and adsorption to sludge (Andersen et al., 2005; Radjenović et al., 2009a), suggesting that quantifying the CEC load partitioned onto sludge is required to identify and quantify the different removal mechanisms involved during treatment (Petrie et al., 2015). Such information is also required to develop models predicting the removal of CECs.

Predicting the fate of CECs by mathematical models in conventional WWTPs constitutes a cost-efficient tool for risk assessment and decision-making. Several state-of-the art models at different levels of complexity were created and calibrated to predict the fate of CECs in activated sludge treatment which is the most widely used biological treatment technology (Cowan et al., 1993; Parker et al., 1994; Plósz et al., 2010; Struijs et al., 1991; Urase & Kikuta, 2005). In some cases, assumptions were made regarding the sorption process, such as ignoring the kinetics of the sorption by assuming that equilibrium is reached rapidly (Abegglen et al., 2009; Suarez et al., 2010; Urase & Kikuta, 2005). Also, in almost all studies, the CEC fate processes (i.e. biodegradation and sorption) were supposed to take place in the aeration tanks only, ignoring the CEC processes in the secondary clarifier. The need to define values for the different parameters involved in the fate equations still pose a difficulty in implementing the developed MP models. This is due to the unavailability of many of these parameters as well as the lack of utility of the available parameters, due to the fact that most of the previous studies did not indicate the level of confidence in these parameters (Pomiès et al., 2013).

This PhD thesis focuses on the removal of specific classes of CECs, including pharmaceuticals and personal care products, drugs of abuse, hormones a nervous stimulant and an artificial sweetener during wastewater treatment and addresses the understudied areas discussed above.

2. LITERATURE REVIEW

2.1. Source, routes and environmental impacts of CECs

Contaminants of emerging concern (CECs) include a wide variety of consumed chemicals that could potentially pose a risk on the environment. A report by the World Health Organization 2011 reported a 23% average volume increase in the consumption of pharmaceuticals across 84 countries of different incomes levels between 2000 and 2008 (Hoebert et al., 2011). Also, The United Nations on Drugs and Crime reported that between 162 and 324 million (3.5 to 7%) of the world population consumed illicit drugs at least once in 2014 (UNODC, 2014). Only pharmaceuticals and personal care products (PPCPs), drugs of abuse and others such as stimulants, hormones and artificial sweeteners are of concern in this project.

These substances that are being consumed at a growing amount are excreted by humans into household discharges and hospitals effluents in both aqueous and particulate phases. Improper disposal of pharmaceuticals and drugs down the drain is another source of these contaminants. Considering that wastewater treatment plants (WWTPs) do not completely remove CECs (Behera et al., 2011; Luo et al., 2014), these contaminants are continuously released with treated wastewater that is discharged into surface water, as well as with treated sludge that is often applied on fields (Ahel & Jeličić, 2001; Heberer, 2002a). The presence of CECs in surface water could cause some contaminants to make their way into drinking water resources (Heberer, 2002b; Heberer et al., 2001; Reddersen et al., 2002; Rodayan et al., 2015) and into ground water (Ahel & Jeličić, 2001; Holm et al., 1995; Reddersen et al., 2002; Sacher et al., 2001). The contamination of surface water with CECs at environmental concentrations was also found to adversely affect the biodiversity of fish and other aquatic species (Purdom et al., 1994) and to cause near extinction of fathead minnow (Kidd et al., 2007). In another recent study, cocaine was observed to induce hormonal changes in European eels (Gay et al., 2016). Besides, as a result of their presence in surface water and manure, contaminants can make their way into other environmental compartments, as illustrated in a review article by Heberer (2002a).

2.2. Classes of CECs studied

The classes of contaminants included in this PhD project are presented and discussed in the following subsections along with rationale for their selection. In general, the target contaminants have been selected based on their known persistence in WWTPs, their common occurrence in surface water, their potential toxic effects, and their previous use as indicators of treatment efficiencies. Metabolites of some of the pharmaceuticals and drugs have been added to the list to evaluate the impact on the material balances for specific compounds in various treatment units.

2.2.1. Pharmaceuticals

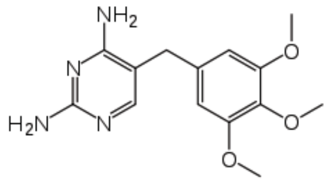
A comprehensive database based on 236 published studies has reported the presence of over 200 pharmaceuticals in inland surface waters globally, with the most frequently detected pharmaceuticals being antibiotics, antiepileptics, pain killers and cardiovascular drugs, which together constituted 86% of the database (Hughes et al., 2013). These classes were also identified to be the most frequently prescribed or over-the-counter purchased drugs (Gu et al., 2010; National Health Service). General information regarding the medical uses and modes of actions of these pharmaceuticals are provided below (Egton Medical Information Systems; Goodman et al., 2006).

Antibiotics are medications used to fight infections due to bacteria or parasites by killing the bacteria or germs or preventing them from reproducing in the body. They are often prescribed for more serious infection by germs. One type of antibiotics is sulfonamides that are derived from a sulfur containing chemical, namely sulfanilamide. These starve the bacteria by disrupting the production of folate. The two antibiotics selected for this project are trimethoprim and sulfamethoxazole from the sulfonamide type. Trimethoprim is mainly used for urinary infections, middle ear infections or traveller's diarrhea. Sulfamethoxazole is often combined with trimethoprim in medications and used for urinary and middle ear infections, as well as prostatitis and bronchitis.

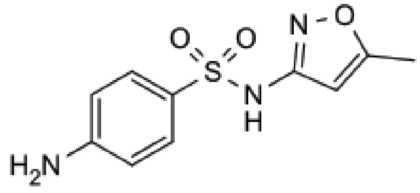
Analgesics are prescribed to relieve pain associated with different conditions, such as sprains, migraines, joint and muscle pain and others. Analgesics selected for this project are ibuprofen, naproxen, acetaminophen and codeine. Both ibuprofen and naproxen are also non-steroidal anti-inflammatories (NSAIDs) that could reduce inflammation related to conditions such as rheumatoid arthritis. However, the effect of naproxen is known to last longer than ibuprofen. Acetaminophen is also widely available, but it does not reduce inflammation and swelling. In general, ibuprofen, naproxen and acetaminophen are used in cases of mild to moderate pain. Ibuprofen is widely consumed around the world, and its widespread consumption accounted in the past for one third of the total consumption of over-the-counter analgesics (Wyeth Consumer Healthcare, 2002).

Anticonvulsant (antiepileptic) medications are often taken daily by epilepsy patients to control seizures. These medications work by diminishing the high electrical activity of the brain that causes the seizure. The antiepileptic selected for this project is carbamazepine, which in addition to its use as an antiepileptic is also prescribed to treat nerve pain and bipolar disorder (2004-2016).

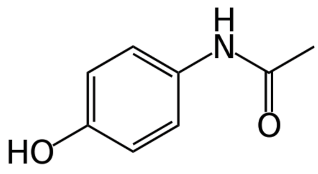
The criteria for selecting the aforementioned pharmaceuticals for monitoring in this PhD project was defined in a study by Dickenson et al. (2011), in which these compounds were identified, amongst others, as suitable indicators of fate of CECs in WWTPs and their transport into surface water. Dickenson et al. (2011) based his conclusion on their detection frequency (DF% > 80%) and detection ratio (DR > 5) (defined as $\frac{Concentration}{limit\ of\ detection}$) in effluent (treated) wastewaters in North America. Although acetaminophen had an average detection frequency of <80% in effluent wastewaters (Dickenson et al., 2011), the widespread non-prescription drug was added to the list due to the fact that it was previously identified as one of 95 contaminants found in wastewater at concentrations as high as 10 ppb in U.S. streams contaminated by wastewater (Kolpin et al., 2002). Characteristics of the studied pharmaceuticals and percentages that are excreted unchanged from human body, as well as information on their occurrence in wastewater effluent and surface water are summarized in Table 2.1. Figure 2.1 shows the chemical structures, the acid dissociation constant (pK_a), the log octanol-water coefficient (Log K_{ow}) and the common trade name of the studied pharmaceuticals.



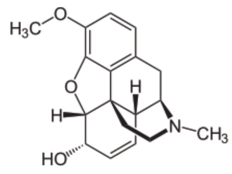
Trimethoprim (MW: 290.3, pK_a: 6.9, Log K_{ow}: 0.91), Proloprim, Monotrim, Triprim



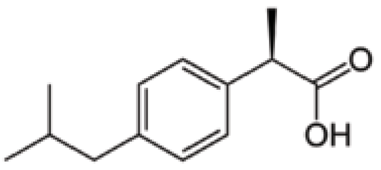
Sulfamethoxazole (MW: 253.3, pK_a: 5.7, Log K_{ow}: 0.89), Gantanol



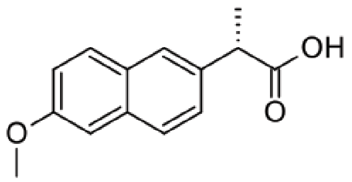
Acetaminophen (MW: 151.17, pK_a: 9.04, Log K_{ow}: 0.46), Tylenol, Panadol



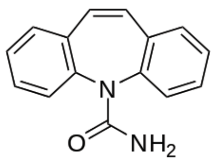
Codeine (MW: 299.36, pK_a: 8.21, Log K_{ow}:1.14), Tylenol Elixir with Codeine (combined with ibuprofen)



Ibuprofen (MW:206.23, pK_a: 4.9, Log K_{ow}: 3.97), Advil, Motrin, Nurofen



Naproxen (MW:230.27, pK_a: 4.2, Log K_{ow}: 3.18), Aleve, Anaprox, Apronax, Naprelan, Naprosyn



Carbamazepine (MW: 263.27, pK_a: 3.19, Log K_{ow}: 2.45), Tegretol

Figure 2.1 Chemical structures of the studied pharmaceuticals, their molecular weight (MW, g/mol), pK_a, log K_{ow} and their common trade names. pK_a and Log K_{ow} values were obtained from the National Center for Biotechnology Information (2004).

Table 2.1 Selected pharmaceuticals and their human excretion percentage, their detection ratio (DR) and detection frequency (DF) in effluent wastewater, as well as their mean concentration in surface water

Type and Subtype	Compound	CAS number	Percentage of unchanged parent compound excreted (in urine)	Effluent wastewaters in North America [1] DF(%), DR	Surface water DF (%), Mean concentration (ng/L) [2]
Antibiotics	Trimethoprim	738-70-5	80-90[3]	86, 24	50, 53
	Sulfamethoxazole	723-46-6	20[4]	94, 426	67, 83
Analgesic	Ibuprofen	15687-27-1	<10[4]	78, 49	63, 504
	Naproxen	22204-53-1	<1[3]	92, 126	69, 98
	Acetaminophen	103-90-2	< 5[5]	<80, 4500	52, 148
Antiepileptic	Carbamazepine	298-46-4	<3[4]	88, 94	85, 174

[1] Dickenson et al. (2011), [2] Hughes et al. (2013), [3] American Hospital Formulary Service (2016), [4] Goodman et al. (1996), [5] Ellenhorn and Barceloux (1988)

In the human body, the studied pharmaceuticals are excreted mainly in the urine in conjugated or free forms (American Hospital Formulary Service, 2016; Goodman et al., 1996; Parke, 1968). As shown from the collected literature in Table 2.1, only a small percentage of the investigated pharmaceuticals is excreted unchanged, except for trimethoprim with 80-90% being excreted unchanged. Considering that negative removals are often reported in the literature for carbamazepine in WWTPs (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Petrovic et al., 2009; Santos et al., 2009), the metabolites of the antiepileptic were included in the monitoring to investigate the effect of accounting for metabolites in fate studies in WWTPs. These metabolites are rac trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine (CBZ-DiOH) and Carbamazepine 10,11-Epoxide (CBZ-EP) that were identified as the major human metabolites by Reith et al. (2000). They are formed through the main route of metabolism that transforms carbamazepine into CBZ-EP, which is further transformed into CBZ-DiOH in the presence of the catalytic enzyme microsomal epoxide hydrolase, and finally CBZ-DiOH is further transformed into 9-hydroxymethyl-10-carbamoylacridan (CBZ-2OH) (Breton et al., 2005; Kitteringham et al., 1996). According to Table 2.1, only 3% of carbamazepine is excreted unchanged (Goodman et al., 1996). Limited information is available concerning the concentration and excretion percentages of the metabolites of carbamazepine in urine (Leclercq et al., 2009). However, CBZ-DiOH is known to be pharmaceutically inactive and was frequently detected at levels three times higher than carbamazepine in surface and drinking water (Hummel et al., 2006; Miao & Metcalfe, 2003; Miao et al., 2005), suggesting the importance of accounting for this metabolite when investigating the removal of carbamazepine during wastewater treatment. The second major metabolite of carbamazepine (CBZ-EP) was detected at lower concentrations than CBZ-DiOH, but was shown to have neurotoxic effects (Rambeck et al., 1993; Semah et al., 1994), justifying its inclusion in the project.

Carbamazepine 10,11-Epoxide (MW: 252.27, pK_a: N.A., Log K_{ow}: 1.26),

rac trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine (MW: 270.28, pK_a: N.A., Log K_{ow}: 0.13)

Figure 2.2 Chemical structure and molecular weight (MW, g/mol) of the two major metabolites of carbamazepine studied. Log K_{ow} values obtained from National Center for Biotechnology Information (2004) N.A.:unavailable data.

2.2.2. Drugs of Abuse

Target compounds classified under this category include drugs that might be prescribed for medical uses but also produce desirable cognitive effects that lead to dependency and recreational uses. The majority of these have been classified as Schedule I substances, according to the Controlled Drugs and Substances Act of Canada. The selected drugs of abuse fall under two main categories: dopamine uptake inhibitors and opioids. General information regarding the medical uses, modes of action and cognitive effects of these drugs is provided below (Goodman et al., 2006; National Institute on Drug Abuse).

Dopamine uptake inhibitors can be prescribed for ADHD and other mental conditions, such as narcolepsy. These drugs prevent the reuptake of the neurotransmitter dopamine, which increases its concentration between synapses. Different types of drugs are capable of increasing the dopamine level, including amphetamine-like drugs, as well as cocaine. The drugs selected are amphetamine, methamphetamine, ephedrine, cocaine and MDA. Both amphetamine and methamphetamine are stimulants of the central nervous system and are mainly used for ADHD. Ephedrine is used to relieve asthma symptoms, such as shortness of breath and wheezing. Although ephedrine has amphetamine-like properties, its effect on the central nervous system is less significant than amphetamines (Munhall & Johnson, 2006). MDMA, also known as Ecstasy, is a recreational drug used for its energizing effect and perception alterations. MDMA has a similar structure as methamphetamine, and its major human metabolite is MDA. In fact, some Ecstasy tablets could be manufactured using MDA instead of MDMA as precursor (Halpern et al., 2011). For these reasons, MDA was also selected for this project.

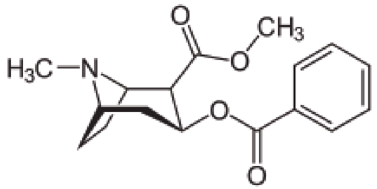
Opioids are used to treat moderate to severe pain and can be used for palliative care. They work by attaching to opioids receptors, altering the perception of pain. Due to their ability to cause dependence, they are available by prescription only, but they are used illicitly for recreational uses around the world. The opioids selected are codeine, dihydrocodeine, tramadol, oxycodone, methadone, morphine, fentanyl and ketamine. Codeine and dihydrocodeine are opioid analgesics that are used to treat mild to moderately severe pain, and their psychological effects include euphoria and anxiety suppression. Tramadol is stronger than both codeine and dihydrocodeine with similar psychological effects. Morphine is an even stronger opioid used to treat severe pain, but also causes euphoria and detachment. Both oxycodone and methadone have similar analgesic and cognitive effects as morphine, but with a longer analgesic effect and less extreme sedative effects. The opioid fentanyl is used medically as a postoperative analgesic, and it has some cognitive euphoria effects; however, to a lower degree than morphine. Ketamine is an aesthetic used before surgeries or other medical procedures, and it has several adverse cognitive effects, such as depersonalization, delirium, consciousness suppression, memory suppression and others.

The drugs of abuse chosen in this PhD project were selected based upon previous studies reporting their presence in effluent wastewaters, as well as in surface water. In particular, two recent studies have indicated the presence of some drugs of abuse in effluent wastewaters and surface water in Canada (Metcalfe et al., 2010; Rodayan et al., 2015; Rodayan et al., 2014a). Table 2.2 shows the selected drugs of abuse and summarizes their average concentrations in the effluent of a Canadian WWTP and downstream their discharge in the river. Out of the 13 studied drugs, 11 were detected in effluent wastewater and 10 were detected in different locations downstream the WWTP. Furthermore, methamphetamine and cocaine were detected frequently (i.e. >50%) in effluents of three Canadian WWTPs (Metcalfe et al., 2010).

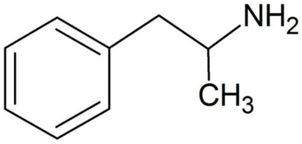
Table 2.2 Selected drugs of abuse and their human excretion percentage, concentrations in effluent wastewater and in surface water.

Subtype	Compound	CAS number	WWTP effluent Concentrations (ng/L)[1]	Surface water (ng/L)[1]	Percentage of unchanged parent compound excreted in urine)
Dopamine uptake inhibitors and their metabolites	Cocaine	50-36-2	18	5	1-9[2]
	Amphetamine	300-62-9	25	<LOD	30-54 (pH dependent)[2]
	Methamphetamine	537-46-2	<LOD	<LOD	10-23 (pH dependent)[2]
	Ephedrine	299-42-3	210	30	Almost all[2]
	MDA	4764-17-4	30	15	Almost all[2]
Opioids	Codeine	76-57-3	64, 50	84,9	10[3]
	Dihydrocodeine	125-28-0	20	10	23-68[4]
	Fentanyl	437-38-7	240	25	<7[5]
	Ketamine	6740-88-1	50	40	4[6]
	Methadone	76-99-3	<LOD	<LOD	5-50 (pH)[7]
	Morphine	57-27-2	35	5	2-12[6]
	Oxycodone	76-42-6	25	5	<19[5]
	Tramadol	27203-92-5	20	5	30[8]

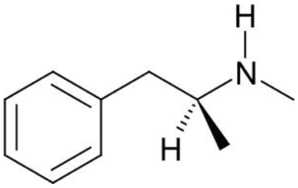
[1]Rodayan et al. (2015), [2] International Programme on Chemical Safety (1999), [3]Parke (1968), [4]Balikova et al. (2001), [5]Physicians' desk reference (2016), [6]Couper and Logan (2004), [7] Goodman et al. (1996), [8]Grond and Sablotzki (2004)



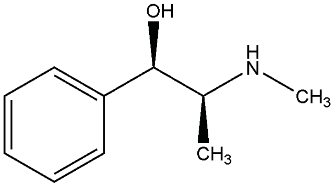
Cocaine (MW: 303.36, pK_a: 8.61, Log K_{ow}: 2.3)



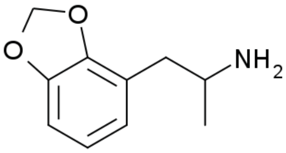
Amphetamine (MW: 135.21, pK_a: 10.1, Log K_{ow}: 1.76)



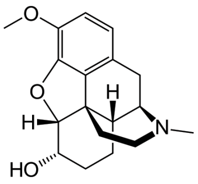
Methamphetamine (MW: 149.2, pK_a: 10.21, Log K_{ow}: 2.07),



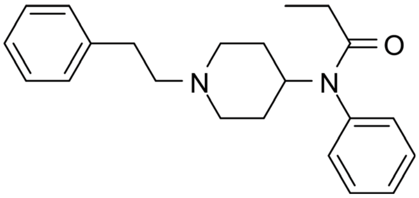
Ephedrine (MW: 165.23, pK_a: 9.65, Log K_{ow}: 1.13),



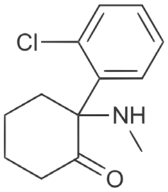
MDA (MW:179.22, pK_a: 9.67, Log K_{ow}: 1.64)



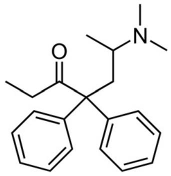
Dihydrocodeine (MW:301.38, pK_a: 8.8, Log K_{ow}: 1.49)



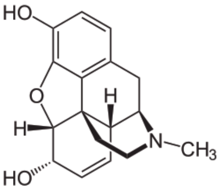
Fentanyl (MW:336.47, pK_a: 8.6, Log K_{ow}: 4.05)



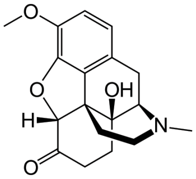
Ketamine (MW:237.73, pK_a: 7.5, Log K_{ow}: 2.18)



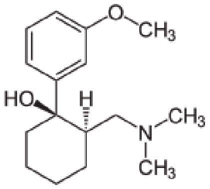
Methadone (MW:309.4, pK_a: 8.94, Log K_{ow}: 3.93)



Morphine (MW:285.34, pK_a: 9.85, Log K_{ow}: 0.89)



Oxycodone (MW:315.37, pK_a: 8.28, Log K_{ow}: 0.66)



Tramadol (MW:263.37, pK_a: 9.41, Log K_{ow}: 2.63)

Figure 2.3 Chemical structure, molecular weight (MW, g/mol) and properties of the studied drugs of abuse. pK_a and Log K_{ow} values obtained from National Center for Biotechnology Information (2004) N.A.:unavailable data.

Regarding the metabolism of drugs of abuse, only 1-9% of an ingested dose of cocaine is excreted as the parent compound, and the remaining dose is excreted as benzoylecgonine (35-54%) and ecgonine methyl ester (32-49%) (Baselt, 1984). Benzoylecgonine, the main metabolite of cocaine, has been detected in all samples (n=36) collected from effluents of three WWTPs in Canada (Metcalfe et al., 2010) at concentrations of 62-775 ng/L, and was added to this study to account for its removal in the treatment train. In fact, benzoylecgonine concentrations were 3-5 times higher than those of cocaine in several studies, as summarized in a review article by Ratola et al. (2012). On the other, hand a higher portion of the ingested dose is excreted unchanged for amphetamine-type drugs (i.e. MDA, ephedrine, amphetamine and methamphetamine) from the human body, making it not as crucial to also monitor the fate of the metabolites (Baselt, 2000; Zuccato et al., 2008). Only a small portion of the intake opioids are excreted unchanged from the human body. However, the main human metabolite of methadone, EDDP, was included in this study, due to its relatively high concentrations observed in the effluent of a WWTP in Canada (110 ng/L) (Rodayan et al., 2015), while methadone itself was not detected in the same study. In other studies, the concentration of EDDP in influent wastewater was found to be more than twice the concentration of methadone (Baker et al., 2012; Castiglioni et al., 2006b). Codeine is partially metabolized in the human body to morphine that is also a synthesized opioid included in this study (International Programme on Chemical Safety, 1999).

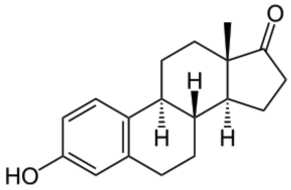
Benzoylecgonine (MW: 252.27 pK_a: N.A., Log K_{ow}: -1.32)

EDDP (2-ethylidene-1,5-dimethyl-3,3- diphenylpyrrolidine) (MW: 270.28 pK_a: 9.64, Log K_{ow}: 4.94)

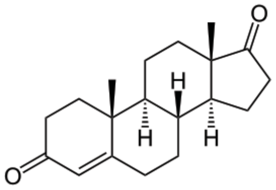
Figure 2.4 Chemical structure, molecular weight (MW, g/mol) and properties of the studied metabolite of cocaine (benzoylecgonine) and methadone (EDDP). pK_a and Log K_{ow} values obtained from National Center for Biotechnology Information (2004). N.A.:unavailable data.

2.2.3. Hormones

The two hormones monitored in this project are androstenedione and estrone. Androstenedione is a natural androgenic steroid produced in the human body by the adrenal glands, testicles and ovaries and used to produce estrogens and testesterone. Androstenedione is also used to produce tablets or sprays that are used by athletes, mostly illegally, to increase the testosterone level in the blood (Murray, 2002). Due to the limited literature investigating its fate during wastewater treatment (Esperanza et al., 2007), it was selected as a model androgen for this project. Estrone is also a natural hormone excreted by the ovaries. Estrone was found to be present in the influent to 18 municipal WWTPs at concentrations of 19–78 ng/L and in the effluent at concentrations of 1–96 ng/L (Servos et al., 2005). Figure 2.5 summarizes the main properties of these two hormones.



Estrone (MW: 270.366 pK_a: 10.3, Log K_{ow}: 3.13)



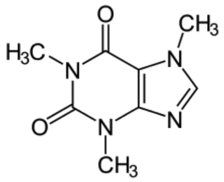
Androstenedione (MW: 286.41 pK_a: N.A., Log K_{ow}: 2.75)

Figure 2.5 Chemical structure, molecular weight (MW, g/mol) and chemical properties of estrone and androstenedione. pK_a and Log K_{ow} values obtained from National Center for Biotechnology Information (2004). N.A.:unavailable data.

2.2.4. Other target contaminants

Three other emerging contaminants that belong to different categories, namely sucralose, caffeine and triclosan were selected for this project. Sucralose is a non-nutritive artificial sweetener, mostly known as Splenda. It has been widely detected in 19 drinking water systems in the USA at concentrations of 47-2900 ng/L and 49-2400 ng/L in the source water and in the finished water, respectively and has been proposed as a tracer of wastewater contamination (Mawhinney et al., 2011). Caffeine is a widely-used central nervous stimulant found in beverages, such as coffee and tea and in some foods, such as chocolate. Caffeine was identified to be one of the potential indicator compounds of wastewater treatment efficiency and the contamination of surface water by wastewater, according to the previously discussed criteria (Dickenson et al., 2011). Triclosan is a common antiseptic agent found in household products and personal care products, such as toothpastes, soaps, detergents and many others. It was selected since it was observed to resist conventional wastewater treatment resulting in its discharge to the environment (Ricart et al., 2010). The main properties of these three compounds are presented in Figure 2.6.

Sucralose (MW: 397.64, pK$_a$: 11.8, Log K$_{ow}$: -1) **Triclosan** (MW: 289.54, pK$_a$: 7.9, Log K$_{ow}$: 4.76)



Caffeine (MW: 194.19, pK$_a$: 14, Log K$_{ow}$: -0.07)

Figure 2.6 Chemical structure, molecular weight (MW, g/mol) and chemical properties of sucralose, caffeine and triclosan. pK$_a$ and Log K$_{ow}$ values obtained from National Center for Biotechnology Information (2004), except for sucralose (Busetti et al., 2015; Subedi & Kannan, 2014a). N.A.:unavailable data.

2.3. Fate in WWTPs

It has been determined long ago that the main route of PPCPs in the environment are discharges from wastewater treatment plants (WWTPs) (Ternes et al., 2004b). WWTPs provide primary, secondary and, in some cases, tertiary (advanced) treatment through biological, physical and chemical processes. The most widely-used technology for biological treatment across the world is the activated sludge process (World Bank Group, 2016). WWTPs are designed to meet regulations on suspended matter, as well as other total organic matter and nutrients but are not specifically designed to treat CECs, considering the absence of regulations governing their discharge. Nevertheless, along with the removal of the traditional pollutants, some removal of the CECs was reported to take place in WWTPs, as summarize in the following sections.

2.3.1. CEC removal mechanisms

According to Rogers (1996), several processes are involved in the elimination of CECs from wastewater during treatment. These include degradation (biological and abiotic), as well as the two physical processes: volatilization and sorption to sludge.

Sorption occurs when a portion of the CEC gets bound to the suspended solids and subsequently settles with the sludge (Ternes et al., 2004b). Adsorption can take place due to one of the following interactions (Jelic et al., 2012; Meakins et al., 1994; Ternes et al., 2004b):

1. Interaction between the hydrophobic groups of the CEC (i.e. aliphatic or aromatic groups) with the lipophilic cell membrane of the microorganisms or the fat contained in the sludge
2. Electrostatic reactions between the positively charged groups of the CEC and the negatively charged surfaces of the microorganism
3. Other interactions: hydrogen bonding, ionic interactions and surface complexation

Due to the different processes that affect the sorption behaviour (Jelic et al., 2012), the extent of adsorption is influenced by a number of conditions and factors, including the treatment conditions and the physico-chemical properties of the CEC. A high log K_{ow} (>4) is associated with higher hydrophobicity and more adsorption to sludge through hydrophobic interactions (Thompson et al., 2011). Not only log K_{ow} of the CEC influences its adsorption, but also its acidic dissociation constant (pK_a). When the pK_a of the CEC has a value that is closer to or higher than the pH of the wastewater, the potential of the CEC to dissociate and occur in the soluble phase decreases, increasing, therefore, its potential to get attached to particulate matter (Thomas & Foster, 2005; Yang et al., 2011). A combination of high log K_{ow} and high pK_a provides suitable conditions for adsorption (Yang et al., 2011). For instance, naproxen and ibuprofen having relatively high log K_{ow} (i.e. >3, Figure 2.1), do not tend to highly adsorb to sludge due to their pK_a (i.e. <5, Figure 2.1) that is much lower than the typical pH of wastewater (i.e. 6.5-8.5) implying their presence mainly as ions in the aqueous phase (Thomas & Foster, 2005).

Biological degradation (biodegradation), which involves the consumption of organic contaminants by biological means, has been reported in literature as the main mechanism of removal for many CECs included in this study. Due to their low concentrations in wastewater (in the range of ng/L to µg/L), however, the microorganisms do not use the CECs in wastewater as a main carbon source (Heberer, 2002a; Ternes, 1998). That is, the CECs get biodegraded with and in the presence of another substrate that constitutes the primary substrate: A process that is known as co-metabolism (Ternes et al., 2004b). A higher sludge age, achieved by a higher solids retention time (SRT), was observed to increase the biodegradation of the CECs (Blair et al., 2013b; Buser et al., 1998). This is either due to a higher diversity in the population of the microorganisms in the aged sludge or to the higher diversity of the metabolic activity in response to a decreasing amount of substrate with time, as explained by Ternes (1998). The existence of nitrifying microorganisms was found to enhance the removal of CECs, due to their enhanced capability to co-metabolise compared to other types of microorganism (Luo et al., 2014). However, a poor correlation was sometimes obtained between the SRT and the CEC removals and linked to the SRT variations at a low range (Santos et al., 2009), indicating that the effect of SRT on CEC removals is not linear and depends on the targeted range. Higher HRT was often linked to higher CEC removals and interpreted by the availability of more time for CECs with slow kinetics to biodegrade or adsorb (Fernandez-Fontaina et al., 2012; Santos et al., 2009). Temperature also plays an important role in influencing the CEC removal processes due to its effect on microbial growth and activity (Hai et al., 2011; Luo et al., 2014).

Volatilization is a physical process involving the transfer of CECs from the surface of wastewater to the air. According to Stenstrom et al. (1989), the tendency of CECs to undergo volatilization is assessed empirically using the air-water partition coefficient (dimensionless Henry's Law constant, H_c) and its relation to the K_{ow}. Volatilization is deemed significant if $H_c > 10^{-4}$ and $H_c/K_{ow} > 10^{-9}$ (Rogers, 1996; Stenstrom et al., 1989). The target CECs are large molecules with low Henry's constants and are not expected to be significantly removed by volatilization (Struijs et al., 1991; Virkutyte et al., 2010).

Abiotic degradation of CECs includes means of degradation that are not biological, such as hydrolysis and photolysis. Hydrolysis in domestic wastewater is slower than other processes and is, therefore, often considered negligible (Schwarzenbach et al., 1993). Pharmaceuticals and drugs are designed for oral intake, which explains their low tendency to chemically degrade in water (Andreozzi et al., 2003). Except for lagoon WWTPs, photolysis under sunlight conditions is also not likely to contribute significantly to the removal of CECs in WWTPs, due to the low exposure to light in the highly-turbid bioreactor, as well as the generally long half life of the photolysis process in comparison to the short retention times of conventional treatment steps (Daneshvar et al., 2010; Deegan et al., 2011; Lishman et al., 2006).

2.3.2. Overall removal of CECs in WWTPs

Table 2.3 shows the range of concentrations observed for CECs in untreated wastewater worldwide. The concentrations of CECs in wastewater are highly variable, depending on the type of the wastewater and the country (Ratola et al., 2012). The majority of the investigated CECs have concentrations in the ng/L range in wastewater, while few of them, namely ibuprofen, sucralose and caffeine, have concentrations higher than 1 μg/L. Data for the occurrence of drugs of abuse in wastewater and their removal in WWTPs is much more limited than for the other CECs on the list, such as pharmaceuticals and hormones. Several studies reported the overall efficiency of WWTPs at treating CECs, as also summarized in Table 2.3.

Table 2.3 Concentrations of the target CECs in influent wastewater to WWTPs and the range of their overall removal efficiencies in WWTPs as reported by several past studies carried out in different countries.

Type and Subtype	References	Compound	Influent Concentration (ng/L)	Removal (%)
Antibiotics	*1-11*	Trimethoprim	60–6,800	<0–81.6
		Sulfamethoxazole	<3–980	4–88.9
		Acetaminophen	1,570–56,900	98.7–100
Anti-inflammatory		Codeine	1732–32,295	13-40
	1,3,4,5,7,9,11,12,13,14,20,42	Ibuprofen	<4–603,000	72–100
		Naproxen	<2–52,900	43.3–98.6
Antiepileptic	*1,2,4,5,7,9,10,11*	Carbamazepine	<40–3,780	<0–62.3
Dopamine uptake inhibitors and their metabolites	*15-35,42,51*	Cocaine	4-4700	90–100
		Benzoylecgonine	9-7500	85–95
		Amphetamine	<LOD–5,236	9–100
		Methamphetamine	<LOD–800	0–99
		Ephedrine	100-429	34–88
		MDA	<LOD–1,690	60
Opioids	*15,16,17,18,20,21,22,23,36,37,38,40,41,42*	Dihydrocodeine	<LOQ–16	-
		Fentanyl	-	-
		Ketamine	7–50	35–84
		Methadone	3.4–1,531	9–22
		EDDP		8–33
		Morphine	<LOQ–929	26–98
		Oxycodone	70–500	28
		Tramadol	8508–89,026	35–37
Hormones	*1,14,43,44,45*	Androstenedione	74–87	100
		Estrone	0.01–0.07	75–91
Others	*1,2,5,7,10,11,12,13*	Caffeine	220,000–290,000	50–100
	46,47,48,49	Sucralose	1,700–33,000	<0–45
	1,5,6,7,45,46	Triclosan	0.03–24	71–99

1: Behera et al. (2011); 2: Choi et al. (2008); 3: Gracia-Lor et al. (2012); 4: Kasprzyk-Hordern et al. (2009); 5: Loos et al. (2013); 6:Martin Ruel et al. (2010); 7:Santos et al. (2009); 8:Stamatis and Konstantinou (2013); 9:Terzic et al. (2008); 10:Zhou and Oleszkiewicz (2010); 11: Singer et al. (2010); 12:Stamatis et al. (2010); 13: Yu and Chu (2009); 14:Zorita et al. (2009); 15: Berset et al. (2010); 16:Bijlsma et al. (2012); 17:Castiglioni et al. (2006b); 18:Huerta-Fontela et al. (2008); 19: Kasprzyk-Hordern et al. (2008a); 20:Kasprzyk-Hordern et al. (2009); 21:Postigo et al. (2010); 22: Terzic et al. (2010); 23:Van Nuijs et al. (2011); 24:Yargeau et al. (2014b); 25:Metcalfe et al. (2010); 26:Loganathan et al. (2009); 27:Bones et al. (2007); 28:Gheorghe et al. (2008); 29: González-Mariño et al. (2010); 30:Hummel et al. (2006); 31:Karolak et al. (2010); 32:Kasprzyk-Hordern et al. (2008b); 33:Van Nuijs et al. (2009a); 34:Van Nuijs et al. (2009b); 35:Zuccato et al. (2005); 36:Boleda et al. (2009); 37:Martínez Bueno et al. (2009); 38:González-Mariño et al. (2012); 39:Huerta-Fontela et al. (2008); 40:Van Nuijs et al. (2009c); 41:Pedrouzo et al. (2011); 42:Rodayan et al. (2014a); 43:Janex-Habibi et al. (2009); 44:Nie et al. (2012); 45:Esperanza et al. (2007); 46:Brorström-Lunden (2008b); 47:Pasquini et al. (2013); 48:Rodayan et al. (2015); 49:Subedi and Kannan (2014a); 50:Kumar et al. (2010); 46:Pothitou and Voutsa (2008); 51:Boles and Wells (2010)

As shown in Table 2.3, while some compounds have high removals (up to 100%), others are persistent (i.e. carbamazepine, codeine, methadone and others). The removals in Table 2.3 also varied for each compound among the different studies due to the differences in the treatment trains, operating conditions and other conditions. This highlights the importance of further understanding the removal process of CECs during wastewater treatment in order to be able to predict their removal and improve their removal in WWTPs. Although these overall removals of CECs convey an important message on the efficiency of the existing WWTPs at removing CECs, they do not distinguish between the efficiency of the different treatment steps, limiting as well the understanding of the contribution of different removal mechanisms.

2.3.3. CEC removal per treatment step

Although there is abundant literature on the overall efficiency of WWTPs at removing CECs (Table 2.3), fewer studies looked at the efficacy of each treatment step separately. Evaluating the individual efficiencies is essential for determining the contribution of distinct processes playing a role in the removal and, eventually, optimizing and designing efficient treatment steps. For instance, Carballa et al. (2005) suggested that evaluating the removals of the primary treatment could provide important information for the optimization of the removal of CECs in primary settlers. Enhancing the CEC removal in primary settlers could minimize the cost of upgrading secondary and tertiary steps (Carballa et al., 2005) and is generally overlooked. Furthermore, obtaining CEC data per treatment step is essential for the calibration of fate models of CECs that provide a valuable and cost-effective tool for predicting the fate of CECs in different treatment systems.

2.3.3.1. Primary settling and activated sludge

Table 2.4 summarizes results from previous studies that investigated the removal of the studied CECs in primary settlers and activated sludge treatment, separately. Primary treatment by primary settling concentrates and removes settleable organics from the wastewater. Typically, primary settlers remove 25 to 35% of the biological oxygen demand (BOD) and 40% to 60% of the total suspended solids (TSS) (Office of Water Programs, 1980). However, poor removal of CECs (<50%) was observed in primary settler for most of the CECs included in the list of this project, as shown in Table 2.4. This could be due to the low tendency to adsorb onto primary sludge that subsequently settles in the clarifier. The low affinity of a contaminant to primary sludge can be explained by its low hydrophobicity manifested by a low log K_{ow} or a high pKa (Figure 2.1-2.6) (Behera et al., 2011). Owing to its hydrophobic nature (Winkler et al., 2007), triclosan was shown to have a positive removal during primary treatment in different studies (Table 2.4), suggesting its high adsorption on the fat-rich primary sludge. It was previously demonstrated that coagulant additives, such as ferric chloride ($FeCl_3$) and aluminum sulfate ($Al_2(SO_4)_3$) frequently added for enhanced particulate and/or phosphorus precipitation, could improve the removal of some pharmaceuticals, such as naproxen during primary clarification (Clara et al., 2005b).

Biological treatment removes BOD at higher levels than primary treatment (Spellman, 2007). Prior to 1950, activated sludge processes were designed to remove only the BOD, after which further developments in their configuration and additives were introduced to achieve nitrogen and phosphorus removal (Ternes et al., 2004b). However, activated sludge units are still not designed for the removal of CECs, also referred to as micropollutants (MPs). Unlike primary treatment, activated sludge units, for which more CEC removal studies are available, removed up to 100% of the incoming load for some of the CECs (Table 2.4). The high variations between the efficacy of the different activated sludge systems investigated in different studies could be attributed to the different operating conditions that significantly impact the biodegradation process of CECs. Other factors causing the variations could be related to the monitoring techniques, which will be discussed later in Section 2.4. In some cases, studies reported the CEC removal as a range, due to the fact that different operating conditions, mainly SRT, were investigated. As shown in the Table 2.4, some CECs, in particular carbamazepine and sucralose seem to be of recalcitrant nature during biological treatment, as reported by several studies. By contrast, ibuprofen, naproxen, benzoylecgonine, acetaminophen and caffeine exhibit consistently high removals from the aqueous phase during activated sludge treatment. Most of the studies listed in Table 2.4 did not distinguish between different mechanisms contributing to elimination of CECs from the aqueous phase (i.e. biodegradation and sorption) due to the unavailability of data concerning the CEC load sorbed onto sludge.

Table 2.4 Removal of CECs obtained in different activated sludge units and primary clarifiers, evaluated separately.

Compound	Primary clarifier (%)	References	Activated sludge (%)	References
Trimethoprim	15	*3*	70; 40; 7; -30; 53	*3; 19; 5; 16; 17*
Sulfamethoxazole	-10	*2*	65; 56; -138–60; 90; 70; 54; 74; 42	*2; 7; 5; 6; 3; 18; 16; 5; 20*
Acetaminophen	0; 22	*3; 4*	86; 99.9	*7; 16*
Codeine	4	*12*	45; 9	*6; 12*
Ibuprofen	-7; 5; 88	*2; 3; 4*	75; 70; 83; 90; 100; 99; 98	*8; 2; 7; 6; 3; 19; 20*
Naproxen	3; -10; <0	*2; 3; 4*	78; 45; 85; 80; 90; 72	*8; 2; 7; 6; 3; 19*
Carbamazepine	26; -10	*14; 3*	<10; -20; 25; <10; -11– -43	*7; 6; 3; 16; 20*
Gemfirozil	0	*3*	39; 5	*7; 16*
Cocaine	70; 0	*11; 12*	90; 40	*12; 11*
Benzoylecgonine	50; 2	*11; 12*	83; 40	*12; 11*
Amphetamine	60; 5	*11; 12*	40	*11*
Methamphetamine	-120; 3	*11; 12*	79; -20	*12; 11*
Ephedrine	9	*12*	25	*12*
Dihydrocodeine	-	-	50	*13*
Fentanyl	-	-	-	-
Ketamine	-	-	-	-
Methadone	14; -110	*11; 12*	-5	*11*
EDDP	-40; 0	*11; 12*	26	*12*
Morphine	0; 5; 25	*3; 11; 12*	95	*13*
Oxycodone	-4	*12*	28	*12*
Tramadol	21	*12*	35	*6*
Androstenedione	-8	*1*	<100	*1*
Estrone	40; - 8; -59	*2; 3; 4*	40; -49–99; -40–20; 70; 85	*2; 15; 5; 6; 3*
Caffeine	17	*3*	100	*3*
Sucralose	-	-	-40–10	*10*
Triclosan	53; 32	*9; 4*	75	*3*

1: Esperanza et al. (2007); 2: Carballa et al. (2004); 3: Behera et al. (2011); 4: Blair et al. (2013b); 5: Göbel et al. (2007); 6: Kasprzyk-Hordern et al. (2009); 7: Radjenović et al. (2007); 8: Stumpf et al. (1999); 9: Winkler et al. (2007), 10: Brorström-Lunden (2008a),11: Subedi and Kannan (2014b), 12: Rodayan et al. (2014a), 13: Wick et al. (2009); 14: Zhou et al. (2009); 15: Joss et al. (2004); 16:Lindberg et al. (2006); 17:Batt et al. (2006); 18:García-Galán et al. (2011); 19:Radjenović et al. (2009b); 20: Clara et al. (2005b)

2.3.3.2. Tertiary (Advanced) treatment

Tertiary treatment is an optional final polishing step for wastewater before discharge into the environment. Tertiary treatment units are added mostly in order to meet regulations with regards to traditional pollutants discharge and are often associated with a higher cost. Different forms of tertiary treatment for wastewater exist, such as sand filtration, membrane filtration and granular activated carbon (GAC). Disinfection is also implemented for the effluent wastewaters in order to reduce the amount of microorganisms in the discharged effluent. The main disinfection method is chlorination. Other disinfection processes implemented include ozone (O_3) and ultraviolet (UV) disinfection. Tertiary treatment provides extra removal of CECs that are persistent during biological secondary treatment. The capacity of membrane tertiary processes (micro- and ultrafiltration), as well as UV and ozone disinfection processes at treating CECs has been evaluated in several pilot- and full-scale studies (De la Cruz et al., 2012; Gerrity et al., 2011; Hernández-Leal et al., 2011; Jermann et al., 2009; Röhricht et al., 2009; Sui et al., 2010; Yangali-Quintanilla et al., 2011), but these technologies are not investigated in this PhD project. The two tertiary treatment steps evaluated in this project are rotating biological contactors and sand filtration.

The rotating biological contactors technology (RBCs) was first developed in Germany in the 1960s. Although rotating biological contactors (RBCs) are often used as the main biological treatment step considered as secondary treatment, they could be coupled to activated sludge units as a tertiary step for the reduction of BOD_5 and nitrogen. In RBCs, the biomass growth is attached to rotating discs, compared to the suspended biomass in activated sludge units. Attached growth processes are known for their simple operation and higher energy efficiency, and by 2007, around 600 RBCs plants were implemented in the US for the treatment of wastewater (Water Environment Federation, 2008). A small number of studies investigated the removal of our target CECs by full-scale RBCs (Batt et al., 2007; Gabet-Giraud et al., 2010; Kanda et al., 2003; Simonich et al., 2002; Thompson et al., 2005) and another study examined bench-scale RBCs (Vasiliadou et al., 2014). Vasiliadou et al. (2014) reported that caffeine had high removal in RBCs that ranged from 39% to 100%, with the higher removals being linked to higher HRT. Batt et al. (2007) observed reduction in concentration of sulfamethoxazole and trimethoprim of 36% and 75%, respectively in full-scale RBCs with HRT of 4 h. The removal of triclosan by RBCs was reported to be 25-30% by Thompson et al. (2005). In all of the aforementioned studies, RBCs were used as a secondary treatment process.

Tertiary sand filtration provides a simple and cost-effective means for the reduction of TSS and ammonia in secondary effluents to meet stringent regulations as it operates by retaining particulate matter. The performance of sand filters in terms of removing CECs was previously examined, and they were found to have variable efficiencies, depending on the compound and the operating conditions (Gao et al., 2012; Göbel et al., 2007; Matamoros et al., 2007; Nakada et al., 2007; Zorita et al., 2009). The pain-killers ibuprofen and naproxen were monitored in sand filters, and removals of naproxen were found to range from negative values to 50% at different seasons (HRT=0.5 h) by Nakada et al. (2007), while no removal was observed by Zorita et al. (2009) (HRT=1 h). Ibuprofen, on the other hand, had removal ranging 20-100% in the same studies (Nakada et al., 2007; Zorita et al., 2009). Nakada et al. (2007) also observed that estrone had variable removals in a sand filter (i.e. <0 – 60%), depending on the season and that triclosan was poorly removed (i.e. <50%). Carbamazepine was also observed to have poor removals (i.e. <15% with some negative removals) by sand filtration in multiple studies (Gao et al., 2012; Nakada et al., 2007; Sui et al., 2010). Considerable removal (over 60%) of trimethoprim was observed previously (Nakada et al., 2007; Sui et al., 2010), while Göbel et al. (2007) reported variable removal efficiencies (i.e. 15-74%) of trimethoprim in sand filters of different WWTPs. Sand filtration was also reported to remove 19% of the incoming sulfamethoxazole by Gao et al. (2012) and around 30% by Nakada et al. (2007), whereas Göbel et al. (2007) reported insignificant change in the loads of sulfamethoxazole when passed by sand filtration. Nakada et al. (2007) attributed the observed poor removal of CECs by sand filtration to their hydrophilic nature (manifested by a low log K_{ow}) and observed that a combination of ozonation and sand filtration enhances the system's removal of CECs. Removal in sand filters is attributed to adsorption to solid particles that are retained by the sand filter. However, previous studies also indicated that biodegradation can contribute to CEC removal due to the formation of biofilm on sand particles (Göbel et al., 2007).

2.4. Hydraulics and sampling techniques

It was previously suggested that the transport of the CECs in treatment steps is highly affected by the hydrodynamics of the treatment unit (Majewsky et al., 2011; Ort et al., 2010). Hence, a measured difference in the micropollutant load between the sampled inlet and the outlet of a full-scale treatment unit is not necessarily due to removal. It could simply mean that the incoming contaminant load has not yet reached the outlet by the time the sample was collected due to the hydraulic delay. This could result in a mismatch of measured influent and effluent loads that are used to calculate removal efficiencies. Most of the studies investigating the CEC overall removal in WWTPs and the removal per treatment step (results in Table 2.3 and Table 2.4, respectively) did not adequately account for the hydraulics of the WWTP in the removal calculations. In a review paper, Petrie et al. (2015) argued that sampling is fundamentally the most important step in obtaining high quality data on CEC but still one of the most overlooked aspects. Ort et al. (2010) showed that of 87 peer-reviewed articles examining CEC fate in WWTPs, only 10% provided justification for the sampling strategy used. Negative removals and variable removal levels compiled in Table 2.4 could be attributed to the overlooking of the hydrodynamics factor for sampling and removal calculation.

2.4.1. Composite vs grab samples

Collecting grab samples was deemed to overlook the temporal variations in concentrations. Compared to 24-h composite samples, grab samples ignore the diurnal variation in the contaminant loads that generally peaks at 7:00-9:00 and influences the match between the sampled inlet and outlets samples (Petrie et al., 2015). Hence, 24-h composite samples provide a more representative overview of the load that enters at a certain time. Table 2.5 shows the sampling technique used by the studies that investigated the CEC removal in primary and secondary treatments, separately. It could be observed from Table 2.5 that the implementation of the 24-h composite sampling strategy is becoming widespread.

2.4.2. Time-proportional vs flow proportional and frequency of sampling

Ort et al. (2010) emphasized the need for collecting flow-proportional samples in order to avoid bias of sampling due to the flow variations and showed that only 23% of 83 peer-reviewed articles on CEC removal described their collected samples as flow-proportional. This could be attributed to the generally more complex sampling instruments required for flow-proportional samples. From Table 2.4, out of the 11 studies reporting removal per treatment step, three studies collected time-proportional samples, while eight studies collected flow-proportional samples, and one study collected both types of samples. The frequency of the sample collection was also suggested to play an important role in the quality of the sample and the degree it represents reality. In a proposed sampling guide, Ort et al. (2010) established rules of thumbs to estimate the minimum required frequency of the sampling in order to obtain representative samples, depending on the target CECs and their consumption information. In general, higher frequency is associated with a higher resolution of the sampling; hence, continuous sampling was claimed to be the ideal precautionary sampling mode (Ort et al., 2010). However, several issues arise when implementing continuous sampling, in particular with regards to the sampler tubes that are normally purged from one time to another to avoid clogging, as well as storage capacity issues;

hence, only 1% of 83 reviewed articles used continuous sampling (Ort et al., 2010). Therefore, a compromise needs to be established between the operational feasibility and the high frequency of sampling.

2.4.3. HRT and RTD

As seen in Table 2.5, three out of 11 of the studies lagged their effluent sampling by a time period equivalent to the HRT of the treatment unit investigated to account for the retention time. Petrie et al. (2015) suggested that the approach of temporally shifting the effluent sampling by the HRT is impractical for systems with a higher HRT, such as activated sludge (normally HRT >6). In fact, accounting for the HRT solely assumes plug flow characteristic, which is not necessarily the case in units with a high degree of mixing. Hence, not only accounting for the HRT is essential in CEC fate studies but also taking into consideration the unequal distribution of residence time between fluid elements due to mixing, as suggested by Majewsky et al. (2011). To reduce the measurement uncertainty due to short sampling periods that do not account for the residence time distribution (RTD), prolonged sampling campaigns are required to cover most of incoming contaminant load, which often implies higher cost for sampling and analysis. An approach combining prior hydraulic modelling and defined sampling strategy was developed by Majewsky et al. (2011) to account for the distribution of the residence time in CEC fate studies and that aims at achieving a compromise between cost minimization and error reduction by the determination of the necessary number of sampling days. Once the hydraulic model of the treatment unit has been defined (further discussed in section 2.7.1), it can be used to obtain load fractions of the influent that describe the load exiting on a specific day. These fractions are used to calculate a hypothetical output load that would leave the treatment unit on a specific day in the case of no biodegradation as fractions of input contaminant load of each previous day (also called fractionated load), as illustrated in Figure 2.7. For example, on the fourth day of sampling the effluent in Figure 2.7 contains 64% of the CEC load entering on the same day, 31% of the load entering the previous day and 0.7% of the load entering two days ago, assuming no biodegradation. Comparing this load with the actual effluent loads gives a reliable insight of the contaminant load lost in the system due to biodegradation without the bias of hydraulic effect. In only one study in Table 2.4 this method was applied to account for RTD in calculating CEC removals in activated sludge (Rodayan et al., 2014a).

Obtaining reliable measured data on the fate of CECs is essential for the calibration of mathematical models describing their fate, as emphasized by Ort et al. (2010). Alternative sampling techniques, not discussed above, include passive sampling techniques that have several shortcomings, and developments are still needed to investigate the uptake of CECs by passive samplers (Mills et al., 2014). Passive sampling techniques; however, are not used in this thesis.

Table 2.5 Sampling techniques used for the studies shown in Table 2.4, including the type of sample (grab or 24-h composite, flow-proportional or time-proportional), the consideration of residence time distribution (RTD) or hydraulic residence time (HRT) and the inclusion of sludge samples in the analysis.

Studies on CEC removals in primary, secondary, separately	Grab or composite	Flow- or time-proportional	RTD/ HRT considered	Sludge collected
Esperanza et al. (2007)	N.A	N.A.	No	Yes
Carballa et al. (2004)	24-h	Time	HRT	No
Behera et al. (2011)	Grab	-	No	No
Blair et al. (2013b)	24-h	Flow and Time	No	No
Göbel et al. (2007)	24-h	Flow	No	Yes
Kasprzyk-Hordern et al. (2009)	24-h and grab	N.A.	No	No
Radjenović et al. (2007)	24-h	Time	HRT	No
Stumpf et al. (1999)	24-h	N.A.	No	No
Winkler et al. (2007)	N.A.	N.A.	N.A.	Yes
Brorström-Lunden (2008a)	24-h	Flow	Yes	No
Subedi and Kannan (2014b)	24-h	N.A.	No	Yes
Rodayan et al. (2014a)	24-h	Flow	RTD	No
Wick et al. (2009)	24-h	N.A.	HRT	Yes
Zhou et al. (2009)	N.A.	N.A.	N.A.	No
Joss et al. (2004)	24-h	Flow	No	No
Lindberg et al. (2006)	24-h	Flow	No	Yes
Batt et al. (2006)	24-h	Flow	No	No
García-Galán et al. (2011)	24-h	Flow	No	No
Radjenović et al. (2009b)	24-h	Flow	No	Yes
Clara et al. (2005b)	24-h	Time	No	No

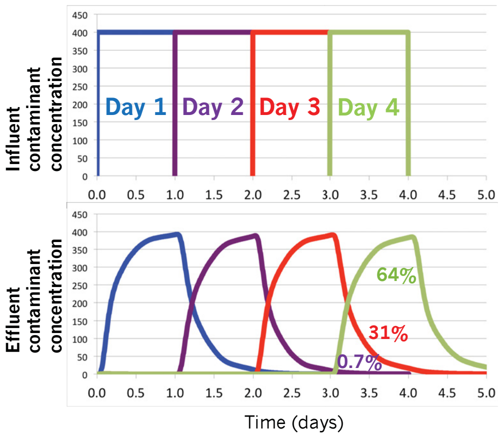


Figure 2.7 Illustration of the concept of the residence time distribution and its impact on sampling strategies.

2.5. Fate pathways

2.5.1. Sludge and K_d values

The majority of studies investigated the fate of CECs by analyzing aqueous samples from the input and the output of units and overlooking the contaminant load that might be sorbed onto suspended particulate matter in the streams. In 2009, Miège et al. (2009) reviewed 117 peer-reviewed articles examining CEC fate in WWTPs and found that only 15 of these reported CEC concentrations in sludge and only one considered the CEC concentration in both aqueous and particulate phase for removal calculations. Table 2.4 and Table 2.5 also confirm that most of the studies analyzing CEC removal per treatment steps did not consider the sludge samples. Petrie et al. (2015) attributed the scarcity of studies considering CEC loads in biosolids to the fact that it is experimentally demanding to analyze for CECs in biosolids. This is due to the large sample volumes required and the multiple extraction methods involved (Baker & Kasprzyk-Hordern, 2011; Edwards et al., 2009; Radjenović et al., 2009a).

As discussed earlier, adsorption is one important fate pathway of some CECs during treatment. Ignoring the adsorption behaviour of CECs when investigating their removal in WWTPs leads to bias due to a number of factors. Firstly, the concentrations of pharmaceuticals in the particulate matter could be of significance and have values as high as 26-296 ng/L (Petrie et al., 2014). In the same study by Petrie et al. (2014), it was found that triclosan load within the suspended matter in the secondary effluent comprised >20% of its total load, despite the low total suspended solids (TSS) concentration in that stream. Hence, calculations of CEC removal using loads based solely on the aqueous phase could lead to erroneous results. This is explained by the fact that the CEC removal from the aqueous phase does not necessarily indicate the CEC was transformed and will not be discharged into the environment, but it could also suggest that the CEC was transported to the particulate compartment. Hence, ignoring the potential transfer of CECs to sludge might result in underestimation of the risk of discharge and reuse of this residue of WWTPs. Before discharge, the generated sludge is treated, after which it is often used for agricultural purposes. To date, there is no legislation controlling the CEC concentrations in treated sludge that is applied to agricultural lands (Petrie et al., 2015). In fact, triclosan was shown to persist in biosolids-amended soils for up to eight months at concentrations of 800-1000 ng/g, after which its transformation product methyl-triclosan is formed and was detected (Butler et al., 2012). The partitioning behaviour of CECs onto biosolids can be quantified from CEC measurements by calculating the distribution coefficient ($\mathrm{K_d}$) that is the ratio between the CEC concentration in the particulate and aqueous phases at equilibrium. Table 2.6 lists the previously obtained log $\mathrm{K_d}$ values for the target CECs whose adsorption was studied in both primary and secondary sludge.

Table 2.6 $\boldsymbol{Log\ K_d}$ values reported in the literature for the target CECs for primary and waste secondary sludge.

Compound	Primary sludge	Secondary sludge
Acetaminophen	2.77[10]	1.26[10]
Trimethoprim	3[3]; 2.63[4]; 2.59[14]	<2[3], 2.4[4]
Sulfamethoxazole	0.5[4]; 2.31[10]	2[3], 1.89[4]; 2.21[10]
Ibuprofen	1.66-2.33[5]; 0.98[4]; 1.51[10]; 0.99-1.16[12]	1.82-3.01[5]; 2.55[1]; 1.17[10]
Naproxen	0.97-1.33[5]; 1.38[10]	1.21-1.59[5]; 2.34[10]
Carbamazepine	1.6-2.34[5]; 2.5[4]; 1.73[10]	3.24-3.66[5], 2.13[4]; 1.23[6]; 2.01[10]
Cocaine	1.53[7]; 2.65[11]	2.45[11]
Benzoylecgonine	0.85[7]; 1.74[11]	2.04[11]
Amphetamine	3.09[7]	-
Methamphetamine	-	-
EDDP	2.55[7]; 3.08[11]	2.80[11]
Ephedrine	-	-
Codeine	2.21[11]	1.95[9]; 1.08[6]; 2.54[11]
Dihydrocodeine	-	1.08[6]
Methadone	2.76[7]; 2.65[11]	1.88[6]; 2.86[11]
Morphine	1.94[7]	1.08[6]
Oxycodone	-	1.15[6]
Tramadol	2.04[14]	1.67[6]
Ketamine	-	-
Fentanyl	-	-
Androstenedione	2.19[10]	2.24[10]
Estrone	2.77[1]; 2.63[10]	2.5[2], 2.90[1]; 2.80[10]
Caffeine	1.75-3.08[5]; 1.48[10]	1.48[10]
Sucralose	2.88[8]	1.98[8]
Triclosan	3.25[13]	3.1[3]

References: [1]Carballa et al. (2007), [2] Nie et al. (2012), [3]Okuda et al. (2009),[4]Radjenović et al. (2009b), [5]Martín et al. (2012), [6]Wick et al. (2009), [7]Subedi and Kannan (2014b), [8]Tran et al. (2015), [9]Wick et al. (2011), [10]Berthod et al. (2016), [11]Senta et al. (2013), [12]Yan et al. (2014), [13]Stasinakis et al. (2013), [14]Hörsing et al. (2011)

In alignment with the data compiled in Table 2.6, Ternes et al. (2004a) demonstrated that pharmaceuticals have different K_d in primary and activated sludge of a WWTP and attributed these differences to the variable sludge composition and pH that affect the adsorption process (Section 2.3.1). Table 2.6 shows a log K_d range of 0.5–3.08 in primary sludge and <2–3.66 in secondary sludge for the CECs included in the present PhD project. It is important to note that adsorption, presented as log K_d values in Table 2.6, is not necessarily proportional to the log $\mathrm{K_{ow}}$ values of Figure (2.1-2.6), further emphasizing that several different factors play a role in influencing the adsorption behaviour. The table demonstrates the data gap in log K_d values of CECs, especially for drugs of abuse (Chiaia et al., 2008; Subedi & Kannan, 2014b).

2.5.2. Mass balances on treatment units

Quantifying CECs in the particulate phase allows for a more detailed diagnosis of the CEC removal because concentrations in the particulate phase provide the missing data for a complete mass balance required to quantify the contribution of the two main removal pathways (i.e. sorption and degradation) (Petrie et al., 2015). In most of the previous studies undertaking CEC mass balancing in activated sludge, the difference between the incoming load and the combined outgoing load (i.e. discharged with effluent and sorbed to sludge) was assumed to be equivalent to the load lost due to biodegradation (Clara et al., 2005a; Göbel et al., 2007; Jelic et al., 2011; Joss et al., 2005; Petrie et al., 2014; Suarez et al., 2010). Results for these mass balances of target CECs in activated sludge are shown in Table 2.7.

The mass balance results in Table 2.7 reflect the percentage of the incoming load that is observed in each fate compartment (i.e. effluent, sludge or biodegraded). Based on several studies listed in Table 2.7, it is evident that biodegradation is the dominant route of removal in activated sludge for the target CECs of the present project. Some CECs, such as caffeine, ibuprofen and acetaminophen, were constantly reported to be readily biodegrade at an efficiency of >90% under different SRT conditions, as shown in Table 2.7. Unlike other CECs on the list, triclosan and trimethoprim showed a higher potential to sorb onto sludge (Table 2.7 and 2.6). For carbamazepine, the biodegraded fraction was repeatedly low <40%, indicating its resistance to microbial biodegradation in activated sludge. The load in the effluent was either equal to or above 100% and the biodegraded load was <0% in some cases. The explanation for the observed output load being >100% of input load will be further elaborated on in the next section. As drugs of abuse are not often monitored in sludge, information labout their fate pathways during wastewater treatment in the literature is scarce and so will be investigated in the present project. These data of fate pathways were also often based on samples that were collected without accounting for the RTD and in some cases the HRT of the WWTP, increasing the bias of the results, which is one of the aspects addressed in this project.

Table 2.7 Contribution of the different fate pathways: sorption and transformation or discharge in the effluent (%) for target CECs in activated sludge processes with different SRTs.

Compound	Sludge age (days)	Transformation (%)	Sorption (%)	Effluent (%)	Ref
Acetaminophen	-	>99	<0.1	<0.1	*6*
	30	98	<1	1	*7*
Trimethoprim	<50	–90	<5	-10	*5*
	<20	<10	<5	>90	*5*
	6	40	<5	<60	*4*
	16	38–40	5–10	50–55	*4*
	<20	18	-	72	*3*
Sulfamethoxazole	4–12	50–90	<5	10–50	*5*
	<20	20	0	80	*3*
	-	63	<0.1	37	*6*
Ibuprofen	4–60	90–100	<5	0–10	*1*
	2	<5	<5	95–100	*2*
	10–55	95–100	<5	0–5	*2*
	<20	35–40	0	60–65	*3*
	30	91	<1	3	*7*
Naproxen	10–30	55–58	<5	15–45	*1*
	6	77	0	23	*4*
	16	95–98	0	<5	*4*
	<20	5	0	95	*3*
	>50	85–90	-	10–15	*3*
	30	66	<1	10	*7*
Carbamazepine	4–60	<40	<5	>60	*1*
	6	22	3	75	*4*
	16	-	5	95	*4*
	-	<0	<0.1	>100	*6*
	30	<0	<1	>100	*7*
Estrone	10–30	35–97	<5	5–60	*1*
Caffeine	-	>99	<0.1	<0.1	*6*
Triclosan	30	44	3	53	*7*

References: 1:Joss et al. (2005); 2:Clara et al. (2005b); 3:Suarez et al. (2010); 4:Jelic et al. (2011); 5:Göbel et al. (2007); 6:Gao et al. (2012); 7:Petrie et al. (2014);

2.6. Metabolite in fate studies

Some of the CEC removal studies (including most studies in Table 2.3 and Table 2.4) focused on the parent compound without considering the removal of the metabolites, which is mainly due to the lack of commercial availability of analytical standards for these metabolites, as well as the distinctive analytical methods required for quantifying these metabolites (Heidler & Halden, 2008; Petrie et al., 2015). In a review, Farré et al. (2012) stated that more research is needed to determine the fate of metabolites, as well as to develop analytical techniques capable of detecting both parent compounds and their metabolites, simultaneously. A number of other studies investigated the removal of the metabolites of our target drugs of abuse and pharmaceuticals in WWTPs (Bijlsma et al., 2012; Bones et al., 2007; Huerta-Fontela et al., 2008; Kasprzyk-Hordern et al., 2009; Metcalfe et al., 2010; Miao et al., 2005; Nefau et al., 2013; Postigo et al., 2010; Rodayan et al., 2014a; Subedi & Kannan, 2014b; Terzic et al., 2010; Terzic et al., 2008; Yargeau et al., 2014b), and in many cases, different removal efficiencies were observed for the CEC and the metabolite. As shown in Table 2.4, the main metabolite of cocaine (i.e. benzoylecgonine) seems to have similar removal as cocaine in each of the studies, whereas no correlation was observed between the trends of methadone and its major metabolite (EDDP). No results were included in Table 2.4 on the removal of carbamazepine metabolites investigated in this project, due to the lack of data from the literature.

Metabolites have potential tendency to transform back during wastewater treatment forming the parent compound, which could result in low or negative misrepresentative removal efficiency values (Miao et al., 2002). Some negative removals presented in Table 2.3 and Table 2.4 have been, hypothetically, attributed to these back-transformations. To explore this hypothesis, the fate of metabolites needs to be monitored, along with the fate of the parent compound. In the present project, some major metabolites were selected. Due to the aforementioned limitations, not all metabolites were monitored.

2.7. Fate modelling of micropollutants (MPs)

With the continual development of new pharmaceuticals and the growing public awareness of the risks associated with their presence in the aquatic environment, predicting the load of CECs discharged into water bodies constitutes a growing necessity. Some models have been developed to estimate the discharge of pharmaceuticals into the environment as a risk-analysis and decision-making tool. For instance, Zhang and Geissen (2010) estimated the load of carbamazepine in the effluents of 68 countries and Ort et al. (2009) predicted the concentration of 12 compounds in rivers downstream 742 WWTPs in Switzerland. These models involve predicting the amount of CECs present in sewage first and then the amount discharged from WWTPs. The input to these simplistic models includes data on the regional sale volumes of the CEC, consumption rate, metabolism and in case of pharmaceuticals, excretion and disposal rates. In these models, the removal efficiency of CECs in sewage pipes and WWTPs is required to obtain the discharge load of CECs. This value was taken as the average of removal efficiencies reported previously in the literature (Ort et al., 2009; Zhang & Geissen, 2010). These models provide a feasible tool for the assessment of CEC discharge; however, they contain many uncertainties. One of these is failing to account for the distinct capabilities of individual treatment trains at removing CECs, as well as the effect of operation conditions and seasonal variations in each WWTP. This underlines the need for models that could provide accurate predictions of the fate of CECs in WWTPs to partially eliminate the uncertainty associated with these models. In the next subsections, prerequisites for implementing micropollutant fate models (i.e. hydraulic and conventional pollutant modelling) are discussed, followed by the developed fate models, mathematical equations of the main mechanistic processes and current model applications and limitations. The micropollutant fate modelling in this PhD project focused on the activated sludge process, due to its widespread use and the availability of some models from the literature as compared to other treatment steps.

2.7.1. Hydraulic and conventional pollutant modelling

Prior to predicting the fate of micropollutants in activated sludge, the conventional pollutants must be well predicted, as they influence the processes that determine the fate of CECs. For instance, the amount of biomass available affects the biodegradation process of CECs, and the amount of solids affects their adsorption. There are several main steps in the modelling of conventional pollutants in activated sludge, as illustrated by previous studies (Coen et al., 1996; Hulsbeek et al., 2002; Petersen et al., 2003), including the definition of the objective of the modelling exercise, the selection of a suitable activated sludge model, the identification of a hydraulic model, collection of required data and finally, the calibration of the model parameters to fit measurements.

The definition of the objective of the modelling is essential in deciding the level of complexity and tolerated error. The three main objectives are learning or research, design and optimization (Gernaey et al., 2004). The modelling of conventional pollutants in the case of this PhD project is said to have a “service role”, that is once solved it leads to numerical values that are used for different analysis, as explained by Russel et al. (2002). The ultimate goal of the analysis is the prediction of the fate of micropollutants, but in order perform this, values of conventional pollutants must be well predicted.

Accurate hydraulic representation of the activated sludge process is necessary since errors in the hydraulic modelling of the activated sludge process could result in incorrect calibrated parameters that significantly deviate from the default parameters (Hulsbeek et al., 2002). That is because the hydraulics influences the transport of the pollutants in the activated sludge process, which could bias the output of the model, altering therefore the fit of the different parameters. Hydraulic modelling has been established decades ago due to its various applications in chemical engineering (Levenspiel, 1972). The application of hydraulic modelling in WWTPs was practiced many years ago, mostly for the modelling of conventional pollutants (De Clercq et al., 1999; Gujer et al., 1999). Tracer tests used for the investigation of the hydrodynamics in full-scale WWTPs are labour-intensive and the substance used as a tracer might pose a hazard to the environment (Ahnert et al., 2010). Therefore, Ahnert et al. (2010) demonstrated that alternative measurements, such as the temperature of the wastewater that is often monitored online in WWTPs has the capability to inform about the hydrodynamics, yielding results that are consistent with conventional tracer tests. Electrical conductivity too could be used to describe the hydrodynamics of WWTPs (Ahnert et al., 2010) but is not routinely measured and requires additional measurements to be collected.

Regarding the step involving the selection of an activated sludge model, several models have been developed more than two decades ago (Gernaey et al., 2004). The well-known activated sludge model No. 1 (ASM1) was developed by Henze et al. (1987) in order to predict organic carbon and nitrogen removal, oxygen consumption and sludge production in activated sludge processes. The ASM3 model developed later (Gujer et al., 1999) has the same objectives as the ASM1, except for it models the storage of readily biodegradable substrate in the internal cell structure of heterotrophs. The ASM3 shows a better performance for industrial wastewater or in activated sludge processes that have non-aerated zones (Gernaey et al., 2004). The ASM2 model was also developed to further extend the capabilities of the ASM1 model by predicting the biological phosphorus removal as well (Henze et al., 1995). The ASM2d model is a minor extension of the ASM2 model that includes additional processes to take into account the fact that phosphorus accumulating organisms can use cell internal organic storage products for denitrification (Henze et al., 1999). Selecting the most suitable model to implement is dependent upon the goal of the application.

Regarding calibration, multiple systematic protocols for the calibration of activated sludge models have been developed in the early 2000's, such as the STOWA, BIOMATH, WERF and HSG protocols (Hulsbeek et al., 2002; Langergraber et al., 2004; Melcer et al., 2003; Roeleveld & Van Loosdrecht, 2002; Vanrolleghem et al., 2003). Among the four protocols listed above, the Dutch foundation for applied water research (STOWA) protocol is particularly simple and cost effective (Sin et al., 2005). In addition to calibration of model parameters, these protocols included instructions for the characterization of wastewater. Wastewater characterization involves using measurements of influent wastewater composition to define the model input as fractions of the model components. This is an essential step in the modelling of activated sludge units, as the influent significantly impacts the output of the model. Apart from the four protocols listed above, methods for influent characterization were provided in the previous studies introducing the ASM models (Gujer et al., 1999; Henze et al., 1987; Henze et al., 1995; Henze et al., 1999).

Several simulation softwares exist to simulate the behaviour of a WWTP with defined configuration and input using the mathematical models described above. Some of these simulators are general-purpose simulators, such as MATLAB software, whereas others are specifically designed for WWTP modelling with libraries that include developed models and common process units. Examples of these include GPS-X, WEST, SIMBA, AQUASIM, BioWin, SIMBA and STOAT. A detailed overview of different simulation environments was previously provided by Olsson and Newell (1999).

2.7.2. Developed MP models

Many years ago, Mackay (1979) developed a mathematical model to predict the fate of CECs in the environment and treatment systems using a basic concept of thermodynamics, fugacity. This model hypothetically divides the environment into different compartments in which CECs are present and assumes that all these compartments are at equilibrium. Following the introduction of the fugacity-based fate MP models, Struijs et al. (1991) applied the concept to primary settling and activated sludge systems that were divided into nine compartments for MP estimation. This spread-sheet based model required minimum input and it was mainly applied for volatile organic compounds. Due to the equilibrium assumption, fugacity models ignore the impact of the incoming natural biological and physical disturbances to the environmental compartments (Mackay, 1979).

Shortly after the development of the equilibrium-based MP fate models, other more mechanistic MP fate models were developed to explicitly account for the different processes involved in describing the fate of MPs in treatment units. The mechanistic models were first introduced by Cowan et al. (1993), who proposed new mechanistic algorithms for predicting the fate of surfactants in primary and activated sludge treatment steps. These algorithms used biodegradation rate constant, the Henry's constant and the partition coefficient of the MP between the aqueous and particulate phases. Later, another similar model (TOXCHEM) was developed for the description of metal transport in primary and activated sludge treatment systems and validated by dynamic data from full-scale WWTPs (Monteith et al., 1993; Parker et al., 1994). The TOXCHEM model included a separate model for the secondary clarifier where complete mixing in terms of MP concentration was assumed in order to take into account the processes of sorption and volatilization that occur in the secondary settling (Parker et al., 1994). Later, the TOXCHEM model was further extended to describe the fate of VOCs and pesticides (Melcer et al., 1994; Monteith et al., 1995). More fate models were later formulated based on the same concepts and calibrated to estimate the removal of different types of micropollutants in WWTPs, mostly metals, VOCs, pesticides and PHAs (Byrns, 2001; Dionisi et al., 2006; Kemp et al., 2002; Lindblom et al., 2009; Rittmann et al., 2003; Wang et al., 1999), as well as other MPs such as pentachlorophenol (Jacobsen & Arvin, 1996). Some of the developed mechanistic fate models were based upon steady state in order to model the permanent output concentration of micropollutants without considering temporal variations (Byrns, 2001; Cowan et al., 1993; Parker et al., 1994). However, the majority allowed dynamic simulations for the prediction of the temporal changes in the output micropollutant concentration mainly due to dynamic variations in their input concentrations (Jacobsen & Arvin, 1996; Kemp et al., 2002; Lindblom et al., 2009; Melcer et al., 1994; Monteith et al., 1995; Plósz et al., 2010; Rittmann et al., 2003; Urase & Kikuta, 2005).

With the introduction and development of the wide-spread ASM models (Henze et al., 1987; Henze et al., 2000), it became an easier task to integrate the mechanistic equations of the MP removal processes into these activated sludge models. This permitted the simultaneous dynamic prediction of bulk pollutants, including biomass growth and solids with the micropollutant prediction. The ASM1 was previously extended to include the fate of different MPs in activated sludge, namely bisphenol-A and pharmaceuticals (Lindblom et al., 2009; Plósz et al., 2010). More recently, Vezzaro et al. (2014) has developed another ASM-based MP model as a part of the integrated Urban Wastewater and Stormwater System (IUWS) modelbase, developed under the Source Control Options for Reducing Emissions of Priority Pollutants (ScorePP) project. Unlike the model developed by Plósz et al. (2010), the Activated Sludge Model No. 2d (ASM2dmodTemp) was selected as a basis for this model as it was found to provide an optimum compromise between the goodness of fit and the complexity of the model (Vezzaro et al., 2009). Further, unlike the ASM2d, the ASM2dTemp, used as a basis, includes the temperature correction factors for kinetic equations, which allows considering the effect of temperature in the MP process equations. The MP processes added to the ASM model included sorption, desorption, aerobic and anoxic biodegradation, hydrolosis and volatilization. Although the IUWS modelbase was applied for MP fate prediction in stormwater detention ponds multiple times (Vezzaro et al., 2011a; Vezzaro & Mikkelsen, 2012; Vezzaro et al., 2011b), it was applied for a WWTP only once by Cloutier et al. (2012) to model the fate of a hormone, a plasticizer and a volatile organic compound but without calibration with measurements.

Although the majority of the MP fate models focused on investigating the fate of metals, surfactants, VOC, pesticides, PHAs, fewer models have also considered the fate and transport of pharmaceuticals and hormones. Kikuta and Urase (2002) introduced a two-phase mechanistic model to estimate biodegradation and sorption of estrogens in order to monitor the change in their concentrations in both aqueous and particulate compartments. Later, the model was calibrated and verified with batch experiments to describe the removal of hormones and 10 pharmaceuticals (Urase & Kikuta, 2005). Another model, based on ASM1 as discussed earlier, was developed and calibrated by batch experiments for a suite of three antibiotics and was validated by data from full-scale WWTPs (Plósz et al., 2010). This model was further used for other pharmaceuticals namely carbamazepine and diclofenac (Plósz et al., 2012). Past studies attempted to model the fate of a number of the CECs under study in the current PhD project, namely trimethoprim, sulfamethoxazole, acetaminophen, ibuprofen, naproxen, carbamazepine, dihydrocodeine, methadone, morphine, oxycodone, tramadol and estrone (Abegglen et al., 2009; Cloutier et al., 2012; Plósz et al., 2012; Urase & Kikuta, 2005).

2.7.3. Mechanistic processes

Mechanistic fate models for micropollutants include a set of mathematical equations that correspond to the main removal mechanisms, described in Section 2.3.1. However, the type of kinetics utilized could vary from one study to another.

Biodegradation of micropollutants in activated sludge treatment occurs by co-metabolism where the bulk organic matter constitutes the main substrate. The rate is anticipated to be influenced by the concentration of the active biomass that is capable of biodegrading the micropollutant. However, since it is not possible to measure this experimentally, and in some cases it is not predicted by the model, it is taken to be equivalent to mixed liquor suspended solids (MLSS) or the mixed liquor volatile suspended solids (MLVSS) in the aeration tanks (Byrns, 2001; Dionisi et al., 2006). The kinetics of the biodegradation influencing the soluble micropollutant concentration (S_{MP}) was assumed to be first order with respect to substrate in some studies (Boeije et al., 1998; Byrns, 2001), as formulated in Eq. 2.1 (Cowan et al., 1993; Govind et al., 1991; Melcer et al., 1994; Monteith et al., 1995), below:

$$\frac{dS_{MP}}{dt} = -k_{bio} * S_{MP} * X_{active} \tag{2.1}$$

Where k_{bio} is the first-order biodegradation kinetic rate constant and X_{active} is the concentrations of the active biomass. Since this kinetic equation is the one implemented in this PhD project, the k_{bio} values of different micropollutants is presented in Table 2.8. According to Suarez et al. (2010), numerical values of k_{bio} higher than 1 L/gSS.d correspond to high degradation potential, while values in the range 0.5-1 L/gSS.d correspond to moderate biodegradation and <0.5 L/gSS.d to low sorption potential. These k_{bio} values do not only depend on the degradability of the micropollutant but also on a number of characteristics of the biomass of each study, such as the fraction of the active biomass and the level of its biodiversity, as well as the floc size of the sludge (Ternes & Joss, 2006). The nitrifying conditions, such as the amount of biomass with high nitrifying activity and the nitrogen load, were also found to influence the biodegradation rate constants of MPs (Huerta-Fontela et al., 2008).

Some other studies assumed pseudo first-order kinetics (Eq. 2.2) where the k_{bio} and the X_{SS} are merged as the pseudo rate constant $\acute{k}_{bio}$ (Cowan et al., 1993; Melcer et al., 1994; Monteith et al., 1995). This is believed to be a fairly acceptable assumption when the active biomass concentration remains almost constant (Ternes & Joss, 2006).

$$\frac{dS_{MP}}{dt} = -\acute{k}_{bio} * S_{MP} \qquad (2.2)$$

In other cases, biotransformation of micropollutants was assumed to follow Monod-type kinetics, as in the expression of Eq. 2.3 (Lindblom et al., 2009; Plósz et al., 2010; Siegrist et al., 1989).

$$\frac{dS_{MP}}{dt} = \frac{1}{Y_{MP}} * \mu_{max,MP} * \frac{S_{MP}}{K_{MP}+S_{MP}} * \frac{S_O}{K_{O,MP}+S_O} * X_{active} \qquad (2.3)$$

Where Y_{MP} is the conversion yield, $\mu_{max,MP}$ is the maximum growth rate, S_O is the oxygen concentration and K_{MP} and $K_{O,MP}$ are the micropollutant and oxygen half saturation coefficients, respectively. Due to the additional parameters included in the Monod-type kinetic equation, accounting for the influence of operating conditions, such as oxygen, phosphorus and nitrogen on the biodegradation kinetics is possible. In addition, the kinetics of the reaction from the Monod-type kinetics varies depending on the concentration of the micropollutant. Pomiès et al. (2013) suggested that more research is needed to identify the most representative kinetic formulation for the different micropollutants in activated sludge treatment. Most studies did not adopt the Monod-type kinetics due to the extra parameters required to define the kinetics and the fact that at considerably low concentrations of the micropollutant (i.e. lower than the half saturation coefficient of the Monod equation), the Monod-type kinetic simplifies to first order kinetics. The equations of biodegradation assume that biodegradation takes place in the soluble compartment, which is an assumption that is commonly made (Lindblom et al., 2009; Melcer et al., 1994; Monteith et al., 1995; Plósz et al., 2010; Rittmann et al., 2003; Struijs et al., 1991). One justification concerning that was provided by Rittmann et al. (2001) in a study where it was experimentally proven that once the MP adsorb to sludge, it becomes non biodegradable. Other studies examined the biodegradation in the particulate compartment and reported high biodegradation of some MPs (not included in the current project) in the particulate compartment (Cowan et al., 1993; Lee et al., 1998). Urase and Kikuta (2005) also demonstrated that some pharmaceuticals, such as carbamazepine, biodegrade to some extent in the particulate phase.

Table 2.8 Values of $\boldsymbol{k_{bio}}$ of the some target CECs studied in the literature.

Compound	k_{bio} (L/gSS.d)
Acetaminophen	58–80[6]
Trimethoprim	0.05–0.22[1,3,4]
Sulfamethoxazole	0.19–7.6[1,2,4,6]
Ibuprofen	1.33–38[1,3,4,6]
Naproxen	0.08–4.2[1,3,4,6]
Carbamazepine	<0.005–5[1,2,3,4,5]
Codeine	4.8[5]
Dihydrocodeine	1.8[5]
Methadone	0.12–0.24[5]
Morphine	11.1–16.9[5]
Oxycodone	0.06–0.36[5]
Tramadol	<0.13[5]
Estrone	137–187[1,8]
Triclosan	30–288[9]

[1]Abegglen et al. (2009); [2]Plósz et al. (2012); [3]Fernandez-Fontaina et al. (2013) [4]Suarez et al. (2010); [5]Wick et al. (2009); [6]Joss et al. (2006) [7]Xue et al. (2010); [8]Joss et al. (2004); [9]Stasinakis et al. (2010)

Adsorption and desorption are also major fate pathways for micropollutants during wastewater treatment that take place in the particulate and aqueous compartments, simultaneously. The effect of the kinetic sorption/desorption on the soluble micropollutant concentration (S_{MP}) is often described by the expression in Eq. 2.4 (Urase & Kikuta, 2005), where k_{sor} and k_{des} represent the sorption and desorption kinetic rate constant, respectively. Values for k_{sor} for the target MPs from the literature are limited since equilibrium conditions were assumed in most MP models supposing that sorption happens instantaneously and equilibrium is reached rapidly. Only eight out of the 18 studies models by Pomiès et al. (2013) accounted for the kinetics of sorption. For example, for ibuprofen, k_{sor} was reported to be 3.2-63 L/gSS.d (Urase & Kikuta, 2005). At equilibrium, a single parameter K_d (partition coefficient of the MP between aqueous and particulate phases) is defined and is often obtained experimentally, as described in Section 2.4.2. In the model, based on Eq. 2.4, the partition coefficient can be written in terms of k_{sor} and k_{des} at equilibrium, as shown in Eq. 2.5 (Urase & Kikuta, 2005). The $K_d$values are often obtained experimentally as discussed earlier and values for logK_d are summarized in Table 2.6 for the target MPs of this project.

$$\frac{dS_{MP}}{dt} = -k_{sor} * S_{MP} * X_{SS} + k_{des} * X_{MP} \qquad (2.4)$$

$$K_d = \frac{k_{sor}}{k_{des}} \qquad (2.5)$$

2.7.4. Model applications and issues

MP fate models are often used to understand the removal mechanisms of MPs in conventional treatment units. This helps to get insights on potential factors that could be manipulated for enhanced performance of conventional treatment units to better remove MPs. Adaptation of these models to advanced and evolving treatment units, as well as to regulatory discharge targets further maximizes the benefit of these fate models (Clouzot et al., 2013). In a review article, Pomiès et al. (2013) pointed out that the limited availability of data on sorption and biodegradation kinetic parameters of MPs and the lack of standard experimental procedures used to obtain the available data as major information gaps in the field of micropollutant modelling. Validation of the micropollutant models, as explained by Pomiès et al. (2013), must be carried out using experimental data that are different from the data used for calibration. Out of the 18 reviewed articles, 10 only carried validation, but this was not always performed properly (Pomiès et al., 2013). Evaluating the sensitivity of the micropollutant fate models to the values of different paramaters was also identified as another area with little or no information in the literature (Pomiès et al., 2013).

2.8. Summary of the literature

To sum up, the presence of CECs in wastewater and their poor removal during wastewater treatment has triggered numerous studies examining the overall removal of CECs in WWTPs. However, fewer reports are available on the CEC removals per treatment step. In addition, many of these were based on sampling methods that do not account for hydraulics, providing less reliable data. Due to the differences in the investigated treatment trains among studies, there is a lack of information on the efficacy of each treatment step at removing CECs and limited understanding on ways to improve their removal. Studies examining the removal of CECs often ignored or simplified the influence of hydrodynamics on the reported CEC removal results, in particular the influence of the residence time distribution. Recently, the detailed influence of the residence time distribution on CEC removal data was pointed out, and an improved sampling and calculation approach was suggested to account for that. This approach had been applied and reported in two studies in the literature, but both studies were limited to investigating the removal of CECs in activated sludge treatment. In addition, although some studies determined the concentration of CECs in sludge and performed mass balances, only a limited number of CECs were examined, with very scarce data on sorption of drugs of abuse. Also, none of these studies carrying out a mass balance accounted for the residence time distribution in determining a sampling strategy and calculating removal. These limitations and the fact that the majority of the studies focused on the CEC removal from the aqueous phase without analyzing the sludge, created a gap in the understanding of the fate of CECs in wastewater treatment plants. Several models were created to predict the fate of CECs in the widespread activated sludge processes, mostly for pesticides and metals removal. To a lesser extent, models were developed and calibrated for the fate prediction of pharmaceuticals, hormones and personal care products. However, almost all of these fate models were limited to describing the fate of CECs in bioreactors without integrating these fate processes into the secondary clarifier model coupled to the bioreactor model. In addition, there is still a lack of information on the sensitivity of the fate model outputs to model parameters, leading to uncertainty of the degree of confidence in the obtained numerical values of the calibrated parameters.

3. OBJECTIVES

The main research hypotheses of this project are:

1- Different WWTPs and different units within a WWTP have variable hydraulic properties that require distinctive sampling strategies and CEC removal calculations.

2- Removal of CECs takes place mostly in the biological treatment step of WWTPs and to a lower, but not negligible, degree in primary and tertiary treatment steps.

3- A portion of the target CECs, including drugs of abuse, pharmaceuticals, hormones and personal care products sorbs onto sludge in wastewater treatment facilities, while the majority of the CEC load is either degraded or discharged with the effluent.

4- The fate of the target CECs in activated sludge processes can be well predicted by mechanistic equations describing the kinetics of biodegradation, as well as the kinetics and equilibrium of sorption. In addition, parameters of the mechanistic fate equations influence the predicted output to different extents for each CEC, requiring more attention to the calibration of specific parameters than of others.

This PhD project thus aims at monitoring the fate of a group of CECs at different steps of the wastewater treatment train and at predicting the fate of these contaminants in the activated sludge process. The specific objectives formulated to validate the research hypotheses are:

1- To model the hydraulic behaviour of treatment steps in multiple wastewater treatment plants and obtain suitable sampling strategies and removal calculations for the determination of reliable aqueous removal data for the selected CECs.

2- To analyze the CECs' concentrations in primary and secondary sludge and consequently perform a mass balance analysis of the chosen CECs in activated sludge and primary treatment of a full-scale WWTP in order to quantify the contribution of the various fate routes of CECs in wastewater, including biodegradation, sorption and discharge along with the treated wastewater.

3- To develop a mathematical model describing the fate of several CECs in activated sludge treatment including the settler, to identify the most influential parameters on model outputs for the target CECs and to calibrate the model using soluble and particulate CEC measurements at different locations of a full-scale activated sludge unit.

4. ESTIMATING REMOVAL OF CONTAMINANTS OF EMERGING CONCERN FROM WASTEWATER TREATMENT PLANTS: THE CRITICAL ROLE OF WASTEWATER HYDRODYNAMICS

4.1. Preface

The impact of the hydrodynamics of WWTPs on the transport of CECs and the calculation of their removal has been recently demonstrated to be of importance, and the fractionated approach has been proposed to account for the hydraulics. The work presented in this manuscript further expands the application of the fractionated approach to estimate the removal of an extended list of classes of CECs by applying it to primary and secondary treatment steps at two WWTPs with potentially different hydraulic characteristics. The work further addresses one of the objectives of this PhD project i.e. to obtain reliable removals of CECs at different treatment steps.

The two full-scale WWTPs investigated use conventional activated sludge as secondary treatment. In one of the WWTPs (WWTP A), the activated sludge unit is preceded by primary treatment, while the other (WWTP B) implements extended aeration (i.e. with longer hydraulic retention time (HRT)) and is not preceded by primary clarification. As a requirement of the fractionated approach, prior hydraulic modelling of the WWTPs under study was performed using the wastewater temperature and electrical conductivity profiles of as tracers. Target CECs consisted of a list of drugs of abuse, as well as pharmaceuticals, hormones and a commonly-used antiseptic, a stimulant and an artificial sweetener. Samples were collected as 24-h composite before and after primary and secondary treatment steps, after which they were prepared by filtration and solid-phase extraction to measure the concentration of target CECs by LC-HRMS.

Calibrated hydraulic models of the two WWTPs indicated that three days of sampling were required for accurate calculation of CEC removals in WWTP A as compared to four days of sampling required for WWTP B, due to the longer HRT and higher degree of mixing in the activated sludge unit of WWTP B. As expected, results showed that the mixing in the primary clarifier was less than in the activated sludge unit. This study provided evidence that the fractionated approach leads to different removal calculations for different WWTPs and treatment steps with distinct mixing regimes and demonstrated the importance of using such an approach to obtain reliable removal data. This study also contributed to the very limited literature providing improved data by eliminating the bias of the residence time distribution of the treatment units.

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Estimating removals of contaminants of emerging concern from wastewater treatment plants: The critical role of wastewater hydrodynamics

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4.2. Abstract

Accurate data are needed to evaluate the capacity of wastewater treatments plants (WWTPs) to remove contaminants of emerging concern (CECs). The variability of CEC removals reported in the literature has raised questions about the methods used to estimate removals. In this study, we used the recently proposed "fractionated approach" to account for the influence of hydrodynamics in WWTPs and applied this method for estimating removals of 23 target CECs. Data on the conductivity and temperature of wastewater at two WWTPs were used to determine the hydraulic model that best described the flow regime of several treatment units. Composite samples (24-h) were collected at different stages of treatment over successive days. The concentrations of the target compounds in wastewater were determined by liquid chromatography mass spectrometry. Different hydraulics models were necessary to define the hydrodynamics at the two WWTPs, resulting in different load fractions to be used in calculations of removals. For WWTP A, that has a primary clarifier, all target CECs, except triclosan, were poorly removed during this step at efficiencies <30%. On the other hand, the activated sludge treatment unit at both WWTPs removed most target CECs at >70%. This study expanded the application of the fractionated approach to compare the hydraulics of two treatment trains of different configurations, including primary and secondary treatment. It demonstrated the sensitivity of the method to account for variations between the different treatment units. Reliable removals of an extended list of CECs in primary and secondary treatment were also provided in this study.

Keywords: Micropollutants, Fractionated approach, Hydraulic model, Removal efficiency, Activated sludge, Primary clarification

4.3. Introduction:

Contaminants of emerging concern (CECs), which include pharmaceuticals and personal care products (PPCPs), drugs of abuse and steroid hormones have been detected globally in the aquatic environment. The sources of these CECs are industrial and domestic wastewater, including hospital effluents (Luo et al., 2014). There is evidence that discharges of CECs into surface waters may have adverse effects on aquatic organisms (Gay et al., 2016; Kidd et al., 2007; Purdom et al., 1994). In addition, CECs may contaminate sources of drinking water (Kleywegt et al., 2011; Metcalfe et al., 2014; Rodayan et al., 2015), although it is not clear whether the low concentrations typically detected in drinking water are a threat to human health (Schwab et al., 2005; Webb et al., 2003).

Municipal wastewater treatment plants (WWTPs) are not designed to remove micropollutants, thus many CECs are not removed effectively during treatment (Luo et al., 2014; Onesios et al., 2009; Verlicchi et al., 2012). Most WWTPs in North America treat sewage using secondary treatment technologies, after which wastewater is discharged to the aquatic environment, sometimes without a disinfection step prior to discharge. Removals of CECs reported in literature vary significantly for given compounds, even for WWTPs with comparable treatment processes, which raises questions about the reliability of the data. Ort et al. (2010) reviewed the available data on removals of CECs in WWTPs and concluded that the sampling schemes typically used are inadequate for accurately estimating these removals. "Negative" removals, or concentrations of CECs in final treated effluents that are higher than concentrations in untreated influent are frequently observed, especially for recalcitrant compounds, such as carbamazepine (Yang et al., 2011; Zorita et al., 2009). Some of these negative removals have been attributed to de-conjugation of Phase II metabolites during biological treatment, resulting in generation of the parent compound (Ternes, 1998), as well as to desorption from sludge and particulate matter during treatment (Clara et al., 2005a; Ternes, 1998).

The negative and variable removals of CECs estimated for WWTPs may be due to sampling schemes that do not account for the fact that the daily incoming load is distributed over multiple days in the effluent due to the residence time distribution (RTD) of wastewater. Majewsky et al. (2011) recommended an approach to sampling and removal calculations within WWTPs "the fractionated approach" that compares the mass load of the output stream with a reference mass load of the input stream that takes into account the RTD. This approach requires a more intensive sampling strategy over several days, together with hydraulic modelling of the WWTP, utilizing conductivity as a tracer of the RTD (Majewsky et al., 2011). Ahnert et al. (2010) proposed that also temperature could be used as a tracer to determine the RTD within WWTPs. A subsequent study by Majewsky et al. (2013) using the fractionated approach generated superior estimates of the removals of five pharmaceuticals and four pesticides in a WWTP with activated sludge treatment.

A subsequent application of the fractionation approach for the removal of drugs of abuse in an activated sludge unit of a WWTP demonstrated the value of this method over conventional approaches for removal calculations (Rodayan et al., 2014a). As a further advance over this previous study, the goal of the present study was to evaluate the capacity of the fractionated approach to estimate CEC removals in two WWTPs with different treatment configurations and RTDs. The treatment processes monitored at the two WWTPs were their activated sludge units and, for the first time, a primary clarifier at one of the WWTPs. We applied this method to obtain reliable removals of 23 target CECs that have been widely detected in domestic wastewater, including several drugs of abuse and pharmaceuticals, two steroid hormones (i.e. estrone, androstenedione), a stimulant (i.e. caffeine), an artificial sweetener (i.e. sucralose) and an antibacterial compound found in personal care products (i.e. triclosan). Wastewater samples were analyzed for CECs using liquid chromatography with high-resolution mass spectrometry (LC-HRMS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS).

4.4. Materials and Methods:

4.4.1. CECs, chemicals and reagents

Several of the target compounds in this project were selected according to the criteria identified by Dickenson et al. (2011) in a study that illustrated the value of monitoring a small number of CECs in wastewater treatment facilities in the U.S. Sucralose has been widely detected in drinking water systems in the USA and because of its persistence and ubiquitous presence at high concentrations, has been proposed as a tracer of wastewater contamination (Mawhinney et al., 2011). Our previous studies have shown that drugs of abuse, including cocaine and its major metabolite (i.e. benzoylecgonine), amphetamines and opioid drugs are widely distributed in wastewater in Canada (Metcalfe et al., 2010; Rodayan et al., 2015). For methadone and cocaine, the fate of the metabolites (i.e. EEDP and benzoylecgonine, respectively) was also taken into account. The target CECs selected for monitoring in the two WWTPs are listed in Table 4.1.

The CECs under study are grouped into two classes according to their chemical properties and the methods of extraction and analysis. Class A compounds are drugs of abuse and their metabolites, which are all weak bases, and Class B compounds are all other analytes, which are either weak acids, phenolics or neutral compounds. Class A compounds and their deuterated stable isotope surrogates were purchased from Cerilliant (Round Rock, TX, USA). PPCPs and steroids from the Class B group were purchased from Sigma-Aldrich Canada (Oakville, ON, Canada) and their stable isotope surrogates were purchased from C/D/N Isotopes (Pointe- Claire, QC, Canada), except for androstenedione, for which a deuterated surrogate was provided by Cerilliant. All standards and stock solutions were stored in amber glass vials at -20°C. Methanol, acetonitrile, acetone, formic acid, ammonium hydroxide and sulphuric acid were purchased from Fisher Scientific (Ottawa, ON, Canada). Ultrapure water was generated using a Milli-Q water purification system from Millipore (Bedford, MA, USA).

Table 4.1 Target compounds and their physicochemical properties, internal standards, LODs & LOQs and class for extraction and analysis.

Type and Subtype	Compound	CAS No	Molecular Formula	Molecular Weight	pK_a[a,b]	Log K_{ow}[a,b]	Class	Internal Standard	LOD, LOQ (ng/L)
Antibiotics	Trimethoprim	738-70-5	$C_{10}H_{11}N_3O_3S$	290.3	6.8	0.91	B	Trimethoprim-13C3	7,22
	Sulfamethoxazole	723-46-6	$C_{14}H_{18}N_4O_3$	253.3	5.7	0.89	B	Sulfamethoxazole-13C6	4,14
Analgesics	Acetaminophen	103-90-2	$C_8H_9NO_2$	151.2	9.0	0.46	B	Acetaminophen -D3	11,35
	Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	8.2	1.14[a]	A	Codeine-D3	8,28
	Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.2	4.9	3.97	B	Ibuprofen-13C3	6,21
	Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.3	4.2	3.18	B	Naproxen-13c1-d3	4,14
Antiepileptic	Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	263.3	3.2	2.45	B	Carbamazepine-D10	2,5
Antilipidemic	Gemfibrozil	25812-30-0	$C_{15}H_{22}O_3$	250.3	4.7	4.77	B	Gemfibrozil-D6	5,16
Dopamine uptake inhibitor (cocaine and its metabolite and amphetamine-type stimulants)	Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.4	8.6	2.3	A	Cocaine-D3	38,126
	Benzoylecgonine (cocaine metabolite)	519-09-5	$C_{16}H_{19}NO_4$	289.3	NA	-1.32	A	Benzoylecgonine-D3	6,21
	Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1	1.76	A	Amphetamine-D5	6,28
	Ephedrine	299-42-3	$C_{10}H_{15}NO$	165.2	9.7	1.13	A	Ephedrine-D3	11,37
	MDA	4764-17-4	$C_{10}H_{13}NO_2$	179.2	9.7	1.64	A	MDA-D5	12,41
Opioids	Dihydrocodeine	125-28-0	$C_{18}H_{23}NO_3$	301.4	8.8	1.49[c]	A	Dihydrocodeine-D6	12,41
	Methadone	76-99-3	$C_{21}H_{27}NO$	309.4	8.9	3.93	A	Methadone-D9	6,20
	EDDP (methadone's metabolite)	30223-73-5	$C_{20}H_{23}N$	291.4	9.6	4.94	A	EDDP-D3	8,26
	Morphine	57-27-2	$C_{17}H_{19}NO_3$	285.3	9.9	0.96	A	Morphine-D3	7,25
	Tramadol	27203-92-5	$C_{16}H_{25}NO_2$	263.4	9.4	2.63	A	Tramadol-13C-D3	11,36
Disinfectants	Triclosan	3380-34-5	$C_{12}H_7Cl_3O_2$	289.6	7.9	4.76	B	Triclosan-13C12	6,19
Steroid hormones	Androstenedione	63-05-8	$C_{19}H_{26}O_2$	286.4	NA[e]	2.75	B	Androstene-3,17-dione-2,3,4-13C3	2,5
	Estrone	53-16-7	$C_{18}H_{22}O_2$	270.4	10.3	3.13	B	Estrone-3,4-13C2	2,5
Stimulant	Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	14.0	-0.07	B	Caffeine-13C3	4,14
Artificial sweetener	Sucralose	56038–13–2	$C_{12}H_{19}Cl_3O_8$	397.6	NA[e]	-1[d]	B	Sucralose-D6	7,22

[a] National Center for Biotechnology Information (2004), [b] Ratola et al. (2012), [c]U.S. National Library of Medicine (2005), [d] Subedi and Kannan (2014a), [e] NA: not available

4.4.2. WWTPs characteristics

Two WWTPs that both utilize activated sludge (secondary) treatment, but with different treatment trains were selected for this study. WWTP A, which has a design capacity of 68,200 m^3/d, receives wastewater that is primarily domestic at an average flow of 43,146 m^3/d. This WWTP serves a population of 78,700 The treatment train includes aerated grit tanks, primary clarifiers, and two activated sludge trains, each of which contains two three-pass integrated-film activated sludge (IFAS) bioreactors and two secondary clarifiers. WWTP A employs UV for disinfection, after which the effluent is discharged to a nearby river. The main characteristics of these WWTPs, including the solids retention time (SRT), hydraulic retention time (HRT), and the temperature conditions during sampling are summarized in the supplementary material (Table 4.5).

WWTP B, which has a design capacity of 105,000 m^3/d, receives an average wastewater flow of 50,250 m^3/d and serves a population of 54,000. The influent flow is composed of 26% industrial wastewater, with the remaining amount being domestic wastewater. Unlike WWTP A, this plant contains no primary clarifier and the raw sewage undergoes preliminary treatment by screens, followed by activated sludge treatment. The activated sludge treatment comprises four step-feed aeration tanks followed by four secondary clarifiers, and the effluent is discharged into a nearby river without disinfection.

4.4.3. Hydraulic model

4.4.3.1. Electro-conductivity and temperature measurements

The electrical conductivity and temperature of the wastewater were monitored with HOBO conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada), which were deployed to take high-frequency measurements (every five minutes). At WWTP A, the probes were deployed at the exit of the grit removal, primary clarifier, aeration tank and secondary clarifier points over two weeks prior to the sampling campaign. For WWTP B, the probes were deployed at the exit of screens, aeration tank and secondary clarifier points in the treatment train over four days, as an earlier calibration of the plant was available. A universal optic-USB base station and Onset HOBOware Pro Version 3.2.2 software (Hoskin Scientific, St-Laurent, QC, Canada) were used for transferring and reading the data, respectively. Conductivity was utilized as a tracer for the investigation of the residence time distribution in WWTP A. However, due to several outliers in the conductivity data for WWTP B, temperature was used as the tracer for residence time in that plant.

4.4.3.2. Hydraulic model calibration and load fractions

WEST software (Mike Powered by DHI, Hørsholm, Denmark) was used to determine the hydraulic model that best captures the mixing regimes of the treatment units of the two WWTPs, as described by Majewsky et al. (2011). Briefly, the hydraulic model was built using the actual flow conditions and volumes obtained from the WWTP operators, in addition to the actual conductivity (WWTP A) and temperature (WWTP B) profiles as an input. Calibration of each treatment unit was performed by varying the number of aeration tanks in series and the feed layer of the clarifiers. The best-fit hydraulic model was defined to be the model that minimizes the root mean square error (RMSE) between measured and simulated output profiles. In order to study the transport of CECs by hydraulic effects only, model simulations were run using the best-fit hydraulic model, the actual flow rates and a 24-h step input of the soluble inert component available in WEST to represent a CEC affected by hydraulics only. The output of the model was used to determine the load fractions that best describe the effluent load exiting the treatment unit on a given day as fractions of the incoming loads entering on each of the previous days, as described by Majewsky et al. (2011).

4.4.4. Wastewater sampling

Since obtaining an hourly resolution of the CEC removals is not crucial for the present study, 24-h composite samples were collected in both the influent and the effluent simultaneously over multiple days. Collecting effluent samples over multiple consecutive days eliminates the need for shifting the effluent sampling by an optimum offset after the beginning of the influent sampling. The obtained hydraulic models of the two WWTPs under study indicated that in order to measure most of the influent load material that exits on a single day in the effluent, sampling periods of three and four consecutive days are required at WWTP A & WWTP B, respectively. The explanation of this will be expanded further in the results section. Samples (24-h composite) were collected from the WWTP A at the exit of the grit removal, primary clarifier, aeration tank and secondary clarifier points in the treatment train on September 10-12, 2013. For WWTP B, samples were collected at the exit of screens, aeration tank and secondary clarifier points in the treatment train on September 23-27, 2013. Dry weather conditions prevailed during both sampling periods. The 24-h composite samples were collected using ISCO 6712 samplers (Avensys, St-Laurent, QC, Canada) equipped with 24 collection bottles and packed with ice. These samplers were programmed to collect equal-volume time-proportional samples every 15 minutes. Samples were then placed in 1-L amber HDPE bottles (Fisher Scientific, Ottawa, ON, Canada) and stored at -20°C until extracted.

4.4.5. Sample preparation

Prior to extraction, wastewater samples were filtered using 1-μm glass-fiber filter (Fisher Scientific, Ottawa, ON, Canada). All compounds were extracted using solid phase extraction (SPE) techniques using Oasis cartridges purchased from Waters (Milford, MA, USA). Sample volumes extracted were 100 mL for untreated wastewater influent and 200 mL for all other wastewater samples. The extraction method for Class A compounds was described by Metcalfe et al. (2010) and modified by Yargeau et al. (2014a). For Class B compounds, the extraction method was previously described by Li et al. (2010) and Metcalfe et al. (2014). Table 4.6 summarizes the methods and the instruments used for extracting the two classes of compounds. Prior to extraction, samples were spiked with 100 μL of 500 ng/mL of the stable isotope internal standards listed in Table 5.1. Recoveries of Class A compounds by the SPE procedure were in the range of 55% to 110%, while for Class B compounds, SPE recoveries ranged from 71% to 130%, as reported by Li et al. (2010).

4.4.6. Analysis

Analysis of the Class A compounds was conducted by LC-HRMS using an Accela LC system coupled to a LTQ Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was achieved using the method described by Rodayan et al. (2014b). Ionization was operated using heated electrospray ionization source (HESI) in positive mode using the method described earlier (Rodayan et al., 2014a). Acquisition was performed in full scan mode (50–400 m/z) at high resolution (RFWHM = 41,000). The ions of interest were extracted using an m/z window of ±0.01.

Class B compounds were analyzed by LC-MS/MS using an Agilent 1100 HPLC (Mississauga, ON, Canada) coupled to a Q-Trap 5500 instrument (AB Sciex, Concord, ON, Canada) with a turbo spray ionization source. The chromatographic method was previously described by Metcalfe et al. (2014). Acquisition was performed using the precursor and product ion transitions for multiple reaction monitoring (MRM) of the target analytes and their corresponding deuterated surrogates. The MRM transitions for the target compounds are also listed in the same study (Metcalfe et al., 2014). Negative ionization mode was used for the analysis of sucralose, ibuprofen, gemfibrozil, estrone, naproxen, and triclosan, whereas, positive ionization mode was used for carbamazepine, sulfamethoxazole, androstenedione, acetaminophen and caffeine.

A linear calibration curve of seven concentration points from 5 ng/mL to 150 ng/mL and from 0.78 ng/mL to 200 ng/mL was used for quantification for compounds of class A and B, respectively. To quantify target compounds with concentrations beyond these limits, dilutions were performed on the sample prior to analysis until the concentration of the target compound was within the concentrations range of the calibration curve. The Limits of Detection (LODs) were determined based upon analysis of serial dilutions of standards of each target analyte. The limits of quantification (LOQs) were defined as 3.3xLODs. The LODs and LOQs for each target compound are listed in Table 4.1.

4.4.7. Removal calculations

The obtained concentrations of CECs at the influent of each treatment unit, along with the load fractions were used to calculate a reference influent load, using Eq. 4.1. This reference load was compared to the effluent load of the treatment unit that is based on one day in order to obtain reliable removal calculations, using the so-called fractionated approach (Eq. 4.2). Considering that samples from the effluent of each treatment unit were collected on multiple consecutive days, it was possible to calculate CEC removal values using different effluent loads as the basis for calculations. This is opposed to the commonly used day-by-day approach that calculates the removal by comparing input and output loads from the same day. Concentrations of CECs used for calculations of loads were based on the dissolved portion only of the CECs in the water phase in the present study.

$$L_{ref} = \sum_{i=1}^{i=n} f_i * L_{inf,i} \tag{4.1}$$

$$R = \frac{L_{ref} - L_{eff}}{L_{ref}} * 100\% \tag{4.2}$$

- L_{ref}: Reference mass load of the contaminant in the aqueous phase of the input stream (mg/d) based on several days of sampling (from the i^{th} to the n^{th}). Where "n" is the number of days of sampling.
- $L_{inf,i}$: Mass load of the contaminant in the input stream on the i^{th} day of sampling or "day i" (mg/d)
- f_i : Load fraction or fraction of the incoming load on the i^{th} day of sampling (day i) that is contained in the effluent load on the n^{th} day of sampling (day n)
- L_{eff} : Mass load of the contaminant in the effluent on n^{th} day of sampling (mg/d)
- R: Removal rate of contaminant based on the fractionated approach

4.5. Results and Discussions

4.5.1. Hydraulic model

4.5.1.1. Calibration

Conductivity and temperature were considered to be conserved tracers for hydraulic calibration. However, the measured conductivity of wastewater exhibited a reduction following aeration tanks and a slight increase following primary and secondary clarification, as shown in Figure 4.1 and is consistent with a previous study (Maurer & Gujer, 1995). As a result, it was necessary to rescale the predicted effluent conductivity to account for the actual decline or increase in conductivity observed within the WWTPs. This correction was necessary prior to evaluating the fit of the models, as it decreased the bias of the RMSE between predicted and measured conductivity due to the natural changes of the measured conductivity. The correction was based on the calculated percentage change of the average measured conductivity between the influent and the effluent of each treatment unit, which was assumed to be constant throughout the monitoring period. The effluent conductivity predicted by the model was multiplied by the observed percentage change to obtain a rescaled predicted conductivity. The best-fit models for WWTP A and WWTP B were determined based on the corrected predicted conductivity. The natural change of temperature through treatment units was not significant and did not require correction.

4.5.1.2. Model selection

For WWTP A, the primary and secondary clarifiers were each modelled by a single clarifier, with a volume equivalent to the actual volume. The 10-layered model of the clarifier assumes that the influent of the clarifier enters in the mid layer. Adjusting the feed layer to one layer below the mid layer and one layer above it for primary and secondary clarifiers respectively was shown to optimize the fit of the hydraulic models. The model produced predictions of effluent conductivity that are in agreement with the observed conductivity, as illustrated in Figure 4.1(a) (dashed curves). With regards to the aeration tanks, increasing the number of aeration tanks in series to five tanks with the same actual total volume proved to optimize the fit of the hydraulic model. Generally speaking, a model describing WWTP with a large number of tanks in series exhibits a behaviour that approaches a "plug" flow. WWTP A employs three-pass aeration tanks with a feed that enters the first pass, which is well represented by the semi-plug flow behaviour.

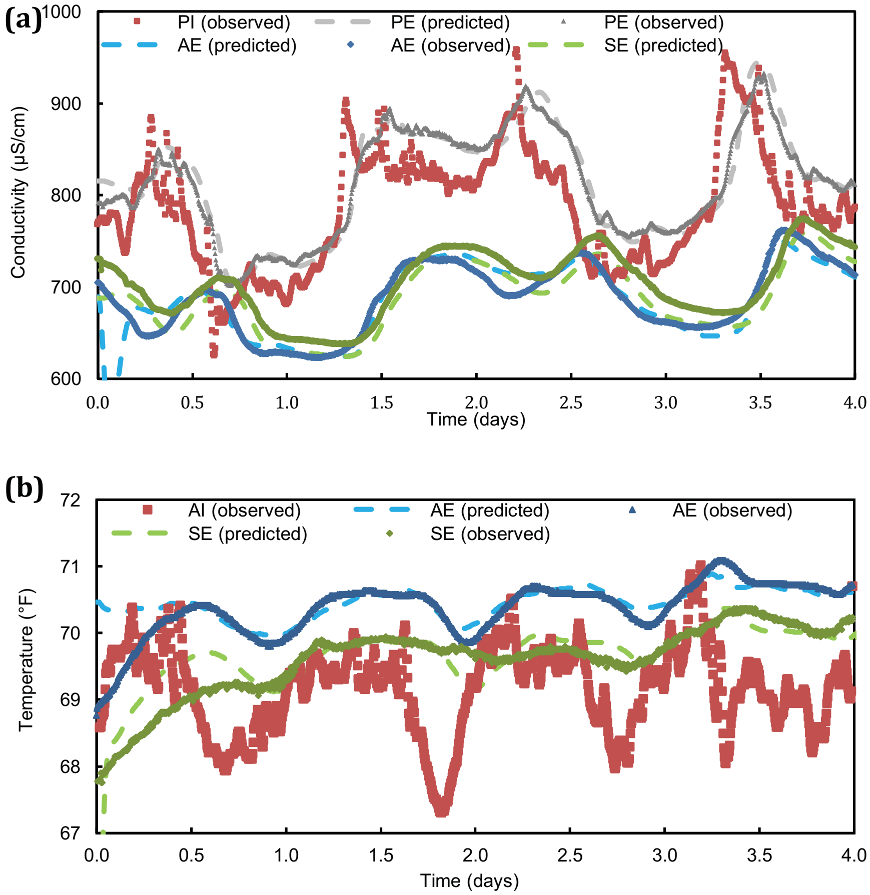

Figure 4.1 Observed (recorded) and predicted (simulated) conductivity trends in (a) WWTP A and (b) WWTP B at different locations (PI: influent to primary clarification, PE: effluent of primary clarification, AE: effluent of aeration, SE: effluent of secondary clarification).

For WWTP B, the best-fit hydraulic model that minimizes the RMSE between predicted and observed temperature for the aeration tanks was observed to be a single aeration tank. Unlike aeration in WWTP A, step-feed operation is employed at the WWTP B. That is, the feed is entering at different points of the multi-pass aeration tanks, which causes the content of the aeration tank to be more homogeneous, thus making the system behave more as mixed tank. The effluent temperature profiles in Figure 4.1(b) show a wider residence time distribution (more broadening of the peaks) than profiles in Figure 4.1(a), suggesting that more substantial mixing occurs in the activated sludge unit of WWTP B when compared to that of WWTP A. The secondary clarifier was represented by a single clarifier with the actual total volume.

4.5.1.3. Load fractions

Table 4.2 summarizes the load fractions determined for each treatment unit, where "f_i" represents the fraction of influent load entering on the ith day that is contained in effluent of day 4, 3 or 2, depending on which day in the effluent was taken as a basis. It is important to note that the load fractions based on the effluent on single day do not necessarily add up to 100%, since each fraction is normalized against the influent load of a different sampling day (Eq. 4.1). As shown in Table 4.2, the effluent of the activated sludge unit of WWTP A on the last day of sampling (Day 3) contained influent material from the same and two previous days. This explains the need for three days of sampling at WWTP A to characterize the reference load that corresponds to the effluent load on the last day. The load fractions for the activated sludge unit of WWTP B were more widely distributed than those from WWTP A, as shown in Table 4.2, so four days of sampling were required. Figure 4.2 provides extra illustration of the response loads obtained from model simulations of the activated sludge treatment trains and used to calculate load fractions. Comparing the activated sludge unit to the primary clarifier, the load fractions obtained from the activated sludge are distributed over a larger number of days than the primary clarifier (i.e. three days), but with low contribution from the first sampling day of sampling (i.e. load fraction of 0.3%). This is linked to the higher HRT and the greater extent of mixing that occurs in the activated sludge as compared to the primary clarifier. The load fractions were also evaluated for aeration tanks and secondary clarifier, separately. The secondary clarifier of WWTP A showed more distribution of load fractions over the days than that of WWTP B, while the opposite was observed for the aeration tanks.

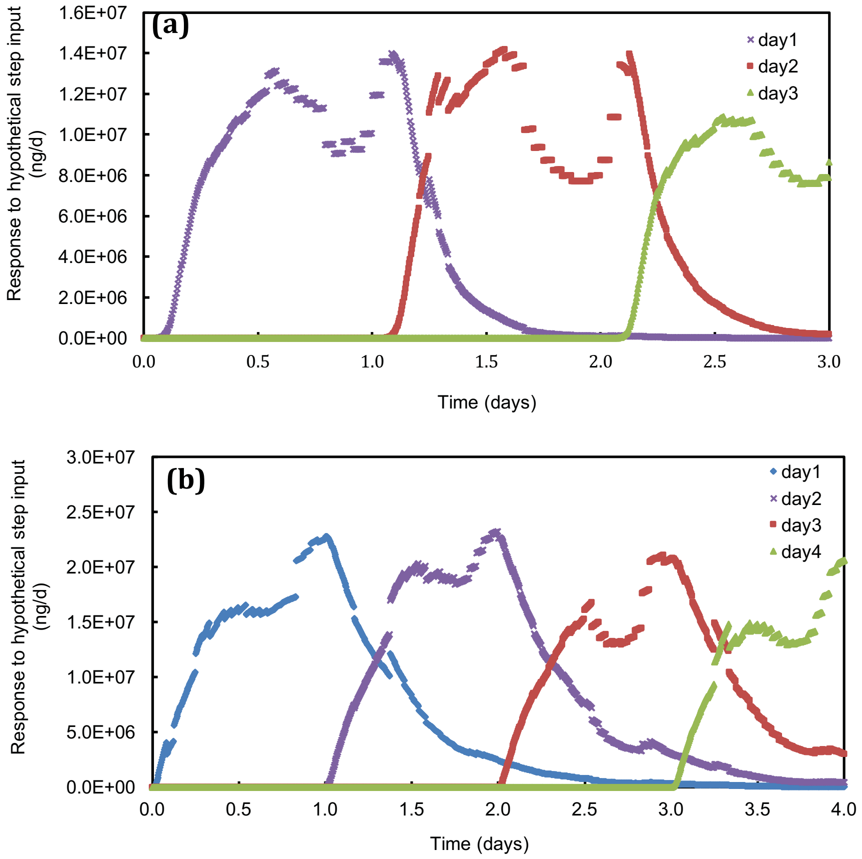

Figure 4.2 Simulated response to a hypothetical CEC 24-h step input (a) Input applied on first, second and third days of sampling separately to the best-fit hydraulic model of the activated sludge unit in WWTP A; (b) Input applied on first, second, third and fourth sampling days separately to the best-fit hydraulic model of the activated sludge unit in WWTP B.

The main factors causing the variations in load fractions between the different treatment units and the similar units between the two WWTPs (Table 4.2) are variations in volumes and flow rates, as well as the differences in the modes of operations. The different load fractions that are used to calculate the reference influent load (Eq. 4.1) eventually affect the calculation of the CEC removals (Eq. 4.2). Furthermore, due to the daily variation in the influent flow rates, as well as daily variations in the diurnal pattern of flow, slight daily variations are observed in the curves representing RTD of activated sludge on different days, as shown in Figure 4.2. Therefore, as shown in Table 4.2, slightly different load fractions obtained for each treatment unit when different days are taken as the basis of the effluent load for removal calculation. Seasonal changes in flow rates would also be expected to have a significant effect on these calculations. This exercise illustrates the sensitivity of the fractionated approach to changes in the operating conditions within the WWTP. Eliminating this bias in CEC removal estimates by accounting for hydraulics is essential, especially when comparing the efficiency of different treatment stages or trains in removing CECs. The CEC removal efficiencies in primary and secondary treatment obtained by applying the fractionated approach are discussed in Sections 4.5.3 and 4.5.4.

4.5.2. Concentrations of target contaminants

The average measured concentrations and the calculated mass loads of CECs in the influent and effluent streams of both WWTPs are summarized in Table 4.3. In the influent streams for both WWTPs, the average concentrations of the target CECs ranged from 6 ng/L for estrone to 4,400 ng/L for sucralose at WWTP A and from 7 ng/L for estrone to 40,800 ng/L for caffeine at WWTP B. Trimethoprim was not detected in the influent of both WWTPs, while MDA and dihydrocodeine were not detected in the influent for WWTP A, but were detected in the influent at WWTP B. Codeine, morphine and sulfamethoxazole were not detected in the influent of WWTP B only (Table 4.3). These data illustrate the potential impact of the different populations on loadings of CECs into domestic wastewater. In general, no significant variations were observed for any of the CECs in the influent of WWTP A during the sampling period. The ratio of benzoylecgonine to cocaine concentrations was calculated to be 4.1 in WWTP A and 3.1 in WWTP B. According to Ratola et al. (2012), the majority of studies report a ratio of 3.1-3.5 in wastewater. It was suggested that a ratio (benzoylecgonine:cocaine) lower than 5 indicates that some cocaine was discharged directly into sewage without being consumed and metabolized (Karolak et al., 2010).

In the effluent of secondary treatment of both WWTPs investigated, some compounds were <LOD or <LOQ, although they were detected in the influent, including sulfamethoxazole, cocaine and androstenedione for WWTP A and acetaminophen, ibuprofen, benzoylecgonine, amphetamine, MDA, dihydrocodeine, estrone and triclosan for WWTP B. The concentrations of sucralose were the highest of all target CECs in the effluent of both WWTPs, with average concentrations of around 5600 ng/L and 1100 ng/L in WWTP A and B, respectively.

Table 4.2 Load fractions of influent loads composing the effluent on a given day for the different treatment units in WWTP A and WWTP B. “fi” denotes the fraction of influent load entering on day i that is contained in effluent of day 4, 3 or 2 (second column).

WWTP	Based on	Load fractions of primary clarifier				Load fractions of aeration tanks				Load fractions of secondary clarifier				Load fractions of activated sludge			
		f_1	f_2	f_3	f_4	f_1	f_2	f_3	f_4	f_1	f_2	f_3	f_4	f_1	f_2	f_3	f_4
WWTP A	Day 3 effluent	0.0	9.9	90.1	-	0.0	10.5	90.1	-	0.0	12.4	88.2	-	0.3	31.2	69.1	-
	Day 2 effluent	10.2	90.1	-	-	10.6	89.5	-	-	9.7	90.0	-	-	32.9	68.5	-	-
WWTP B	Day 4 effluent	-	-	-	-	0.0	1.0	28.4	70.7	0.0	0.0	3.1	97.3	0.5	4.8	38.8	55.0
	Day 3 effluent	-	-	-	-	0.7	25.7	70.5	-	0.0	2.8	96.9	-	3.8	35.5	55.1	-

Table 4.3 Mean concentration (ng/L ± STD) and mean mass loads (mg/d ± STD) of target CECs in the influent to the primary treatment and effluent of the secondary treatment at WWTP A and WWTP B. STD is standard deviation based on different days samples and their replicates (n=9 for WWTP A and n=12 for WWTP B).

	Concentrations (ng/L ± STD)				Mass loads (g/d ± STD)			
	WWTP A		WWTP B		WWTP A		WWTP B	
	Influent	Effluent[c]	Influent	Effluent	Influent	Effluent[c]	Influent	Effluent
Trimethoprim	<LOQ[b]	<LOD[a]	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
Sulfamethoxazole	16 ± 6	<LOD	<LOD	<LOD	0.620 ± 0.224	<LOD	<LOD	<LOD
Acetaminophen	NA	NA	7475 ± 1012	<LOD	NA	NA	447 ± 61	<LOD
Codeine	2518 ± 77	1278 ± 30	<LOD	<LOD	98.9 ± 3	50.1 ± 1.2	<LOD	<LOD
Ibuprofen	2488 ± 100	114 ± 7	249 ± 13	<LOQ	97.6 ± 3.9	4.46 ± 0.27	14.7 ± 0.8	<LOQ
Naproxen	2140 ± 91	343 ± 19	237 ± 15	63 ± 3	84.1 ± 3.5	13.6 ± 0.737	14.0 ± 0.9	3.73 ± 0.18
Carbamazepine	90 ± 2	91 ± 5	23 ± 8	26 ± 3	3.53 ± 0.09	3.59 ± 0.20	1.39 ± 0.46	1.54 ± 0.16
Gemfibrozil	19 ± 0.5	22 ± 2	<LOQ	18 ± 3	0.727 ± 0.018	0.859 ± 0.068	<LOQ	1.04 ± 0.19
Benzoylecgonine	1165 ± 35	112 ± 3	554 ± 29	<LOD	45.7 ± 1.3	4.40 ± 0.13	32.2 ± 1.6	<LOD
Cocaine	284 ± 16	< LOD	181 ± 24	133 ± 12	8.14 ± 0.75	<LOD	10.8 ± 1.3	7.96 ± 0.69
Amphetamine	178 ± 5	21 ± 0.5	56 ± 8	<LOD	7.03 ± 0.19	0.825 ± 0.019	3.30 ± 0.45	<LOD
EEDP	428 ± 29	448 ± 15	155 ± 18	14 ± 4	16.8 ± 1.2	17.6 ± 0.6	9.14 ± 1.01	0.862 ± 0.217
Ephedrine	1672 ± 49	88 ± 5	902 ± 70	106 ± 6	65.6 ± 1.9	3.47 ± 0.20	53.5 ± 3.9	6.23 ± 0.344
MDA	<LOD	<LOD	41 ± 5	<LOD	<LOD	<LOD	2.44 ± 0.30	<LOD
Dihydrocodeine	<LOD	<LOD	467 ± 66	<LOD	<LOD	<LOD	28.4 ± 3.7	<LOD
Methadone	170 ± 4	168 ± 6	18 ± 2	11 ± 0.3	6.67 ± 0.17	6.58 ± 0.25	1.09 ± 0.13	0.645 ± 0.016
Morphine	259 ± 18	33 ± 2	<LOD	<LOD	10.2 ± 0.7	1.29 ± 0.08	<LOD	<LOD
Tramadol	189 ± 7	286 ± 16	43 ± 6	77 ± 3	7.45 ± 0.27	11.2 ± 0.6	2.54 ± 0.34	4.53 ± 0.17
Androstenedione	32 ± 1	<LOQ	55 ± 7	15 ± 5[d]	1.27 ± 0.05	<LOQ	3.25 ± 0.39	0.297 ± 0.159
Estrone	6 ± 1	5 ± 1	7 ± 2	<LOQ	0.239 ± 0.041	0.196 ± 0.035	0.393 ± 0.121	<LOQ
Triclosan	281 ± 8	28 ± 5	27 ± 3	<LOQ	10.9 ± 0.3	1.08 ± 0.20	1.57 ± 0.18	<LOQ
Caffeine	NA	NA	40899 ± 6558	73 ± 21	NA	NA	2434 ± 391	4.24 ± 1.25
Sucralose	4362 ± 223	5578 ± 351	3866 ± 305	1097 ± 147	170 ± 9	218 ± 14	228 ± 14.5	65.0 ± 8.83

[a,b] <LOD and <LOQ: indicates that the compound concentrations were below the limits of detection or quantification, respectively, [c] refers to the secondary effluent of WWTP A before the UV disinfection, [d] Androstenedione’s concentration in the effluent was based on a single day, as on the rest of the days, the concentration was <LOQ

4.5.3. Removals of CECs during primary clarification

Removals of CECs during primary clarification were only determined for WWTP A, since WWTP B does not have a primary clarifier unit. The mass loads of the influent to the primary clarifier of WWTP A were calculated using the fractionated approach and using both day 2 and day 3 effluent as the basis of calculation. Results for reference (influent) and effluent mass loads of all CECs are presented in Figure 4.3, and average removal efficiencies in the primary treatment process are summarized in Table 4.4. The reference mass loads were generally higher when based on day 2 effluent than when based on day 3 effluent, which can be explained by the higher concentrations of most CECs observed in the influent of the second day of sampling. On the other hand, the effluent mass loads observed on the second and the third days of sampling (day 2 and day 3) were comparable.

Triclosan was estimated to have the highest removal (94%) in the primary clarifier, which is in agreement with previously reported results (Behera et al., 2011; Blair et al., 2013b; Winkler et al., 2007). The high removals in the primary clarifier are likely due to the high log K_{ow} of this compound (i.e. 4.76), which contributes to partitioning to sludge during the primary treatment step (Rogers, 1996). In addition to adsorption, some biodegradation of CECs is expected to take place in the primary clarifier, contributing to removal from the aqueous phase (Jones et al., 2005; Petrie et al., 2014). Androstenedione, carbamazepine, gemfibrozil, ibuprofen, naproxen and sucralose had poor removals of 10-20% in the primary clarifier. Poor removal of these compounds during primary clarification was also reported in previous studies (Behera et al., 2011; Blair et al., 2013b; Ternes & Joss, 2006; Zorita et al., 2009). It is notable that androstenedione, which is an intermediate in the biosynthesis of testosterone, was poorly removed by primary clarification. There are only limited data for this compound in the literature (Esperanza et al., 2007). In the case of ibuprofen and naproxen that have similar pK_a (<4) and log K_{ow} values (3-4), their removals in the primary clarifier were almost equivalent at 13-15%. Estrone, which is a metabolite of estradiol, showed negative removal in the primary clarifier. Negative removals of estrone during primary clarification have also been reported by others (Andersen et al., 2003; Behera et al., 2011; Carballa et al., 2004; Kirk et al., 2002; Ternes et al., 1999). These observations were attributed to transformation of estradiol to estrone during primary clarification (Andersen et al., 2003; Carballa et al., 2004; Verlicchi et al., 2012).

Table 4.4 Removal efficiencies (%) of target compounds in primary clarifier and activated sludge treatment steps in WWTP B and WWTP A.

Compound	Removal in primary clarifier (%)	Removal in Activated sludge (%)	
	WWTP A	WWTP A	WWTP B
Trimethoprim	<LOD[a]	<LOQ[b]	<LOD[a]
Sulfamethoxazole	16[c]	71[c]	<LOD[a]
Acetaminophen	NA[d]	NA[d]	100
Codeine	1	54	<LOD[a]
Ibuprofen	15	95	92
Naproxen	14	77	72
Carbamazepine	13	15	7
Gemfibrozil	20	-39	-37
Benzoylecgonine (cocaine metabolite)	3	90	99
Cocaine	-4	77[c]	42
Amphetamine	6	91[c]	90[c]
EEDP (methadone's metabolite)	-8	32	93
Ephedrine	-2	95	88
MDA	<LOD[a]	<LOD[a]	75[c]
Dihydrocodeine	<LOD[a]	<LOD[a]	95[c]
Methadone	-20	25	44
Morphine (codeine metabolite)	3	91	<LOD[a]
Tramadol	15	-47	-73
Triclosan	94	61	38[c]
Androstenedione	11	93[c]	74
Estrone	-31	34	24[c]
Caffeine	NA[d]	NA[d]	100
Sucralose	20	-50	68

[a,b] <LOD and <LOQ: indicates that the compound concentrations were below the LOD or LOQ in both the influent and effluent and that removals could not be calculated, [c]Denotes that the compound concentrations were either <LOQ or <LOD in the effluent of the corresponding treatment stage and that removal was calculated assuming a concentration equal to LOQ & LOD, respectively, indicating that the removal might actually be higher than the reported values, [d]NA: indicates compounds that were not analyzed, such as acetaminophen and caffeine that were later added to the list of target CECs and were not quantified in the samples from WWTP A.

Among the drugs of abuse monitored, the highest removal in the primary clarifier was 15% for tramadol, demonstrating that some sorption of tramadol to sludge is occurring. This can again be explained by the relatively high hydrophobicity of tramadol compared to the other drugs of abuse (Table 4.1). Some target compounds, namely methadone, EDDP, ephedrine and cocaine showed negative removals in the primary clarifier, even using the fractionated approach. Subedi and Kannan (2014b) reported negative removals for methadone and its metabolite, EDDP in the primary treatment process, as we observed in the present study. It is possible that desorption of these compounds from sludge takes place during primary clarification. The trends in the removal of drugs of abuse during primary clarification are in agreement with the limited literature available for these compounds (Rodayan et al., 2014a; Subedi & Kannan, 2014b).

Overall, these results demonstrate that primary clarification has limited capabilities for removing many of the target CECs, with 14 out of the 17 compounds showing removals < 20% at this step in the treatment train at WWTP A. This is consistent with these compounds being relatively hydrophilic and having a low tendency to partition onto solids due to their low log K_{ow} (<3) and low pK_a.

4.5.4. Removal of CECs during activated sludge treatment

As with the primary clarifier, the matching mass loads of CECs in the aqueous phase of the influent and effluent of the activated sludge unit were calculated, as illustrated in Figure 4.4 and Figure 4.5 for WWTP A and WWTP B, respectively. In WWTP A, the effluent mass loads used for calculating removals were based on both the second and third sampling days (as for the primary clarifier), while for WWTP B, it was based on the fourth and third sampling days. The average removals of target compounds in the activated sludge treatment step for the two WWTPs are also presented in Table 4.4.

The majority of the removals for most of the target compounds took place in the activated sludge step, when compared to the low removals in the primary clarifier of WWTP A (Table 4.4). Amphetamine and benzoylecgonine exhibited removals of >90% during activated sludge treatment, consistent with previous research on the removal of these two compounds in WWTPs with activated sludge treatment (Bijlsma et al., 2012; Postigo et al., 2010; Rodayan et al., 2014a). Acetaminophen and caffeine were shown to undergo substantial removal (WWTP B) of almost 100%, probably due to biodegradation, as suggested in previous studies (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Yang et al., 2011). Acetaminophen and caffeine were also shown to undergo substantial removal (WWTP B) of almost 100%, which is in agreement with previous studies (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Yang et al., 2011). Removal efficiencies of 92-95% and 72-77% were observed for ibuprofen and naproxen, respectively in the activated sludge process for both WWTPs, which is in good agreement with previous findings (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Sim et al., 2010; Yang et al., 2011). Removals of androstenedione were >93% in WWTP A and 74% in WWTP B. Almost complete removal of androstenedione from wastewater was previously reported by Esperanza et al. (2007).

Low removals of <40% were observed for estrone and carbamazepine during the activated sludge treatment at both WWTPs, indicating that these compounds are persistent. These observations for carbamazepine are consistent with the many studies and databases showing that carbamazepine is very persistent in WWTPs (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Miège et al., 2009; Yang et al., 2011). Estrone was previously reported to be persistent in some studies (Castiglioni et al., 2006a; Zorita et al., 2009), although other studies reported high removal (Behera et al., 2011; Miège et al., 2009). The wide range of the CEC removal efficiencies reported in the literature illustrate the value of the fractionated method for generating reliable and consistent data on removals of CECs.

Negative removals were observed for tramadol and gemfibrozil in the activated sludge step of both WWTPs under study, which may be due to the presence of conjugated metabolites in the influent of the unit that are converted to the parent compound due to the activated sludge treatment, as was suggested by other authors (Jelic et al., 2011; Lishman et al., 2006). There was also negative removal of the artificial sweetener, sucralose in the activated sludge unit at WWTP A, while a positive removal of 68% was calculated for this compound in WWTP B. Among the limited studies on the fate of sucralose in WWTPs, low removals of sucralose in activated sludge were previously reported (Pasquini et al., 2013; Subedi & Kannan, 2014a), and a number of negative removals of sucralose were observed in Norwegian and Swedish WWTPs (Brorström-Lunden, 2008a; Green, 2007). These were linked to the possibility of the presence of conjugated and complex forms of sucralose in the influent load, which might transform back to the parent form during the biological treatment. Negative and variable removals of carbamazepine were obtained in WWTP A and WWTP B using the day-by-day approach of removal calculation (results not shown here); however, the fractionated approach yielded positive and consistent removals.

4.6. Conclusions

In order to obtain reliable data on the removal of CECs during treatment of wastewater, it is essential to account for mixing regimes that affect the transport of CECs in the WWTP. In this study, we applied the fractionated approach to characterize CEC removals in two WWTPs.

WWTP B had a longer HRT, as well as a higher degree of mixing deduced from the fractionated approach than the activated sludge unit in WWTP A, resulting in the need for data over more sampling days for reliable quantification of removal in WWTP B, as well as different calculations of CEC removal. The removals of target CECs was generally low or negative in the primary clarifier and ranged from negative removal to 100% removal over the entire activated sludge treatment train. This study contributes to advancing the study of the fractionation approach by applying the approach to treatment steps not investigated before and demonstrating that the method is able to detect differences in the hydrodynamics of each of the units investigated at the two treatment plants, resulting in different removal calculations for each unit. Further, the reliable removals of the extended list of target CECs, obtained in this study by taking into account hydrodynamics, are key for calibrating fate models of CECs that can be used to improve our understanding of their fate during treatment and to provide insights for limiting their discharge.

4.7. Acknowledgements

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4.8. Supplementary materials:

Table 4.5 Summary of the operating characteristics of the WWTPs and average temperatures during the sampling campaigns.

Characteristic	Treatment stage	WWTP A	WWTP B
Hydraulic retention time - HRT (h)	Primary clarifier	5.97	-
	Aeration tanks	4.52	12.3
	Secondary clarifier	3.36	10.6
	Total	13.85	22.9
Solid retention time - SRT (days)		10.5	27.6
Average wastewater T (°C)		21	20

Table 4.6 SPE extraction methods and instruments used for compounds of Class a and Class B.

Extraction aspect	Class A	Class B
Instrument	Gilson GX-271 ASPECTM automated	Manual manifold
Cartridge	6 mL-150 mg Oasis MCX cartridges	6 mL-500 mg Oasis MAX cartridges
Sample pH	pH 2.5 using sulfuric acid	pH 8.0 using sodium hydroxide
Cartridge	6 mL of acetone and 6 mL of water (pH 2.5)	6 mL of methanol, 6 mL of 0.1 M
Elution	3x3 mL of 5% ammonium hydroxide in	2 mL methanol and then 3x3 mL of 2%
Reconstitution	25% methanol/75% water	50% methanol/50% water

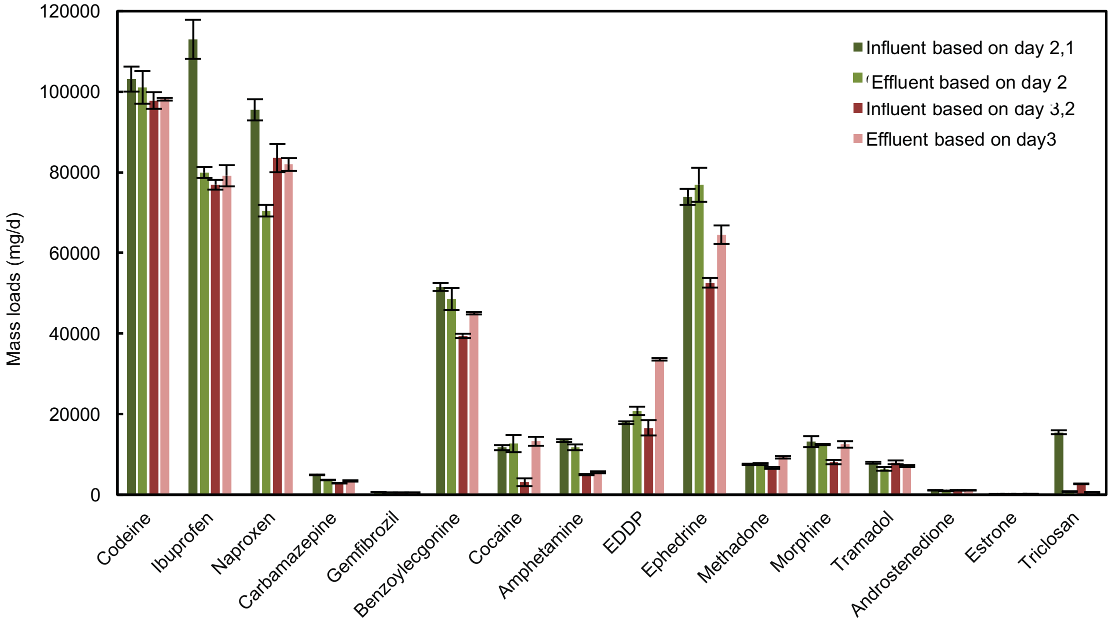

Figure 4.3 Mass loads of target CECs in influent (based on load fractions of two days) and effluent (load on the last day) of the primary clarifier of the WWTP A. Day 1,2,3 represent first, second and third sampling day, respectively. Error bars = one standard deviation of 3 replicates. Sucralose is not shown in this graph, as it has a significantly higher range of mass loads values.

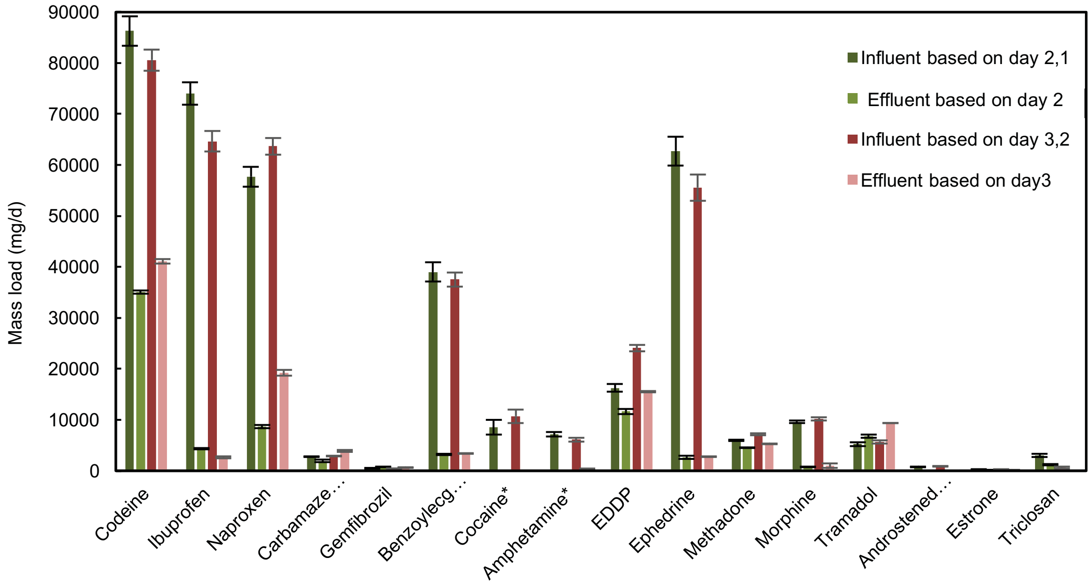

Figure 4.4 Mass loads of target CECs in influent (based on load fractions of two days) and effluent (load on last day) of the activated sludge unit at the WWTP A. Day 1,2,3 represent first, second and third sampling days, respectively. Error bars = one standard deviation of 3 replicates. * denotes compounds with effluent concentration < LOD or LOQ, where mass loads were considered 0 in this graph. Sucralose is not shown in this graph, as it has a significantly higher range of mass loads values.

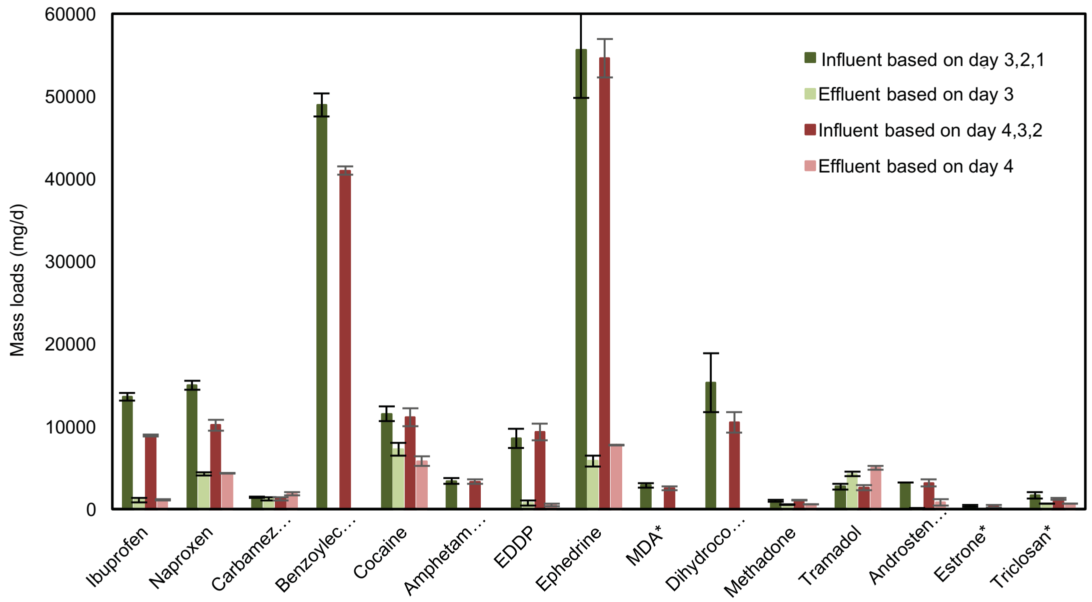

Figure 4.5 Mass loads of target CECs in influent (based on load fractions of 3 days) and effluent (load on last day) of the activated sludge unit at the WWTP B. Day 1,2,3,4 represent first, second, third and fourth sampling days, respectively. Error bars = one standard deviation of 3 replicates. * denotes compounds with effluent concentration < LOD or LOQ, where mass loads were considered 0 in this graph. Caffeine, sucralose and acetaminophen are not shown in this graph, as they have a significantly higher range of mass loads values.

5. FATE AND MASS BALANCE OF CONTAMINANTS OF EMERGING CONCERN DURING WASTEWATER TREATMENT DETERMINED USING THE FRACTIONATED APPROACH

5.1. Preface

In the previous manuscript (Chapter 4), the CEC removal efficiencies from the aqueous phase in primary and secondary treatment steps were investigated taking into consideration the effect of hydrodynamics. In this manuscript, the hydraulics characteristics were also accounted for to examine the CEC removal in primary and secondary treatment but in a mass balance context to eventually estimate the CEC load degraded. In addition, the efficiency of tertiary treatment steps (i.e. rotating biological contactors and sand filters) at removing CECs was assessed for the first time using the fractionated approach, taking into account the hydraulics of the units. Besides measuring CECs in the aqueous phase, the portion of CECs sorbed to sludge was quantified in primary and secondary treatment steps in order to distinguish sorption from other removal mechanisms. This manuscript addresses two of the objectives of this project: To quantify the contribution of different fate pathways on the removal of input loads of CECs in primary and secondary treatment steps and to quantify the removal in tertiary treatment processes while accounting for the effect of hydraulics.

The WWTP selected for this study uses primary clarification, secondary treatment by activated sludge and tertiary treatment by both rotating biological contactors and sand filtration. As in the previous manuscript, an optimum hydraulic model for each treatment unit was identified prior to sampling. Aqueous samples (24-h composite) were collected in between all the treatment steps, and grab sludge samples were collected from primary and secondary treatment steps. The target CECs monitored in this manuscript as well as extraction and chemical analysis procedures were similar to those in Chapter 4. Mass balance analysis was performed to calculate the difference between the incoming CEC load and the load sorbed to sludge; which represents the load transformed by other means, mostly biodegradation.

The optimum hydraulic model structures further demonstrated the unique mixing characteristic of each treatment unit, resulting in each unit requiring different strategies of calculation of the

CEC removal. Results of the mass balance calculations suggested that each target contaminant partitioned differently in each fate compartment depending on its physico-chemical properties, further emphasizing the value of quantifying the contribution of the individual fate pathways. The majority of the target CECs were observed to have a low tendency to adsorb onto sludge with the highest affinity to both primary and secondary sludge observed for the antiseptic triclosan. Removal of CECs by tertiary rotating biological contactors and sand filters, which was investigated for the first time for some of the target CECs, was generally observed to be low.

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Fate and mass balance of contaminants of emerging concern during wastewater treatment determined using the fractionated approach

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5.2. Abstract

Contaminants of emerging concern (CECs) are often poorly removed from wastewater using conventional treatment technologies and there is limited understanding of their fate during treatment. Inappropriate sampling strategies lead to inaccuracies in estimating removals of CECs. In this study, we used the “fractionated approach” that accounts for the residence time distribution (RTD) in treatment units to investigate the fate of 26 target CECs in a municipal wastewater treatment plant (WWTP) that includes primary, secondary and tertiary treatment steps. Prior hydraulic calibration of each treatment unit was performed. Wastewater and sludge samples were collected at different locations along the treatment train and the concentrations of target CECs were measured by liquid chromatography mass spectrometry. The most substantial aqueous removal occurred during activated sludge treatment (up to 99%). Removals were <50% in the primary clarifier and tertiary rotating biological contactors (RBCs) and up to 70% by sand filtration. Mass balance calculations demonstrated that (bio)degradation accounted for up to 50% of the removal in the primary clarifier and 100% in activated sludge. Removal by sorption to primary and secondary sludge was minimal for most CECs. Analysis of the selected metabolites demonstrated that negative removals obtained could be explained by transformations between the parent compound and their metabolites. This study contributes to the growing literature by applying the fractionated approach to calculate removal of different types of CECs across each wastewater treatment step. An additional level of understanding of the fate of CECs was provided by mass balance calculations in primary and secondary treatments.

Keywords: Adsorption, Fractionated approach, Hydraulics, Micropollutants, Pharmaceuticals

5.3. Introduction:

Contaminants of emerging concern (CECs), including pharmaceuticals and personal care products (PPCPs), drugs of abuse (DOAs), hormones and stimulants are present in the aquatic environment (Blair et al., 2013a; Hughes et al., 2013; Rodayan et al., 2015), and these CECs were found to have adverse effects on aquatic organisms (Gay et al., 2016; Kidd et al., 2007; Kümmerer, 2008; Purdom et al., 1994). Discharges of wastewater treatment plants (WWTPs) have been identified as the primary source for CECs introduced into surface waters (Luo et al., 2014). Therefore, accurate determination of the fate of CECs during wastewater treatment is required in order to facilitate risk assessments and to identify strategies to improve their removal.

Unexplained temporal variations in the removals of CECs in activated sludge units and in some cases negative removals (i.e. higher concentration in the effluents than in the influents) have often been observed in WWTPs, which has raised questions about the appropriateness of the protocols for sampling wastewater (Majewsky et al., 2011; Ort et al., 2010). In a critical review, Ort et al. (2010) suggested that sampling strategies that fail to account for the residence time distribution (RTD), which affects the transport of CECs in treatment units, could result in a mismatch between the sampled influent and effluent. This mismatch results in biased and unreliable data on removal efficiencies. Majewsky et al. (2011) proposed a strategy for sampling and removal calculation referred to as the "fractionated approach" to account for the RTD within the units of the WWTPs and match the mass loads of the influent with the effluent. This approach requires prior hydraulic modelling of the WWTP followed by a tailored sampling strategy where composite samples are collected on several consecutive days. The fractionated approach has shown promise for evaluating the removal of pharmaceuticals, pesticides and drugs of abuse during activated sludge treatment (Majewsky et al., 2013; Rodayan et al., 2014a). However, the fractionated approach has not yet been applied to a complete WWTP treatment train to identify and quantify the mechanisms of CEC removal at each stage of treatment.

To date, most studies on the removal of CECs did not investigate the contribution of different removal mechanisms, as they ignored the distribution of CECs between the aqueous and the particulate compartments (Petrie et al., 2015). A limited number of previous studies have taken a mass balance approach to determine removals of CECs in conventional primary and secondary treatment steps through the processes of adsorption and (bio)degradation that were the major removal mechanisms for the CECs investigated (Carballa et al., 2007; Gao et al., 2012; Heidler & Halden, 2008; Jelic et al., 2011; Petrie et al., 2014; Wick et al., 2009; Winkler et al., 2007). The majority of these studies were based on analysis of CECs in grab or 24-h composite wastewater samples collected simultaneously from different treatment stages, while a small number of studies accounted for the hydraulic retention time in the sampling campaign but did not consider the wastewater residence time distribution.

In a recent review, Petrie et al. (2015) identified inadequate sampling approaches, a lack of understanding of adsorption of CECs onto particulates in WWTPs, as well as a lack of data on the fate of the metabolites of pharmaceuticals as information gaps that lead to misrepresentative data of CEC removals. In the present study, advanced sampling strategies that account for the hydraulic behaviour of treatment units were combined with mass balance analysis to monitor the fate of 26 target compounds in a WWTP in order to gain insights into the mechanisms of removal of CECs. The target CECs were contaminants that were reported to be present in surface water or persistent during wastewater treatment. The list included pharmaceuticals and some of their metabolites, hormones, drugs of abuse, a stimulant (i.e. caffeine), an artificial sweetener (i.e. sucralose) and an antibacterial agent (i.e. triclosan). The WWTP monitored in this study employs primary treatment, secondary treatment by activated sludge, tertiary treatment by both rotating biological contactors (RBCs) and sand filtration and disinfection by chlorination/dechlorination. This study contributes to the literature on the use of the fractionated approach for reliable determination of CEC removals, and for the first time, uses this approach to estimate removals during the tertiary treatment steps with RBCs and sand filtration. Further, the predominant removal mechanisms during primary and secondary treatment were identified by the mass balance analysis.

5.4. Materials and Methods

5.4.1. Chemicals and other supplies

The target CECs included several pharmaceuticals identified by Dickenson et al. (2011) in a study that illustrated the value of monitoring a small number of CECs in WWTPs in the USA.

Sucralose was added to the list as a tracer of wastewater contamination due to its persistence and ubiquitous presence at high concentrations (Mawhinney et al., 2011). The drugs of abuse were selected based upon our previous studies indicating their presence in wastewaters in Canada (Metcalfe et al., 2010; Rodayan et al., 2014a). Androstenedione was selected as a model androgen because of the lack of data in the literature investigating its fate (Esperanza et al., 2007), and estrone was selected as a model estrogen because of its widespread occurrence in wastewater (Servos et al., 2005). To illustrate the importance of including metabolites in mass balance calculations, two metabolites of carbamazepine, rac trans-10,11-dihydro-10,11-dihydroxy carbamazepine (CBZ-DiOH) and carbamazepine 10,11-epoxide (CBZ-EP) were monitored because these compounds have been previously detected in wastewater and sludge (Hummel et al., 2006; Miao & Metcalfe, 2003; Miao et al., 2005). Other metabolites monitored in this study included EDDP, the primary metabolite of methadone, and benzoylecgonine, the primary metabolite of cocaine.

The target CECs, along with their physicochemical properties and the suppliers from which they were purchased are listed in Table 5.1. The analytes were classified as Class A and B compounds (Table 5.1) according to the extraction and analysis procedures. Class B compounds are drugs of abuse and some of their metabolites, which are mainly weak bases, in addition to carbamazepine and its two metabolites. Class A compounds are all other target compounds that are weak acids, neutral or phenolic compounds. The internal standards used for each compound are listed in Table 5.1. 10,11-dihydrocarbamazepine was used as a surrogate for CBZ-DiOH, as performed in previous studies (Leclercq et al., 2009; Miao & Metcalfe, 2003). All analytical standards and stock solutions were stored in amber glass vials at -20 °C.

Methanol, acetonitrile, and water of liquid chromatography–mass spectrometry (LC–MS) grade, as well as other chemicals used for sample preparation were purchased from Fisher Scientific (Ottawa, ON, Canada). Ultrapure water was generated using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Table 5.1: Target CECs and their chemical and physical characteristics, internal standards, class (determining the corresponding extraction and analysis methods), LODs and LOQs in aqueous and biosolids samples and the supplier of the compounds and their surrogates.

Type	Subtype	Compound	Log K_{ow}[1]	pK_a[1]	Internal standard (surrogate)	Class	Aqueous LOD, LOQ (ng/L)[4]	Biosolids LOD, LOQ (ng/L)[4]	Company[5] Compound, surrogate
Pharmaceuticals	Antibiotics	Trimethoprim	0.91	6.8	Trimethoprim-13C3	A	7,22	3,10	S,I
		Sulfamethoxazole	0.89	5.7	Sulfamethoxazole-13C6	A	4,14	2,9	S,I
	Analgesics	Codeine	1.14	8.2	Codeine-d3	B	6,18	3,9	C,C
		Ibuprofen	3.97	4.9	Ibuprofen-13C3	A	6,21	10,32	S,I
		Naproxen	3.18	4.2	Naproxen-13c1-d3	A	4,14	6,19	S,I
	Antiepileptic and metabolites	Carbamazepine	2.45	3.2	Carbamazepine-d10	B	2,5	2,6	C,C
		rac trans-10,11-Dihydro-10,11-dihydroxy Carbamazepine (CMZ-DiOH)	0.13	N.A.	10,11 dihydro carbamazepine	B	4,13	3,9	T,T
		Carbamazepine 10,11-Epoxide (CMZ-EP)	1.26	N.A.	Carbamazepine 10,11-Epoxide-d8	B	2,7	1,5	T,T
Drugs of abuse	Cocaine and metabolite	Benzoylecgonine (cocaine's metabolite)	-1.32	N.A.	Benzoylecgonine-d3	B	4,14	2,5	C,C
		Cocaine	2.3	8.6	Cocaine-d3	B	26,84	11,36	C,C
	Amphetamines	Amphetamine	1.76	10.1	Amphetamine-d5	B	4,13	1,4	C,C
		Methamphetamine	2.07	10.2	Methamphetamine-d9	B	7,23	1,4	C,C
		EDDP (methadone's metabolite)	4.94	9.6	EDDP-d3	B	5,18	4,15	C,C
		Ephedrine	1.13	9.7	Ephedrine-d3	B	7,25	3,9	C,C
	Opioids	Dihydrocodeine	1.49	8.8	Dihydrocodeine-d6	B	8,22	5,14	C,C
		Fentanyl	4.05	8.6	Fentanyl-d5	B	6,20	3,8	C,C
		Ketamine	2.18	7.5	Ketamine-d4	B	8,27	2,8	C,C
		Methadone	3.93	8.9	Methadone-d9	B	4,13	1,4	C,C
		Morphine (codeine metabolite)	0.89	9.9	Morphine-d3	B	5,17	10,32	C,C
		Oxycodone	0.66	8.3	Oxycodone-d3	B	10,22	3,7	C,C
		Tramadol	2.63	9.4	Tramadol-d3	B	7,24	1,3	C,C
Personal Care Products	Antibacterial	Triclosan	4.76	7.9	Triclosan-13C12	A	6,19	6,19	K,M
Steroid hormones		Androstenedione	2.75	NA	Androstene-3,17-dione-2,3,4-13C3	A	2,5	7,25	S,C
		Estrone	3.13	10.3	Estrone-3,4-13C2	A	2,5	6,19	S,I
Nervous stimulant		Caffeine	-0.07	14	Caffeine-13C3	A	4,14	5,16	S,I
Artificial sweetener		Sucralose	-1[2]	11.8[3]	Sucralose-d6	A	7,22	9,30	S,T

[1] National Center for Biotechnology Information (2004), [2] (Subedi & Kannan, 2014a), [3] (Busetti et al., 2015); [4] LODs and LOQs were obtained based on standard deviation of y-intercept of measured concentrations of serial dilution, [5] Companies: **S:** Sigma-Aldrich Canada (Oakville, ON, Canada), **I:** C/D/N Isotopes (Pointe-Claire, QC, Canada), **C:** Cerilliant Corporation (Round Rock, Tex, USA), **M:** Cambridge isotope Laboratories (Tewksbury, MA, USA), **T:** Toronto Research Chemicals (North York, ON, Canada), **K:** KICTeam (Langely, BC, Canada).

5.4.2. Study site

The study site is a municipal WWTP located in Guelph, ON, Canada, serving a population of approximately 134,894, having a design capacity of 64,000 m^3/d and receiving an average incoming flow rate of 50,755 m^3/d of domestic, commercial and industrial wastewater. The WWTP (Figure 5.1) provides preliminary treatment by screening and aerated grit removal, after which the load is split into four activated sludge lines. Each of the lines consists of two primary clarifiers, two aeration tanks and one or two secondary clarifiers in parallel. The WWTP provides tertiary treatment for the recombined effluent of the lines by rotating biological contactors (RBCs), followed by sand filtration. The tertiary treatment step was added to the treatment sequence in order to meet regulations concerning the levels of TSS (i.e. 10 mg/L) and ammonia nitrogen (i.e. 3.4 mg/L) in the effluent. For disinfection, chlorine is added to wastewater after the RBCs as 12% sodium hypochlorite at an average chlorine dosage of 1760 kg/d. The disinfected de-chlorinated final effluent is discharged into the Speed River. Table 5.2 summarizes the main characteristics of line 1 in the WWTP since only line 1 was investigated in in the present study.

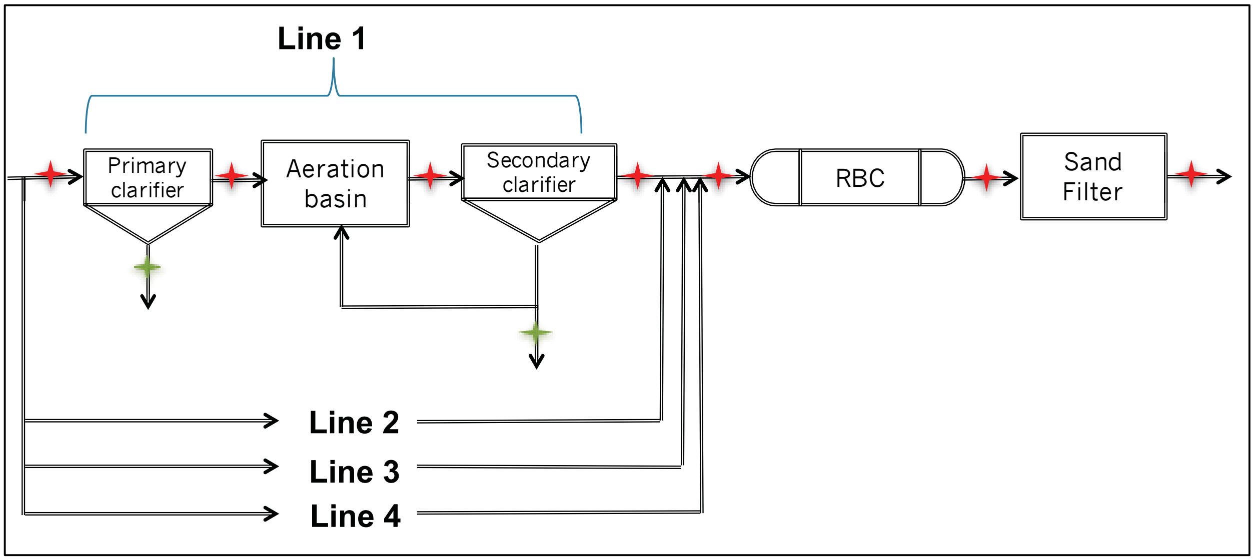

Figure 5.1 Schematic of the WWTP. Lines 1-4 correspond to the four lines of primary and secondary treatment. Red marks represent locations where conductivity probes were deployed and aqueous samples were collected. Green marks represent locations where sludge samples were collected.

Table 5.2 Main characteristics of the WWTP, including hydraulic retention time (HRT), solids retention time (SRT) of treatment units in line 1, average temperature and pH during sampling campaigns.

Characteristic	Location	Value
HRT (h)	Primary clarifier (line 1)	3.72
	Aeration tanks (line 1)	6.3
	Secondary clarifier (line 1)	2.9
	RBCs	0.8
	Sand filter	0.4
SRT (days)[1]	(line 1)	7.76
Average T (°C)		20
Average pH		8.03

[1] The SRT was calculated by dividing total solids in the activated sludge unit of line 1 (volume times sludge concentration) by the wasted sludge (waste flow times waste sludge concentration)

5.4.3. Hydraulic model

Electrical conductivity was utilized as a tracer for the investigation of the residence time distribution as proposed earlier (Ahnert et al., 2010; Majewsky et al., 2011). HOBO conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada) were used to collect electrical conductivity and temperature data (one reading per minute) for hydraulic model calibration. The probes were deployed over 3 weeks (June 12 – July 8, 2014) before and after each treatment unit (red marks in Figure 5.1). A universal optic-USB base station and Onset HOBOware Pro Version 3.2.2 software (Hoskin Scientific, St-Laurent, QC, Canada) were used for data transfer and read out, respectively.

The hydraulic model was created in the simulation software WEST (Mike Powered by DHI, Hørsholm, Denmark). Each part of the treatment plant was modelled separately, using the measured electrical conductivity at the entrance of each treatment step as tracer input for the respective hydraulic model, along with the actual flow conditions and tank volumes. A good fit between the simulated and measured electrical conductivities at the exit of each treatment step was obtained by varying the number of tanks in series (aeration tanks) and the number of layers and feed layer (clarifiers) which determine the flow regime (i.e. from plug flow to fully mixed). The best-fit model was determined on the basis of minimizing the root mean square error (RMSE) and visual inspection of the output graphs. Simulations were run with the calibrated hydraulic model of each treatment unit employing a 24-h step increase of inert tracer as input, along with the actual flow rates during the sampling period. The output was used to obtain the load fractions that describe how the material in the effluent of each treatment stage on a certain day is composed out of fractions of the influent to that treatment step over several days, as illustrated by Majewsky et al. (2011).

5.4.4. Wastewater sampling

Wastewater was collected before and after each treatment unit at the locations shown in Figure 5.1 (red marks). The number of sampling days was based on the best-fit hydraulic model that indicated that the effluent of the activated sludge unit on a given day is composed of influent material entering the WWTP over four days, which will be elaborated upon in the results section. The samples collected during the first sampling campaign (July 21-25, 2014) were analyzed for the Class A compounds, while the samples collected during the second campaign (June 16-20, 2015) were analyzed for the Class B compounds. The samples were collected as 24-h composite using onsite Hach Sigma samplers that collect flow-proportional samples and refrigerate them at 4° C. This is with the exception of the effluent of the RBC, where ISCO 6712 samplers (Avensys, St-Laurent, QC, Canada) equipped with 24 bottles and packed with ice replaced daily were used to collect 24-h time-proportional composite samples. Both samplers collected samples every 15 minutes. Primary and secondary waste sludge samples were collected as grab samples over the four days (green marks in Figure 5.1). At the end of each day of the sampling campaign, the collected aqueous and sludge samples were transferred into 1-L amber HDPE bottles (Fisher Scientific) and stored at -20 °C until extraction was performed (within 3 weeks).

5.4.5. Sample preparation

5.4.5.1. Wastewater samples

Wastewater samples were thawed and filtered using 1-μm glass-fiber filter (Fisher Scientific) prior to extraction. Volumes of 100 mL of raw wastewater influent and 200 mL of all other sample matrixes were spiked with the appropriate internal standards (listed in Table 5.1). Solid phase extraction (SPE) was performed using two different methods. Class A compounds were extracted with Oasis MAX anion exchange cartridges (Waters Corporation), as described by Metcalfe et al. (2014). Class B compounds were extracted with Oasis MCX cation exchange cartridges, as described by Yargeau et al. (2014a). Both methods and instruments used are summarized in the supplementary material (Table 5.6). SPE recoveries of target compounds ranged from 71% to 130% for Class A compounds (average recovery of 75%) and from 60% to

100% for Class B compounds (average recovery of 78%). Glass containers pre-cleaned with hexanes and acetone were used for all sample preparation and analysis experiments.

5.4.5.2. Sludge samples

Approximately 1 g of freeze-dried sludge was placed in accelerated solvent extraction (ASE) stainless steel cells and spiked with internal surrogates (Table 5.1, 100 ng/g) before extraction. Extraction of sludge was conducted by pressurized liquid extraction using a Dionex ASE 350 accelerated solvent extraction system (Thermo Fisher Scientific, Waltham, MA, USA), followed by SPE clean up based on the methods summarized in the supplementary materials (Table 5.6). For the Class A compounds, the two extraction methods used were described by Edwards et al. (2009) as the neutral drug method and the acidic drug method. However, the neutral drugs method with acetone and water (3:7) as the ASE extraction solvent (Table 5.6) gave the highest recoveries in the sludge matrix for this study. For class B compounds (mainly drugs of abuse), the extraction method that achieved the highest recoveries was the one suggested for the beta-blocker atenolol in the same study by Edwards et al. (2009) and is also summarized in Table 5.6. All sludge samples were extracted in triplicates and the extraction efficiencies of target compounds were >70%.

5.4.6. Analysis

Analysis of the Class A compounds was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) using an Agilent 1100 HPLC (Mississauga, ON, Canada) coupled to a Q-Trap 5500 instrument (AB Sciex, Concord, ON, Canada) operated with a turbospray ionization source. The Class A target compounds of all samples were separated chromatographically using the method described by Metcalfe et al. (2014). The analytes were measured in either negative or positive ion mode, depending on the compound. Acquisition was performed using the precursor and product ion transitions for multiple reaction monitoring (MRM) of the target compounds and their corresponding deuterated surrogates. The MRM transitions for the target compounds are listed elsewhere (Metcalfe et al., 2014; Thompson et al., 2011).

Analysis of the Class B compounds was conducted by liquid chromatography with high-resolution mass spectrometry (LC-HRMS) using an Accela LC system coupled to a LTQ

Orbitrap XL (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation and analysis in positive ion mode was achieved using the methods described by Rodayan et al. (2014b). Acquisition was performed in full scan mode (50–400 m/z) at high resolution (RFWHM = 41,000). The ion of interest was extracted using an m/z window of ±0.01. Linear calibration curves of nine points were used for quantification of the concentrations of the target compounds of both classes. Recoveries of the internal standards were used to adjust the concentrations of all target analytes.

5.4.7. CEC removals

The equations used for the calculations of CEC removals from the aqueous phase are presented in Eqs. 1-3 and Figure 5.6 in the supplementary material. Calculation of the CEC removals was first based on the CEC load in the aqueous phase only of the input and output streams of each treatment unit, as described in Eq. 5.1. To account for the residence time distribution, a fractionated input load of the CEC that corresponds to the output load on the fourth day of sampling was calculated (using Eq. 5.2), as proposed by Majewsky et al. (2011). The term “input reference load” will be used throughout the paper to refer to the incoming fractionated load of each treatment unit. The input reference load was compared to the output load to obtain estimates of the CEC removals per treatment unit (Eq. 5.3).

$$L_{aq} = Q * C_{aq} \tag{5.1}$$

$$L_{aq,\,ref} = \sum_{i=1}^{i=4} f_i * L_{aq,\,in,\,i} \tag{5.2}$$

$$R = \frac{L_{aq,ref} - L_{aq,out}}{L_{aq,ref}} * 100\% \tag{5.3}$$

Where L_{aq}: Load of the contaminant in the aqueous phase of a specific stream (mg/d), C_{aq}: Concentration of the contaminant in the aqueous phase of a specific stream (mg/L), Q: Flow rate of the corresponding stream (L/d), $L_{aq,\,ref}$ (mg/d): Input reference mass load of the contaminant in the aqueous phase of the input stream based on several days of sampling, $L_{aq,\,in,\,i}$ (mg/d): Mass load of the contaminant in the input stream on the ith day of sampling, f_i : Fraction of incoming contaminant load on the ith day of sampling that is contained in the outgoing load on the last day of sampling, $L_{aq,out}$ (mg/d): Mass load of the CEC in the aqueous phase of the output stream on the last day of sampling, R (%): Removal of CEC from the aqueous phase in a specific treatment unit using the fractionated approach.

Mass balances were then carried out across the primary and biological treatment steps based on the total contaminant load in both aqueous and particulate phases, according to Eq. 5.4-5.7. The total load of the CEC on each day was obtained in the input stream ($L_{tot,in}$), output stream ($L_{tot,out}$) and sludge stream ($L_{tot,sludge}$) by summing particulate and dissolved loads, as shown in Eq. 5.4. The input reference load was also obtained by the fractionated approach (Eq. 5.6). In both the primary and biological treatment stages, the primary mechanisms of removal of the studied CECs are biodegradation and sorption to solids (Andersen et al., 2005; Li & Zhang, 2010; Radjenović et al., 2009b; Verlicchi et al., 2012). Abiotic removal due to hydrolysis or photolysis was previously investigated for some of the studied CECs and reported to be negligible (Li & Zhang, 2010; Pèrez et al., 2005). Volatilization is also expected to be limited, due to the low Henry constants of the studied CECs (Gao et al., 2012). Therefore, the difference between the total incoming load and the outgoing load (in the output stream or sorbed to sludge) was assumed to be the load that was lost due to (bio)degradation (L_{deg}), as shown in Eq. 5.7. It should be kept in mind that this load could also include experimental or modelling errors that could cause a bias in the results.

$$L_{tot} = L_{aq} + L_s \tag{5.4}$$

$$L_s = Q * C_s * TSS \tag{5.5}$$

$$L_{tot,\,ref} = \sum_{i=1}^{i=4} f_i * L_{tot,\,in,\,i} \tag{5.6}$$

$$L_{deg} = L_{tot,ref} - L_{tot,out} - L_{tot,sludge} \tag{5.7}$$

Where L_{tot}: Mass load of the contaminant in the liquid and particulate phase of a certain stream (mg/d), L_s: Mass load of contaminant measured in the particulate phase of a stream (mg/d), C_s: Concentration of contaminant measured in the particulate phase of a stream on a dry weight basis (mg/g) and TSS: Total suspended solids in the corresponding stream (g/L), which was collected by the WWTP operators following the APHA/AWWA protocol (Rice et al., 2012). L_{aq} is computed using Eq. 5.1, except in the sludge stream where the volume fraction of solids is more significant, so $L_{aq,\,sludge} = Q_{sludge} * C_{aq,\,sludge} * f_w$, f_w: Volume fraction of water in sludge, L_{deg}: Mass load of the contaminant that was (bio)degraded (mg/d).

The measured CEC concentrations were used to calculate the K_d value, defined by Eq. 5.8, to represent the partitioning of CECs between the dry solids and the aqueous phase for primary and secondary waste sludge, separately. Error bars for loads, removals and K_d values were obtained based on standard deviations from lab triplicates using the propagation of error formulas (Ku, 1966). Investigating the statistical significance of the difference between the incoming and the outcoming loads (Section 5.5.7) was performed using the unequal-variance two-sample t-test with a confidence level of 95%.

$$Log\ K_d = \log\left(\frac{C_s}{C_{aq}}\right) \tag{5.8}$$

5.5. Results and Discussion

5.5.1. Calibration of hydraulic model and load fractions

The hydraulic mixing of the aeration tanks in line 1 was best described by three continuous-stirred tanks (CSTs) in series, all having equal volume and each representing perfect mixing. This is consistent with previous studies where the hydrodynamics of the aeration tanks in the activated sludge process were represented by a number of perfectly mixed tanks in series (Majewsky et al., 2011; Rodayan et al., 2014a). The primary and secondary clarifiers were both best modelled with a 10-layer settling tank and the 5th layer as feed layer. Figure 5.7 illustrates that the simulated and the measured output conductivity profiles were in good agreement for all treatment steps, verifying the goodness of the fit of the obtained hydraulic model. The RBC hydraulic behaviour was modelled by a single CST and the sand filter as a series of 2 CSTs. The volumes for the RBCs and sand filters were adjusted until the simulated effluent matched the actual hydraulic behaviour, as it was not possible to estimate the actual hydraulic volume. The obtained volume was small, leading to low retention times, consistent with data obtained from operators at the WWTP. The absence of significant shifts or damping in the dynamics (Figure 5.7) demonstrates the minimal mixing occurring in both the RBCs and the sand filters due to their very short HRTs (see Table 5.2).

The obtained load fractions for the removal calculations are summarized in Table 5.3. For a selected treatment unit, load fractions represent the fractions of CEC incoming load on different days that make their way to the output on the last day of sampling, assuming no removal. Hence, these fractions do not necessarily add up to 100%. For both the primary and secondary clarifier the effluent was comprised of 92% of CEC load in the influent of the last day of sampling and only 8% of the day before. By contrast, the effluent of the aeration tank was composed of 73% of the CEC load on the same day and 12% from the day before, which can be related to the higher degree of mixing that occurs in the aeration tanks. The effluent from the activated sludge unit had even more distributed load fractions (64% from the last day and 36% from the previous day, 1% of three days ago and 0.04% of four days ago) due to the recycling of sludge. The load fractions of the activated sludge unit indicated the need for four days of sampling. For the RBCs and sand filters, only 4% and 3% respectively of CEC load on a previous day was present in the effluent on a given day, which is attributed to their low HRTs (Table 5.2), resulting in low mixing.

Table 5.3 Load fractions for each treatment unit ($\boldsymbol{f_i}$) describing the fraction of incoming CEC load on day i that is contained in the output of day 4 (last day of sampling) assuming no removal (including hydraulics effect only).

Treatment unit	Load fractions (%)			
	f_1	f_2	f_3	f_4
Primary clarifier	0	0	8	92
Aeration tanks	0	0	12	73
Secondary clarifier	0	0	8	92
Activated sludge (combination)	4E-02	1	36	64
RBCs	0	0	4	96
Sand filter	0	0	3	97

5.5.2. Concentrations of CECs in wastewater

The concentrations of the CECs at the different treatment steps are shown in Table 5.4. Most of the target compounds were detected in the influent to the primary clarifier, except for the prescription opioid drugs, fentanyl and ketamine. The lowest concentration of a target compound detected in the influent was observed for estrone (7 ng/L) and the highest mean concentration was for caffeine (28,960 ng/L). Among all target pharmaceuticals, ibuprofen, was present at the highest levels in influent wastewater. Selected metabolites of cocaine, methadone and carbamazepine were monitored as well. In the case of cocaine, only 1-9% is excreted unchanged from the human body, while 35-54% is excreted as benzoylecgonine (Ratola et al., 2012). The ratio between benzoylecgonine and cocaine was calculated to be 1.5. Despite the fact that the majority of studies reported values in the range of 3.1-3.5, lower values similar to the present study were reported in some studies (Bones et al., 2007), suggesting that some cocaine is being discharged into the sewage system without consumption (Karolak et al., 2010; Ratola et al., 2012).

Table 5.4 shows that the CEC concentrations in the combined secondary effluent (i.e. combined output from all the lines, Figure 5.1) were generally in the same ranges as the concentrations in the secondary effluent (i.e. from line 1), indicating that in terms of CEC removal, line 1 had a similar efficiency at removing CECs as the other three lines combined. In the final effluent, some target compounds, nine out of 26 CECs were not detected at concentrations above the limits of detection (LODs) or limits of quantification (LOQs). The highest effluent levels were observed for sucralose (3476 ng/L) followed closely by caffeine (2015 ng/L), unlike in the influent where caffeine had levels one order of magnitude higher than sucralose.

Table 5.4 Concentrations (ng/L ± standard deviation) of target CECs in line 1 of the WWTP at the influent to the primary clarifier (primary influent), effluent of the primary clarifier (primary effluent), effluent of the secondary clarifier (secondary effluent), as well as the combined secondary effluents of all lines (1-4), and effluent of RBCs (RBCs effluent) and sand filter effluent. Standard deviation was based on 3 replicates of sample preparation and analysis.

Compound	Primary influent (Line 1)	Primary effluent (Line 1)	Secondary effluent (Line 1)	Combined secondary effluent	RBCs effluent	Sand filter effluent
Androstenedione	92 ± 5	65 ± 2	42 ± 2	41 ± 2	40 ± 1	44 ± 1
Estrone	7 ± 3	8 ± 2	5 ± 1	7 ± 3	5 ± 1	6 ± 2
Trimethoprim	25 ± 2	25 ± 3	37 ± 3	29 ± 3	22 ± 2	<LOD
Sulfamethoxazole	33 ± 5	31 ± 5	43 ± 16	47 ± 21	37 ± 6	<LOQ
Ibuprofen	3644 ± 105	1690 ± 90	<LOQ	<LOQ	<LOQ	<LOQ
Naproxen	471 ± 36	504 ± 35	36 ± 5	20 ± 3	18 ± 2	<LOQ
Triclosan	166 ± 26	132 ± 11	50 ± 2	31 ± 4	42 ± 3	29 ± 2
Caffeine	28960 ± 4658	28123 ± 4750	1756 ± 226	2148 ± 127	2245 ± 194	2015 ± 270
Sucralose	2437 ± 220	2635 ± 166	4591 ± 291	3178 ± 84	3175 ± 209	3476 ± 129
Cocaine	361 ± 18	287 ± 30	111 ± 26	97 ± 21	<LOQ	<LOQ
Benzoylecgonine	524 ± 14	415 ± 18	83 ± 5	58 ± 4	50 ± 2	47 ± 3
Amphetamine	101 ± 2	77 ± 3	<LOQ	<LOD	<LOD	<LOD
Methamphetamine	300 ± 7	213 ± 10	47 ± 5	43 ± 2	23 ± 0.2	<LOD
EDDP	249 ± 29	299 ± 21	231 ± 55	156 ± 7	169 ± 15	156 ± 4
Ephedrine	1616 ± 62	1053 ± 61	111 ± 8	86 ± 8	72 ± 1	72 ± 3
Codeine	2116 ± 92	1204 ± 40	1281 ± 109	901 ± 16	529 ± 15	130 ± 5
Dihydrocodeine	324 ± 23	459 ± 23	22 ± 1	39 ± 3	28 ± 1	27 ± 1
Methadone	123 ± 3	98 ± 3	48 ± 4	54 ± 2	44 ± 1	42 ± 2
Morphine	295 ± 12	338 ± 61	68 ± 19	24 ± 3	23 ± 4	21 ± 5
Oxycodone	126 ± 10	137 ± 24	64 ± 3	46 ± 3	35 ± 3	27 ± 7
Tramadol	174 ± 6	116 ± 8	177 ± 11	105 ± 5	125 ± 7	105 ± 5
Ketamine	<LOD	<LOD	<LOQ	<LOQ	<LOQ	<LOQ
Fentanyl	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carbamazepine	591 ± 24	416 ± 20	606 ± 28	588 ± 17	532 ± 8	519 ± 13
CMZ-DIOH	1074 ± 54	702 ± 40	553 ± 51	650 ± 26	703 ± 21	648 ± 49
CMZ-EP	192 ± 36	258 ± 40	295 ± 41	315 ± 44	385 ± 62	119 ± 32

5.5.3. Removals by primary clarifier

5.5.3.1. Aqueous phase

The CEC removals (%) based on aqueous phase data at each treatment step were estimated using the fractionated approach, as well as concentrations at the input and output streams (Figure 5.2). The results obtained in the present study are generally comparable to results from the literature summarized in the Supplemental Material (Table 5.7). Poor removals from the aqueous phase of wastewater (<40%) were observed for all target CECs in the primary clarifier, with the highest removals observed for tramadol and codeine (Figure 5.2). Negative removals in the primary clarifier were observed for a number of CECs, namely oxycodone, naproxen, estrone, EDDP and dihydrocodeine (Figure 5.2). Some of these CECs were previously reported to have negative removals (Table 5.7).

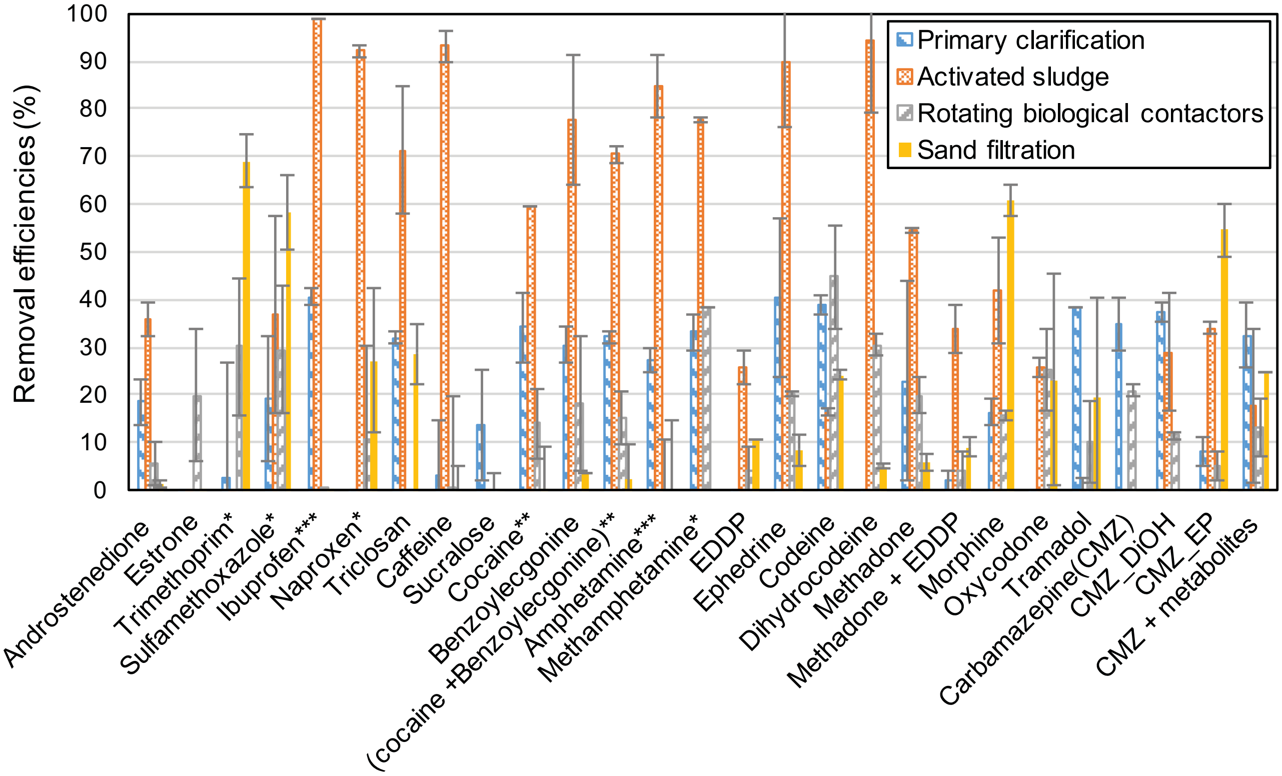

Figure 5.2 Estimated removal efficiencies (%) of target CECs. The removal efficiency was based on the incoming load to each treatment unit. ***, **, * denote compounds that were <LOD or <LOQ in the effluent of the activated sludge, RBC and sand filter, respectively, so removal was based on LOD (for <LOD) or LOQ (for <LOQ) and may be higher than reported here. Absence of a column indicates that either the removal was negative or the compound was <LOD or <LOQ in the influent to the treatment unit.

5.5.3.2. Primary sludge

Concentrations of CECs in primary sludge, along with estimated $log\ K_d$ values for the CECs in primary sludge are summarized in Table 5.5. The average concentration of the selected CECs in the solids of the primary sludge was the highest for caffeine, followed by triclosan and codeine; however, triclosan was found to have the highest $log\ K_d$ value (Table 5.5). High concentrations in primary sludge can be attributed to high incoming concentrations (i.e. codeine and caffeine) in the wastewater, or high partitioning to sludge because of high hydrophobicity (i.e. triclosan, Table 5.5.1). Some CECs (i.e. naproxen, sucralose and ketamine) were not detected in the solids of primary sludge (Table 5.5), which is consistent with previous studies (Brorström-Lunden, 2008b; Jelic et al., 2011; Radjenović et al., 2009b). The data reported in the present study on the levels drugs of abuse in primary and secondary waste sludge are valuable additions to the limited research in this area (Subedi & Kannan, 2015). The estimated $log\ K_d$ values for drugs of abuse indicate that these compounds are poorly sorbed to particulates, and the concentrations detected in the aqueous phase of untreated wastewater can thus be used to estimate community drug consumption using the “sewage epidemiology” approach (Castiglioni et al., 2006b).

The total input reference mass load of the CECs to the primary clarifier was assigned to different fate pathways, and the percentage of each pathway was calculated (Figure 5.3). According to data obtained from operators at the WWTP, the primary clarifier removes 84% of TSS on average. This is higher than normal and can be attributed to the high HRT of 3.72 h as opposed to the usual HRT of 2-3 h in primary clarifiers under dry weather conditions. Also, the chemical addition of ferric chloride for phosphorus removal enhances the settling of solids, contributing to the high TSS removal. Despite the high TSS reduction, the fraction of CECs that is removed with sludge was less than 5% of the total incoming load for 15 out of 22 compounds (Figure 5.3) owing to their hydrophilic properties (Table 5.1). This fraction was previously reported to be less than 0.1% for ibuprofen, naproxen, sulfamethoxazole and caffeine in primary treatment (Carballa et al., 2007; Gao et al., 2012). Ternes et al. (2004a) suggested that compounds with $K_d < 500$ L/Kg or $log\ K_d < 2.70$ often have a negligible fraction sorbed to sludge. In the present study, the estimated $log\ K_d$ values for androstenedione, trimethoprim, triclosan and carbamazepine were above this threshold (Table 5.5), and these compounds were observed to be the only four compounds with more than 10% of their incoming load sorbed to primary sludge.

Table 5.5 Average concentrations of target CECs in primary and activated sludge and the range of concentrations on the four days of sampling represented as average (lowest –highest), along with the estimated $\boldsymbol{log\ K_d}$ ± standard deviation in primary and activated sludge.

Compound	Average Concentrations (Lowest-highest) (ng/L)		$Log\ K_d$ ± standard deviation (Log L/Kg)	
	Primary sludge	Activated sludge	Primary clarifier	Secondary clarifier
Androstenedione	98 (85-113)	39 (15-65)	3.20 ± 0.19	2.92 ± 0.37
Estrone	<LOD	28 (<LOD-53)	<LOD	3.65 ± 0.32
Trimethoprim	31 (24-43)	154 (92-198)	3.07 ± 0.19	3.59 ± 0.18
Sulfamethoxazole	12 (6-17)	<LOQ	2.15 ± 0.49	<LOQ
Ibuprofen[1]	196 (73-496)	39 (21-59)	2.27 ± 0.64	3.36 ± 0.38
Naproxen	<LOD	23 (<LOQ-25)	<LOD	2.95 ± 0.32
Triclosan	599 (340-909)	1335 (1199-1377)	3.68 ± 0.11	4.49 ± 0.27
Caffeine	2828 (1536-4716)	776 (674-953)	2.10 ± 0.37	2.73 ± 0.31
Sucralose	<LOD	152 (110-218)	<LOD	1.53 ± 0.19
Cocaine	27 (16-35)	<LOD	1.93 ± 0.18	<LOD
Benzoylecgonine	5 (<LOD-8)	<LOQ	1.40 ± 0.07	<LOQ
Amphetamine[1]	20 (7-37)	13 (5-20)	2.29 ± 0.38	3.09 ± 0.33
Methamphetamine	14 (11-17)	4 (<LOD-4)	1.83 ± 0.02	1.92
EDDP	89 (73-109)	65 (44-95)	2.46 ± 0.1	2.44 ± 0.17
Ephedrine	47 (22-69)	26 (<LOQ-55)	1.60 ± 0.48	2.16 ± 0.5
Codeine	202 (77-305)	36 (<LOQ-53)	2.17 ± 0.26	1.36 ± 0.4
Dihydrocodeine	29 (10-44)	41 (18-56)	1.71 ± 0.08	3.20 ± 0.31
Methadone	49 (7-125)	46 (<LOQ-125)	2.57	2.55 ± 0.71
Morphine	96 (40-171)	76 (37-117)	2.61 ± 0.4	2.99 ± 0.29
Oxycodone	25 (9-42)	8 (<LOD-13)	2.09 ± 0.45	1.97 ± 0.44
Tramadol	92 (21-232)	7 (<LOD-11)	2.58 ± 0.5	1.49 ± 0.36
Ketamine	<LOQ	<LOQ	<LOQ	<LOQ
Fentanyl	18 (11-27)	23 (15-31)	N.A.	N.A.
Carbamazepine[2]	49 (32-78)	27 (22-34)	2.75 ± 0.21	2.29 ± 0.04
CMZ-DiOH	<LOD	<LOD	<LOD	<LOD
CMZ-EP	<LOD	<LOD	<LOD	<LOD

[1]Denotes compounds that were <LOD or LOQ in the secondary effluent, indicating that the obtained $log\ K_d$ in the secondary clarifier might not be accurate as it was based on the LOD or LOQ of the compound, [2]Carbamazepine solids analysis was based on the 2014 sampling campaign.

Despite the generally low biological activity of primary clarifiers, some of the removal during primary clarification can be attributed to (bio)degradation, as shown in Figure 5.3. The percentage of incoming load degraded during primary clarification was as high as 40-54% for a number of the investigated CECs (i.e. ibuprofen, morphine, codeine, triclosan and ephedrine). Considering the small error bars of the mass loads in Figure 5.3 (except for triclosan), this high (bio)degradation in the primary clarifier is unlikely attributed to measurement bias caused by sample preparation and analysis. McCall et al. (2016) showed in a review article that several studies reported the formation of transformation products of drugs of abuse in the sewers (HRT 30 min to 12 h) under different conditions, and Heuett et al. (2015) reported the detection of transformation products of drugs of abuse in influent sewage to a WWTP. The degradation of some pharmaceuticals and formation of transformation products in a real sewer pipe was also reported by Jelic et al. (2015). Having similar conditions as in sewers, primary clarifiers could also allow (bio)degradation of CECs. In addition, the primary clarifier under study has a higher HRT (3.72 h) than usual, which could explain the high observed CEC (bio)degradation. In fact, most of the compounds with the highest (bio)degradation in the primary clarifier also showed high (bio)degradation potential in the activated sludge (Figure 5.4). It should be noted that the sum of the percentages removed by (bio)degradation and removed with sludge (Figure 5.3) is not equivalent to the percentage removal from the aqueous phase calculated previously (Figure 5.2). This is explained by the fact that the input reference mass load used in the mass balance calculations includes not only CEC loads in the aqueous phase, but also in the particulate phase. The CEC concentrations in the suspended particulate phase of primary inlet load were assumed to be equal to those in primary sludge, making the assumption that equilibrium partitioning of CECs between the particulate and aqueous phases takes place before the primary clarifier.

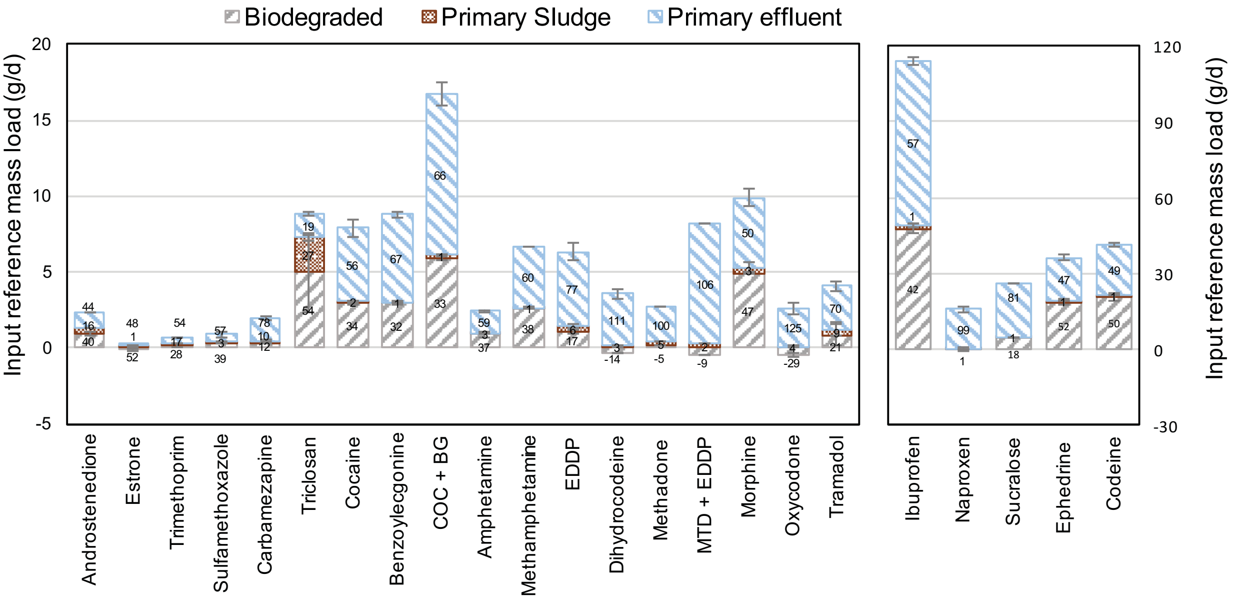

Figure 5.3 Input reference mass loads (g/d) of target CECs into the primary clarifier assigned into three main fate pathways: (bio)degraded, discharged with primary sludge and discharged with the primary effluent with the % contribution of each pathway indicated in the corresponding column (COC: cocaine, BG: benzoylecgonine, MTD: methadone). For caffeine (not shown due to the high mass loads): 14% biodegraded, 2% sorbed to primary sludge and 84% in the effluent.

5.5.4. Removals by activated sludge

5.5.4.1. Aqueous phase

The estimated removals of CECs (%) during the activated sludge treatment step are illustrated in Figure 5.2. In general, for the majority of the compounds, most of the removal takes place in the activated sludge treatment stage when compared to other treatment steps. Amongst the target compounds, the removals varied from >80% for six target CECs, namely ibuprofen, naproxen, amphetamine, ephedrine, dihydrocodeine and caffeine to negative removals for carbamazepine, tramadol, estrone, sucralose and trimethoprim. The variable data on CEC removal by activated sludge treatment reported in the literature is summarized in the supplementary material (Table 5.7).

5.5.4.2. Secondary sludge

The measured concentrations of CECs in the solids of secondary sludge and estimates of the $log\ K_d$ values for secondary sludge are summarized in Table 5.5. Triclosan followed by caffeine and trimethoprim were shown to have the highest average concentrations in secondary sludge. Similar to the analysis of the primary sludge, triclosan had the highest estimated $log\ K_d$ value (i.e. 4.49) for secondary sludge. Caffeine and sucralose ($log\ K_{ow}$ -0.07 and -1, respectively), on the other hand, had high concentrations in secondary waste sludge (Table 5.5) owing to their high loads to the biological treatment (Table 5.4). The differences in the estimated $log\ K_d$ values between primary and secondary waste sludge for each of the CECs can be explained by variable sludge composition (Ternes et al., 2004a).

Figure 5.4 displays the CEC input reference load to the activated sludge unit assigned to different fate pathways. The reference mass loads were calculated based on the assumption that the CEC concentrations in the suspended solids contained in the output of the primary and secondary clarifiers were the same as those measured in the primary and secondary sludge, respectively. This involves making the assumption that the CEC particulate concentration is uniform throughout the primary and secondary clarifiers. Discharge with the secondary waste sludge was found to be the predominant removal mechanism for trimethoprim, triclosan and estrone, with 55%, 51% and 33% of their input reference load, respectively ending up in the secondary waste sludge. For all the remaining compounds, a low fraction (i.e. <5%) of the input reference load was discharged with secondary waste sludge (Figure 5.4), due to their hydrophilic nature ($log\ K_{ow}$, Table 5.1). This is in good agreement with previous research for a number of the target compounds whose fate in activated sludge units has been previously investigated through mass balance (i.e. ibuprofen, naproxen, carbamazepine, sulfamethoxazole and caffeine) (Gao et al., 2012; Joss et al., 2005; Petrie et al., 2014).

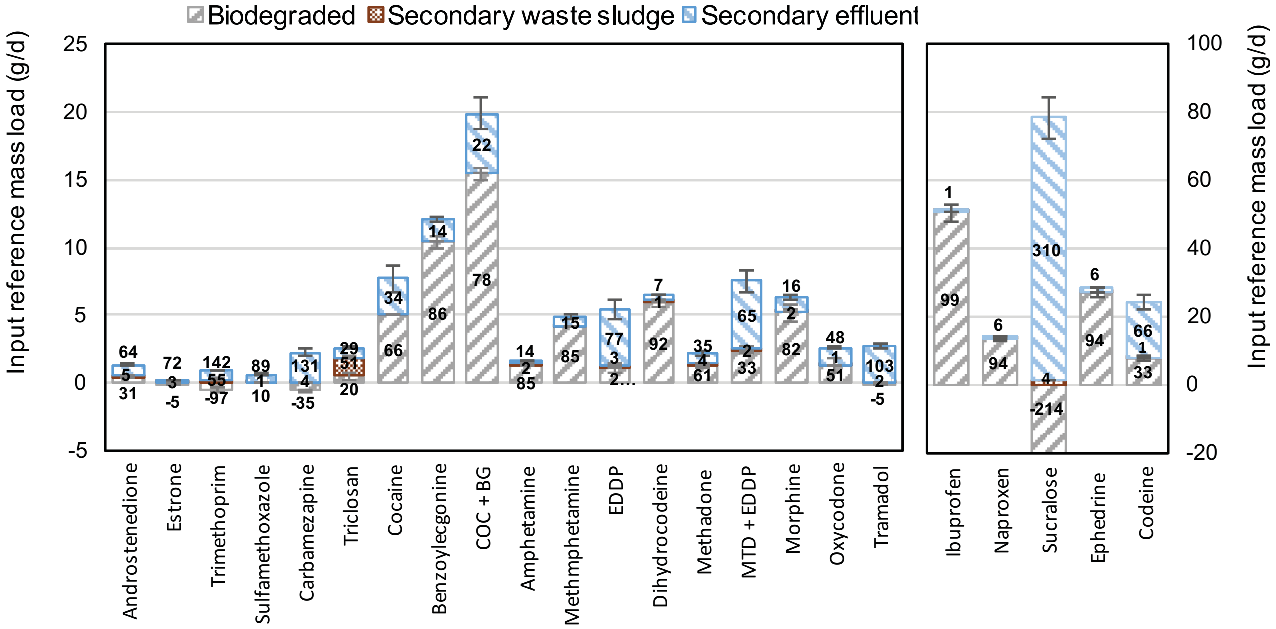

Figure 5.4 Input reference mass loads of target CECs into activated sludge assigned into three main fate pathways: (bio)degraded, discharged with waste activated sludge and discharged with the secondary effluent with % of each pathway in columns (COC: cocaine, BG: benzoylecgonine, MTD: methadone). For ibuprofen and amphetamine, the concentration in the effluent was <LOQ, so the LOQ was used for calculations. For caffeine (not shown due to the high mass loads): 92% biodegraded and 8% in the effluent.

(Bio)degradation was the predominant removal mechanism for 17 out of the 22 target CECs. More than 90% of the incoming load was removed by (bio)degradation in the case of ibuprofen, naproxen, ephedrine, dihydrocodeine, as well as caffeine (not shown in Figure 5.4). Substantial (bio)degradation of ibuprofen, naproxen and caffeine was also reported by other authors (Carballa et al., 2007; Gao et al., 2012; Joss et al., 2005). For the majority of the compounds studied, the mass fraction removed by (bio)degradation (Figure 5.4) and the data on removals from the aqueous phase (Figure 5.2) were comparable. This indicates that (bio)degradation is responsible for the majority of the removal of the studied CECs from the aqueous phase in the activated sludge unit, while removal by adsorption is of insignificant importance. By contrast, the fraction of biodegraded material was negative for several CECs, such as carbamazepine, sucralose, trimethoprim and tramadol, which indicated that the mass loads in the secondary output were higher than the incoming loads, consistent with their negative removal from the aqueous phase (Figure 5.2). The use of the fractionated approach accounting for RTD of the treatment units limited the bias associated with the sampling strategy and removal calculations but some negative removals were still observed. These negative removals are, therefore, likely due to the presence of conjugated forms of the compound that transform into the parent compound during treatment, as well as desorption from particulate matter (Jelic et al., 2011; Ternes & Joss, 2006). For carbamazepine and trimethoprim, the absence of removal by biodegradation obtained in this study is in agreement with previous studies (Gao et al., 2012; Joss et al., 2005; Li & Zhang, 2010; Petrie et al., 2014).

5.5.5. Removals by RBCs

The WWTP was upgraded to include RBCs in order to meet regulations with regards to the ammonia nitrogen levels in the effluent (i.e. maximum concentration of 3.4 mg/L). Although the ammonia nitrogen in the secondary effluent was reported to be <1 mg/L during the summer, indicating full nitrification by the activated sludge process, levels as high as 6.6 mg/L were observed over several days during the winter. The RBCs removed ammonia nitrogen and achieved effluent levels below 2.41 mg/L throughout the year. In spite of their capability to remove ammonia nitrogen, the RBCs were generally ineffective at removing most of the target CECs (i.e. <30%), except for methamphetamine, codeine and dihydrocodeine, as shown in Figure 5.2. Additionally, the CECs whose removal calculation had to be based on LOQ or LOD due to the fact that they were below these limits (marked with two stars) could also have high removals. Among the target CECs, low average removals of <15% were observed for 11 of the 26 target CECs. Negative removals at this treatment stage were observed for sucralose and triclosan, indicating the possible de-conjugation of their conjugates during tertiary treatment, as in the activated sludge treatment stage or possible sampling bias. The fate of a limited number of the target compounds (i.e. trimethoprim, sulfamethoxazole, triclosan, ibuprofen, carbamazepine and caffeine) has been investigated before in RBCs, and their obtained removals were higher than in the present study (Batt et al., 2007; Kanda et al., 2003; Thompson et al., 2005; Vasiliadou et al., 2014). This can be explained by the fact that the RBCs are used as tertiary treatment in this study, with a low HRT (i.e. 0.8 h) and little organic material available for biofilm growth, while in the previous studies, RBCs were used for secondary treatment as an alternative to activated sludge treatment. The contribution of the particulate CEC load to the total load in the RBCs and the sand filter is deemed insignificant, since the TSS load entering these units is low (<20 mg/L). Hence, a detailed mass balance was not performed for these units.

5.5.6. Removals by sand filtration

Similar to the RBCs, the average removal of CECs by sand filtration and chlorination was based on the incoming load in the aqueous phase only, due to the low TSS load. The removal was greater than 50% for only 4 CECs (i.e. trimethoprim, sulfamethoxazole, morphine and CBZ-EP). The incoming loads of trimethoprim and sulfamethoxazole were reduced by more than 69% and 58%, respectively by sand filtration. Data from previous studies reported considerable removal (over 60%) of trimethoprim in sand filters (Göbel et al., 2007; Nakada et al., 2007; Sui et al., 2010), but lower removals (i.e. 20-30%) were reported for sulfamethoxazole (Gao et al., 2012; Göbel et al., 2007; Nakada et al., 2007). With the exception of the four compounds listed above, sand filtration and chlorination were inefficient at removing most of the other target CECs, as less than 30% of their incoming load was removed (Figure 5.2). The observed removal for triclosan was similar to that observed by Nakada et al. (2007) (i.e. 29%). Negative removals (or no removal) were obtained for estrone, sucralose, carbamazepine and CBZ-DiOH during the sand filtration and chlorination steps. For estrone and carbamazepine, the negative removals are in agreement with previous studies (Gao et al., 2012; Nakada et al., 2007; Sui et al., 2010), indicating possible desorption of these compounds during sand filtration. Removal from the aqueous phase in sand filters is attributed to adsorption to solid particles that are retained by the sand filter. However, previous studies also indicated that (bio)degradation may contribute to CEC removal due to the formation of biofilm on sand particles (Göbel et al., 2007). In the WWTP investigated, chlorine was added right before sand filtration, which makes the growth of a biofilm unlikely.

5.5.7. Metabolites

The combined loads of parent compounds and their metabolite(s) were considered for some target CECs when calculating removal efficiencies for each treatment stage (Figure 5.5) to account for possible inter-transformations. Cocaine and its major metabolite had comparable removals in all treatment units. Although EDDP (methadone's major metabolite) alone exhibited negative removal of -17% in the primary clarifier, the removal efficiency based on combined concentrations of EDDP and its parent compound methadone was above zero, indicating that some of the methadone was possibly converted into EDDP. Similarly, removals based on combined loads were obtained for carbamazepine and its two investigated metabolites, CBZ-DiOH and CBZ-EP. A limited number of studies have investigated the removals of carbamazepine and its major metabolites in WWTPs (Hummel et al., 2006; Leclercq et al., 2009; Miao et al., 2005). Only the study by Miao et al. (2005) examined the concentrations of carbamazepine and its metabolites at each treatment step of the WWTP and observed a decline in the concentration of carbamazepine and its two metabolites in the primary clarifier and an increase in carbamazepine and CBZ-DiOH concentrations by activated sludge. In the present study, the calculation was further refined by considering the input and output mass loadings of carbamazepine and its two major metabolites in each treatment unit taking the RTD into account, as shown in Figure 5.5. It should be noted that in the present study, due to use of the fractionated approach, the input reference load a treatment step is not equivalent to the output load of the previous treatment step (Figure 5.5).

A decrease was observed in the loads of carbamazepine and CBZ-DiOH during primary clarification ($p<0.05$), whilst the variations in the loads of CBZ-EP across the primary clarifier were not statistically different ($p=0.26$). Similarly, activated sludge was not shown to cause a decrease in the loads of carbamazepine and CBZ-EP ($p=0.57$ and 0.07, respectively), but it decreased the loads of CBZ-DIOH ($p<0.05$) and, thus, the combined load of carbamazepine and its metabolites. This is possibly explained by partial transformation of CBZ-DiOH back into carbamazepine during both primary clarification and activated sludge causing the persistence of carbamazepine by compensating for the (bio)degraded load. Tertiary treatment in the form of RBCs diminished the load of carbamazepine and CBZ-DiOH significantly ($p<0.05$), but not that of CBZ-EP ($p=0.25$). Sand filtration and chlorination, on the other hand, resulted in a decrease in the average load of only CBZ-EP, accompanied with a load increase of both carbamazepine and CBZ-DiOH ($p<0.05$ for all the compounds). The combined load of carbamazepine and the two metabolites was also increased ($p=0.03$). This might suggest the transformation of CBZ-EP to both CBZ-DiOH and carbamazepine during the sand filtration step. The two metabolites of carbamazepine were not quantified in sludge samples in the present study. However, Miao et al. (2005) concluded that these compounds are present at low concentrations in biosolids (<8 ng/g).

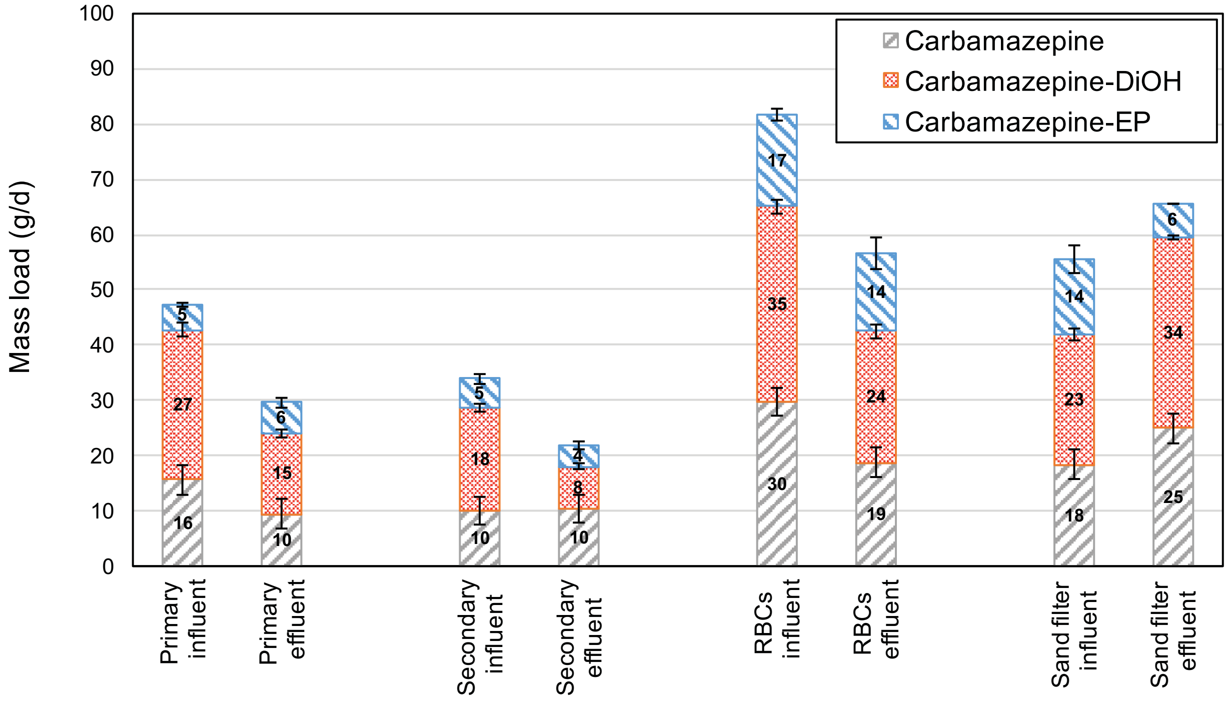

Figure 5.5 Mass loads of carbamazepine and its two investigated metabolites (CMZ-DiOH & CMZ-EP) in both the input (reference load) and the output of the primary clarifier, secondary treatment, RBCs and sand filter. The numeric values of the load are presented in the columns.

5.6. Conclusions

Removal data for the aqueous phase showed that most CECs were poorly removed (i.e. <40%) in the primary clarifier. The greatest removals typically occurred in the activated sludge, while removals were <50% in the RBCs and ranged from no removal to 70% removal during sand filtration. The mass balances, which looked at dissolved as well as adsorbed CECs provided further insight into the predominant removal mechanisms for CECs during primary and secondary treatment. It showed that not only sorption to the primary sludge, but also (bio)degradation contribute to the removal of some of the target CECs in the primary clarifier. In activated sludge, (bio)degradation was found to be the predominant removal mechanism, with sorption accounting for <5% for most CECs. The estimated $log\, K_d$ values (1.40 to 3.68 in primary sludge and 1.36 to 4.49 in activated sludge) also indicated that most CECs are not significantly removed by partitioning onto sludge, with the exception of triclosan. Accounting for the levels of metabolites of the selected CECs (i.e. carbamazepine, methadone and cocaine) explained some of the negative removals that were observed. The current study expands the understanding of the removal pathways of CECs at different treatment steps, for which limited data is available in the literature. It also takes into account the hydraulics following a novel approach applied for the first time to primary clarification, RBCs and sand filtration to provide reliable data of the CEC removals. Reliable data on the fate of CECs are valuable for the calibration of mathematical fate models that can be used to optimize treatment technologies and reduce discharges of CECs into the aquatic environment.

5.7. Acknowledgements

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5.8. Supplementary material:

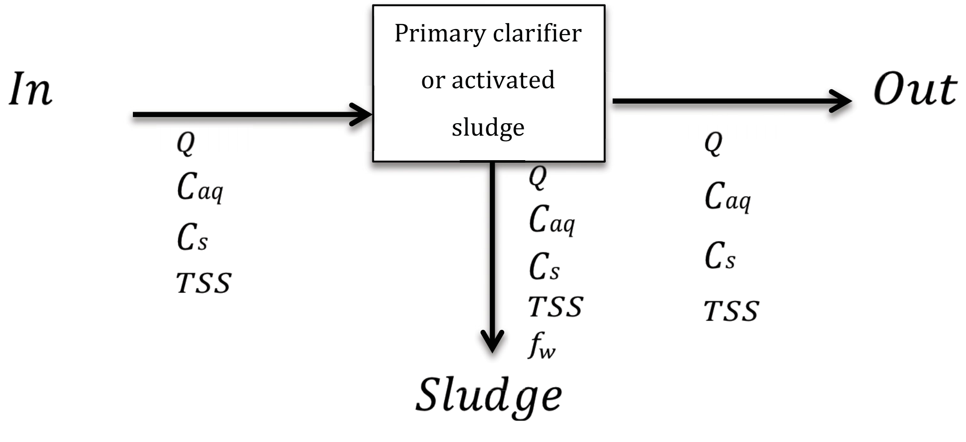

Figure 5.6 Schematic illustrating the information (measurements) of streams used for mass balance calculations.

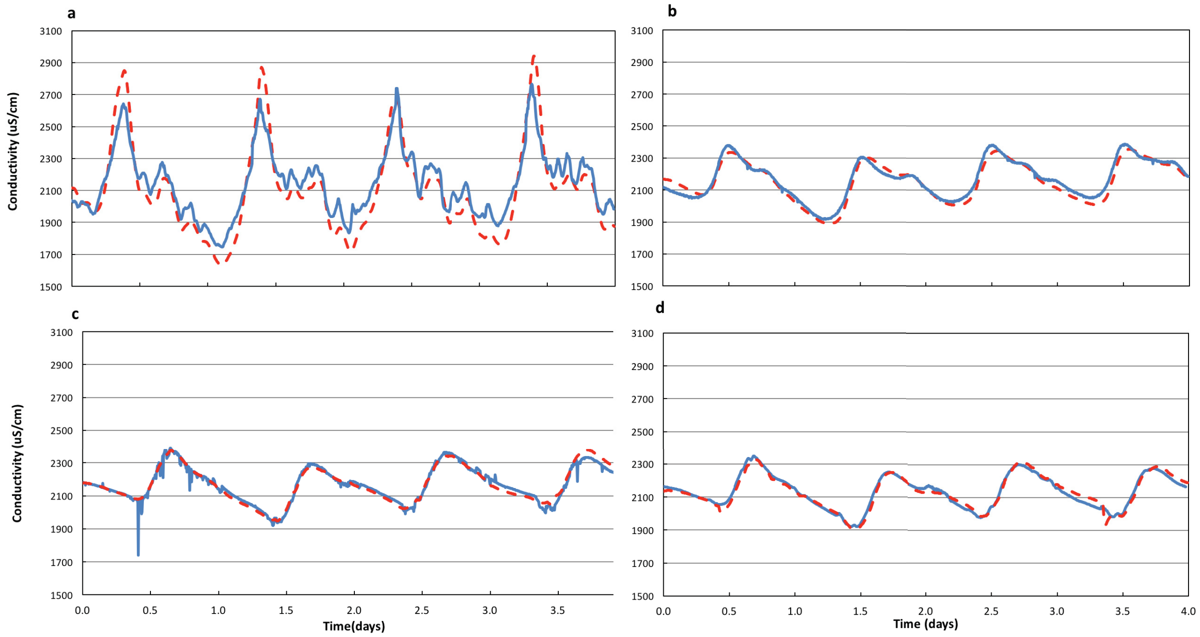

Figure 5.7 Effluent tracer (electrical conductivity (μ S/cm)) trends measured (blue) and predicted by the best-fitting hydraulic model (dashed red) throughout four consecutive days for: a) primary clarifier, b) aeration tanks, c) RBCs and d) sand filter.

Table 5.6 Extraction methods of wastewater and biosolids samples for Class A and Class B compounds using solid phase extraction (SPE) for aqueous samples and accelerated solvent extraction couple to SPE (ASE-SPE) for biosolids.

Sample type	Method	Extraction step	Class A	Class B
Aqueous	SPE	Instrument	Manual manifold	Gilson GX-271 ASPECTM automated instrument
		Cartridge	6 mL-500 mg Oasis MAX cartridges	6 mL-150 mg Oasis MCX cartridges
		Sample pH	pH 8.0 using sodium hydroxide	pH 2.5 using sulfuric acid
		Conditioning	Methanol, 0.1 M ammonium hydroxide and Milli-Q water (pH 8.0)	Acetone and Milli-Q water (pH 2.5)
		Elution	2 mL methanol and then 3x3 mL of 2% formic acid in methanol, 1 mL/min	3x3 mL of 5% ammonium hydroxide in methanol, 1 mL/min
		Reconstitution	50% methanol/50% water to a total volume of 0.4 mL	25% methanol/75% water to a total volume of 0.4 mL
Biosolids	ASE	Instrument	Dionex ASE 350	Dionex ASE 350
		Conditions	Temperature =80 °C Static cycle= 3	Temperature =50 °C Static cycles= 2
		Elution solvent	Acetone: water (3:7)	Methanol:water:acetic acid (49:49:2)
	SPE	Instrument	Manual manifold	Gilson GX-271 ASPECTM automated instrument
		Cartridge	6 mL-500 mg Oasis HLB cartridges	6 mL-150 mg Oasis MCX cartridges
		Sample pH	pH 7.5 using sodium hydroxide	pH 2.5 using sulfuric acid
		Conditioning	Acetone, Methanol and Milli-Q water (pH 7.5).	Acetone and Milli-Q water (pH 2.5)
		Elution	3x3 mL of methanol, 1 mL/min	3x3 mL of 5% ammonium hydroxide in methanol, 1 mL/min
		Reconstitution	50% methanol/50% water to a total volume of 0.1 mL	25% methanol/75% water to a total volume of 0.4 mL

Table 5.7 Average estimated removal efficiencies of target CECs in activated sludge and primary clarifier, separately from previous studies that investigated the removal from the aqueous phase (Please refer to Table 2.4 in the literature review).

6. DYNAMIC MODELLING OF SOLIDS IN FULL-SCALE ACTIVATED SLUDGE PLANT PRECEDED BY CEPT AS A BASIC STEP FOR MICROPOLLUTANT REMOVAL MODELLING

6.1. Preface

The fate of conventional pollutants, such as solids and biomass, as well as the concentration of dissolved oxygen in the activated sludge process significantly influence the extent at which CECs are biodegraded or adsorbed to sludge. Hence, for unbiased prediction of the fate of CECs in activated sludge treatment, which is the last objective of this PhD project, the fate of conventional pollutants must first be accurately predicted. This is addressed in details in the current manuscript.

The full-scale activated sludge unit used for calibration in this manuscript is part of the same WWTP examined in Chapter 7 for the prediction of the fate of CECs. Measurements of conventional pollutants and dissolved oxygen at different locations of the activated sludge unit under study were obtained from the operators over one year. In addition, extra measurements necessary for the characterization of the influent (i.e. the representation of the input in terms of model components) were collected over three days. An Activated Sludge Model that has an extension for the prediction of CEC fate was used in this chapter for prediction of the conventional pollutants' fate and was used as well for the work presented in Chapter 7. Simulation results were matched with the output measurements by calibrating the parameters of the activated sludge model and the settler model coupled to it. The basic model structure was based on the hydraulic model obtained in Chapter 5 for the activated sludge unit under study.

The dissolved oxygen was observed to be in excess in the aeration tanks under study. From influent characterization results, the biodegradable particulate fraction in the primary effluent was found to be lower than typical, which was attributed to the presence of the chemically-enhanced primary treatment that contributes to a higher reduction of the solids concentration. Optimizing the fit between simulations and measurements of output solids from the secondary clarifier required using the more recent Bürger-Diehl settler model that achieves a more realistic prediction of the solids fate compared to the popular Takács model. A satisfactory estimation of nutrient fate was observed without altering the standard Activated Sludge Model No. 2d parameters, further validating the established balance of solids in the calibrated model. In addition to providing insights on modelling a post-CEPT activated sludge system for the first time, this manuscript forms an essential preliminary step for the prediction of the fate of target CECs, which is addressed in Chapter 7.

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Dynamic modelling of solids in a full-scale activated sludge plant preceded by CEPT as a preliminary step for micropollutant removal modelling

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6.2. Abstract

The presence of micropollutants in the environment has triggered research on quantifying and predicting their fate in wastewater treatment plants (WWTPs). Since the removal of micropollutants is highly related to conventional pollutant removal and affected by hydraulics, aeration, biomass composition and solids concentration, the fate of these conventional pollutants and characteristics must be well predicted before tackling models to predict the fate of micropollutants. In light of this, the current paper presents the dynamic modelling of conventional pollutants undergoing activated sludge treatment using a limited set of additional daily composite data besides the routine data collected at a WWTP over one year. Results showed that as a basis for modelling the removal of micropollutants, the Bürger-Diehl settler model was found to capture the actual effluent total suspended solids (TSS) concentrations more efficiently than the Takács model by explicitly modelling the overflow boundary. Results also demonstrated that particular attention must be given to characterizing incoming TSS to obtain a representative solids balance in the presence of a chemically enhanced primary treatment, which is key to predict the fate of micropollutants.

Keywords: Activated sludge modelling, Conventional pollutants, Contaminants of emerging concern, Influent characterization, Solids balance

6.3. Introduction

Conventional pollutant modelling in activated sludge wastewater treatment plants (WWTPs) has been carried out for purposes of design and control (Demey et al., 2001; Mulder et al., 1997), optimization (Printemps et al., 2004; Weijers et al., 2012), process upgrades (Coen et al., 1996) and for evaluation of greenhouse gas emissions (Guo & Vanrolleghem, 2014). Such modelling activity involves purpose-dependent calibration of models representing the bioreactor and the settler, which are the two essential constituents of an activated sludge plant. A series of Activated Sludge Models (ASMs) have been developed by Henze et al. (1999) as a tool to model bioreactors, and the implementation of these models for dynamic modelling of conventional activated sludge units began in the late 1970s (Smith & Dudley, 1998).

In addition to the bioreactor model, a representative secondary settler model is essential for accurate modelling of the bioprocesses in the bioreactors as the return sludge from the settler influences the solids balance in the whole system (Smith & Dudley, 1998). The 1-D 10-layer Takács model (Takács et al., 1991) has long been the most widely used model to describe the settling dynamics in secondary settlers. A more advanced model was developed later, the Bürger-Diehl model (Bürger et al., 2012; Bürger et al., 2011), that calculates the flux between the layers in a more mathematically sound way, as well as allows the addition of compression settling (i.e. settling due to compressive forces) and inlet dispersion (i.e. mixing around the inlet region). This model also explicitly takes the overflow and underflow boundaries into account to better represent the flows leaving the settler (Bürger et al., 2012; Bürger et al., 2011). The Bürger-Diehl model was applied by Torfs et al. (2015) and its benefits over the Takács model were discussed in the same study. Besides the selection of a suitable settler model, proper characterization of incoming solids in terms of model components is essential to achieve a proper solids balance (Rieger et al., 2013). Proper characterization of the influent organics is also essential, as it affects the system's solids mass balance. Characterization of incoming solids can be either based on chemical oxygen demand (COD) or total suspended solids (TSS) measurements (Rieger et al., 2013). There is currently, however, little information in the literature on the impact of the chemically enhanced primary treatment (CEPT) preceding the activated sludge treatment on the solids composition in the input stream to the activated sludge unit.

Investigating the fate of micropollutants of emerging concern, such as pharmaceuticals, personal care products and hormones in conventional wastewater treatment processes has been gaining attention due to their presence in ecosystems and their proven adverse effects on the aquatic environment (Clouzot et al., 2013). Several models have been built to predict the fate of different types of micropollutants in WWTPs (Dionisi et al., 2006; Lindblom et al., 2009; Plósz et al., 2012; Urase & Kikuta, 2005; Vezzaro et al., 2014). Among these efforts is the extension of the ASM No. 2d model, which is among the commonly-used ASMs (Henze et al., 1999), under the ScorePP Project to include the fate of micropollutants (Vezzaro et al., 2014). The main processes considered to influence the fate of micropollutants in WWTPs are biodegradation, sorption, photolysis, volatilization and hydrolysis (Rogers, 1996). The extent to which these processes remove micropollutants from wastewater depends on a number of conditions, including the oxygen supply that affects volatilization, the solids concentration that affects adsorption, as well as the solids retention time (SRT) that influences the diversity of biomass and the presence of slowly growing microorganisms, both of which are associated with a higher degree of micropollutant biodegradation (Kreuzinger et al., 2004; Suarez et al., 2010). For example, the presence of nitrifying biomass, which is one type of slowly growing microorganisms, has been shown to correspond to improved biodegradation of miropollutants (Suarez et al., 2010). The abundance of nitrifying biomass can be indicated by the removal of its main substrate, ammonia. In this context, it is important to stress that if the goal of the modelling exercise is to model micropollutants, it is not necessary to predict the detailed diurnal variations in the concentration of ammonia, but it suffices to capture the general ammonia removal in order to get a proper estimate of the nitrifying biomass. Hence, the emphasis of the conventional pollutants calibration exercise should be adapted to its final goal (i.e. supporting proper modelling of the fate of micropollutants in this context).

This paper presents a procedure for the calibration of a conventional activated sludge model based on standard data available from WWTPs (i.e. with a minimal additional sampling effort) for the specific objective of a subsequent development and calibration of a micropollutant model that is tackled in another study. The conventional pollutant modelling involved hydraulic modelling of the WWTP, selection of a bioreactor and a settler model and subsequent calibration of the submodels. Particular emphasis was put on the prediction of the overall profile of oxygen concentration, the solids inventory and the solids retention time (SRT) as these are the most important variables influencing the micropollutant removal processes. The modelling approach also aimed at providing insights on the characterization of incoming wastewater when modelling activated sludge systems that are preceded by CEPT.

6.4. Methodology

6.4.1. WWTP

The WWTP under study is located in Guelph, Ontario. The main characteristics of this WWTP are summarized in Table 6.1, and the schematic of the WWTP is presented in Figure 6.1. Preliminary treatment is achieved by screening and aerated grit removal, after which ferric chloride (37-45%) is added at a rate of 1 L/min and the load is split into four treatment lines. Each of these lines consists of two primary clarifiers, two aeration tanks and one or two secondary clarifiers in parallel. The incoming flow rate to each of these lines is different, with line 1 receiving the highest flow. For this paper, only the activated sludge unit (aeration tanks and secondary clarifier) of the first line was investigated. Each of the two parallel aeration tanks (East and West) in line 1 contains three passes in series. The combined effluent of both aeration tanks is directed to one secondary clarifier. Following secondary treatment, the recombined effluent of the lines enters rotating biological contactors (RBCs), followed by sand filtration for tertiary treatment. RBCs and sand filtration were installed to ensure that the TSS and ammonia levels at the final effluent meet environmental regulations. The final effluent disinfected by chlorination is discharged into a nearby river.

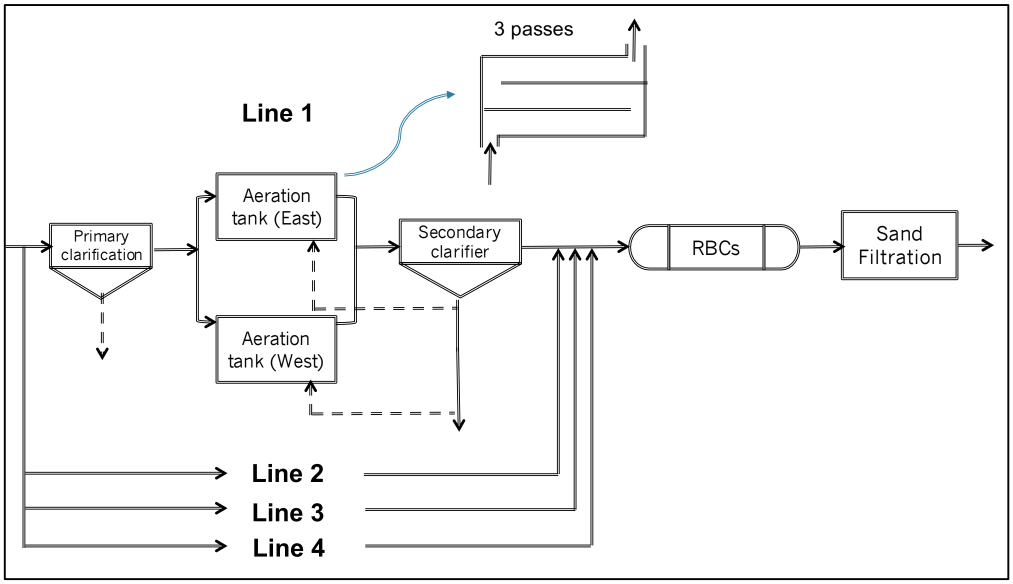

Figure 6.1 Schematic of the Guelph WWTP. Wastewater streams are represented by continuous lines and sludge streams by dashed lines.

Table 6.1 Main characteristics of the studied WWTP, as well as the average hydraulic retention time (HRT), average solids retention time (SRT) and average temperature (T) over July 21-24 2014 in the first activated sludge line (line 1)

Characteristic	Treatment stage	Guelph
Population served		135,000
Design capacity (m^3/d)		64,000
Average incoming flow rate (m^3/d)		50,750
	Primary clarifier (line 1)	3.72
HRT (h)	Aeration tanks (line 1)	6.30
	Secondary clarifier (line 1)	2.90
SRT (days)	Line 1	7.76
Average T (°C)		20

6.4.2. Data collection

Flow rates of aqueous and sludge streams exiting line 1, as well as the volumes of the individual units were obtained from the operators at the WWTP. Table 6.2 gives an overview of the characteristics monitored at the different sampling locations, as well as the frequencies of the measurements. At the primary and secondary effluents, samples were collected by onsite Hach Sigma samplers as daily flow-proportional composites. In the aeration tanks, grab samples were collected for the analysis of the Mixed Liquor Suspended Solids (MLSS) and Mixed Liquor Volatile Suspended Solids (MLVSS). Routine measurements that were performed onsite every few days were acquired from the WWTP over one year and are denoted as "Y" in Table 6.2. These include Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), Total Kjeldahl Nitrogen (TKN) and Nitrite and Nitrate Nitrogen (NO_2 & NO_3, respectively). The remaining measurements, denoted "S", were performed in a sampling and measurement campaign performed over three consecutive dry days in July 2014 and include soluble Chemical Oxygen Demand (COD_s), 5-day Biological Oxygen Demand (BOD_5), Ammonium nitrogen (NH_4), Total Phosphorus (P) and alkalinity. Analysis for all measurements was performed by the onsite lab, following APHA/AWWA protocols outlined by Rice et al. (2012). Online dissolved oxygen concentration (DO) measurements were available from onsite oxygen sensors installed at the first and third pass of both aeration trains. For all output figures (Figures 6.2-6.5), the time axis begins in July and ends in June the following year, with the winter period falling in the middle of the axis.

Table 6.2 Schedule of the monitoring of wastewater characteristics in the primary effluent, the secondary effluent, the waste sludge and inside aeration tanks, including measurements performed frequently over the year (Y) and those performed over a few days in the summer (S) during a more intensive measurement campaign.

Variable	COD	CODs	BOD_5	TSS	TKN	NH_4	P	Alkalinity	NO_2 & NO_3	DO
Primary effluent	Y(1) [a]	S[a]	S	Y (1)	Y (2)	S	S	S	-	-
Aeration tanks	-	-	-	Y (1)[b]	-	-	-	-	-	Online
Secondary effluent	Y(2)	S	-	Y (2)	Y (2)	S	-	-	Y (2)	-
Waste sludge	-	-	-	Y (1)	-	-	-	-	-	-

[a] S: represents measurements collected over three days in the summer of 2014 (July 21-July 24). Y: represents measurements collected from July 2013 to July 2014 with the frequency (times per week) between brackets, [b] This included MLSS and MLVSS

6.4.3. Simulation environment and model bases

Simulations were carried out with the WEST modelling and simulation software (Mike Powered by DHI, Hørsholm, Denmark). The ASM2dTemp modelbase available in the WEST software was used to describe the bioreactor processes. For the secondary settler, the widely-used Takács all fractions propagator model (Takács et al., 1991; Vanrolleghem & Nopens, 2014) and the more recent Bürger-Diehl model (Bürger et al., 2012) were each tested for the system under study.

6.4.4. Hydraulic calibration

Hydraulic calibration of the activated sludge unit under study has been performed earlier, and details on the results can be found elsewhere (Baalbaki et al., 2016). In brief, HOBO conductivity loggers (Hoskin Scientific, St-Laurent, QC, Canada) were deployed over a period of three weeks (June 12 – July 8, 2014) at the primary effluent, aeration tank effluent and secondary effluent to collect electrical conductivity data (one reading per minute). The measured conductivity was used as a tracer for the hydraulic calibration, along with the volumes of the treatment units and flow rates. After varying the number of tanks in series and the influent layer of the settlers, the final layout that describes the hydraulic behaviour of the activated sludge was decided based on minimizing the root mean square error (RMSE) between the predicted and the measured conductivity trends. The best-fit model structure consisted of three aeration tanks in series and one secondary clarifier with the feed stream entering at the mid layer (Baalbaki et al., 2016). In the final model structure, it was necessary to separately consider the two parallel trains of aeration tanks (i.e. two parallel trains of three tanks in series) to capture the distinct hydraulic behaviour of each train, since the incoming wastewater and return sludge flow rates are observed to be different for each train.

6.4.5. Influent characterization

Characterization of the chemical oxygen demand (COD) in the influent was performed following the STOWA guidelines for modelling with ASM (Roeleveld & Van Loosdrecht, 2002; STOWA, 1996). This protocol was chosen over other fractionation protocols (e.g. Melcer et al. (2003)) for its simplicity and reduced need of measurements. The COD was fractionated into four main components, as illustrated in Table 6.3: inert soluble fraction (S_I), biodegradable soluble fraction (S_S), slowly biodegradable particulate fraction (X_s) and inert particulate fraction (X_I). The differentiation between soluble and particulate material for ASM models is based by convention on a filter size of 0.45 µm. Following the STOWA protocol (Roeleveld & Van Loosdrecht, 2002; STOWA, 1996), the S_I fraction was assumed to be equal to the soluble COD measurement that remains in the effluent. The S_S fraction was obtained by subtracting the inert soluble COD (S_I) from the measured soluble COD in the influent. The X_s was obtained from the total biodegradable material, which is estimated by the ultimate BOD (i.e. at time infinity) extrapolated from the measured BOD_5. Finally, the X_I fraction was found as the difference between the total COD measurement and the previously defined fractions. These calculations were performed over the three dry summer days of the sampling campaign and the obtained fractions were applied for the whole year.

Details of the TSS fractionation are also illustrated in Table 6.3. A common approach to fractionate the TSS in the influent to the model is by using the particulate COD concentrations as a basis (e.g. by using a fixed TSS/COD ratio which is by default 0.75 for non-biomass and 0.9 for biomass (Henze et al., 1999)). This will be referred to as the COD-based TSS. The used influent TSS/COD ratios assume a certain fixed amount of inorganic suspended solids (i.e. iSS, such as minerals) that are not measured as COD (Henze et al., 1999). These ratios can be adjusted by the modeller to vary the quantity of iSS that needs to be included to fit the measured output TSS. When the TSS/COD ratios related to the influent fractionation are adapted to account for inorganics, the ratios pertaining to the biological conversion model must not be altered. The inorganic fraction does not take part in the biokinetic processes. It is assumed to be physically incorporated in activated sludge flocs, to remain unchanged during the process and to eventually be removed with the waste sludge after the secondary settling step. As an alternative to the COD-based TSS, characterization of input TSS could also be performed on the basis of

TSS measurements. The standard measurement of TSS for wastewater is, however, known to exclude some organic and inorganic colloids due to the used filter size that is larger than the one used for COD analysis (Table 6.3). Since these colloids attach to activated sludge flocs once inside the bioreactor and thus contribute to the MLSS, the fractionation needs to be adjusted accordingly to account for these colloids. Ideally, both TSS characterization methods (i.e. based on particulate COD and based on TSS measurements) should give the same results but differences can occur when not properly accounting for colloids and inorganics. In this study, both approaches were considered for the characterization of influent TSS. The way the state variable TSS is standardly incorporated in the ASM2d model accommodates both approaches since, next to the organic fraction of TSS, it also allows to introduce the iSS fraction of TSS.

Table 6.3 The relationship between the measurement and the fractionation of COD and TSS, as well as the modelling of particulates in the ASM2d model. X_{AUT} and X_{HET} represent nitrifying and heterotrophic biomass concentration, respectively.

COD fractionation (Filter size: 0.45 µm)	**TSS fractionation** (Filter size: 1.2 µm)	**Modelling particulates** (ASM convention: >0.45 µm)
Soluble (<0.45 µm)	Colloids (0.45 µm – 1.2 µm)	Organic colloids and particulates
Inert (S_I) Substrate (S_S)	Organic: measured as particulate COD (X_s) Inorganic: not measured as COD, nor as TSS	ASM2d state variables implicated: X_S, X_I, X_{HET}, X_{AUT}, TSS TSS/COD ratio: 0.75 – 0.9 Sludge balance (MLSS) changes with consumption of the organic material and subsequent growth of biomass
Particulate (>0.45 µm)	Solids (>1.2 µm)	Inorganic colloids and particulates
Inert (X_I) Substrate (X_S) Biomass (X_{HET}, X_{AUT})	Organic: measured as particulate COD and TSS Inorganic: measured as TSS	ASM2d state variable implicated: TSS The used TSS/COD ratios assume a certain amount of inorganic material The inorganic part of TSS is considered inert. It gets physically incorporated into sludge flocs and is removed via the waste sludge after secondary settling

The characterization of nitrogen (N) and phosphorus (P) in the influent was performed using measurements of total nitrogen and total phosphorus. The nitrogen and phosphorus contained in organic matter was calculated using typical fractions of N and P contents in COD provided for

ASM2d by Henze et al. (1999) and shown in the supplementary materials (Table 6.6). The ammonia was then calculated as the difference between the total nitrogen (TKN) available from the measurements and the calculated nitrogen content in organic matter throughout the year. The calculated ammonia results were successfully verified over the three days of the sampling campaign during which measurements of ammonia were available.

As shown in Table 6.2, data for COD and TKN were available at a frequency of one to two measurements per week for a year. However, to increase the resolution of the model one value per day is needed for the conventional pollutants. For combined sewers, COD and TKN can be correlated with the influent flow, and the correlations are used to increase the time resolution of the characterized influent if frequent flow data are available (Rousseau et al., 2001). However, analysis of the data for the studied WWTP did not show meaningful correlations between the flow rate and the aforementioned measurements (not shown), which is likely related to its separate sewer system. Therefore, the time resolution of the influent measurements was increased by fitting the less-frequently measured data to a best-fit polynomial function. These polynomials captured the yearly profile of the measurements and allowed interpolation for daily values to be used for input generation. The fits are given in the Supplementary Material (Figure 6.6). More detailed diurnal patterns of conventional pollutants in the influent were deemed unnecessary given the modelling goal. Thus, the achieved conventional pollutant model should not be evaluated based on sub-daily dynamics.

6.4.6. Simulation approach and calibration

Preliminary simulations were carried out using the default ASM parameters listed by Henze et al. (2000) and default settler parameters suggested in the COST Simulation Benchmark manual (Copp, 2002). Each dynamic simulation was preceded by a steady simulation for a period equivalent to at least three times the SRT (i.e. 20 days) in order to determine the initial conditions for the dynamic simulation (Rieger et al., 2013).

Model calibration efforts were focused on fitting variables that directly impact the fate of micropollutants, such as dissolved oxygen concentration, solids concentration and nutrients concentration. The first step was the calibration of the oxygen mass transfer coefficient (k_La) to match the measured DO, which was in general observed to be abundant (i.e. average > 5mg/L).

The second step involved achieving a match between the simulated and the measured TSS concentration at different locations in the activated sludge process, such as the waste sludge (i.e. WAS TSS), the aeration tank (i.e. MLSS) and the secondary effluent (i.e. Effluent TSS). The solids balance was first pursued by calibrating the settling parameters. Results of this initial calibration work could give an indication as to whether adjusting the settling parameters is sufficient to obtain the correct solids balance, or if, alternatively, characterization of the input solids is the appropriate approach for achieving the solids balance. The third step involved achieving an agreement with measured nutrients: NH_4 (and its product NO_3), as well as COD. This last step can depend on several biokinetic parameters such as growth rates, decay rates and substrate affinities. However, for a conventional activated sludge process, these parameters are relatively well known (Henze et al., 2000), and large calibration efforts should be avoided. That is because discrepancies between model predictions and measurements that require the calibration of these parameters may indicate other underlying calibration problems related to the hydraulic model or the solids balance.

The parameters that correspond to each step were varied using the scenario analysis tool in WEST software, and the best-fit model was decided upon by visual inspection. While the simulations were set to provide hourly values of the output variables, the measurements of the same variables were based on daily flow-proportional samples. Therefore, it was necessary to calculate the daily flow-average of the simulated results using the equation: $\bar{C} = \sum Q_i C_i / \sum Q_i$, where $\bar{C}$ represents the flow-average concentration, Q_i and C_i represent the flow rate and the simulated output concentration at a certain time point "i". This provided simulated values with similar time scale as the experimental measurements for the purpose of comparison.

6.5. Results and discussions

The first step towards the calibration of the conventional pollutant model is the characterization of the influent wastewater. This was followed by calibration of the model for the prediction of the dissolved oxygen concentration followed by solids concentration, including characterization of influent solids and finally, model calibration with respect to the nutrient concentrations.

6.5.1. Wastewater characterization

The recorded flow rates throughout the year seemed to be stable due to the mostly separate sewer system (i.e. with very few combined sewers), which is expected to reduce the seasonal variations in flow rates due to the seasonal variations in rainfall. The recorded temperatures varied from 15 °C to 22 °C throughout the year. The measured COD concentration trends showed an increase in winter compared to the summer, which might be due to increased degradation of COD in the sewers and primary clarifier at the higher temperatures in the summer. It is important to note that the primary clarifier of this plant has a long retention time of almost 4 hours (Table 6.1), making that degradation in the clarifier more significant than in typical primary clarifiers. On the other hand, the incoming ammonia concentrations to the activated sludge unit under study showed no significant seasonal variation throughout the year. This could be attributed to the absence of nitrogen removing processes in the mostly anaerobic conditions of a sewer system and primary clarifier. In addition to ammonia, the TSS profile did not show variation over the one-year period. The measured TSS values had an average value of 70 g/m^3 in the primary effluent, which corresponds to a TSS reduction of 84% in the primary clarifier. This TSS reduction is larger than the typical reduction of 40-60% (Office of Water Programs, 1980) in primary clarifiers, likely because of the chemically enhanced settler that is known to achieve high TSS removal. The incoming TSS characterization is further discussed in Section 6.5.3 as part of the calibration for solids.

Table 6.4 summarizes the fractions of the incoming COD obtained over the three days of the sampling campaign and applied for the whole year. It should be noted that the extra measurements used for obtaining these fractions over three days in the summer fall on the same fitted polynomial that describes the data over the full year (i.e. Figure 6.6). This indicates that there were no special events on these days that could affect the wastewater composition. Typically, the biodegradable material contained in the primary effluent is predominantly particulate (i.e. slowly biodegradable material) rather than soluble (Henze et al., 2000); however, the characterization results shown in Table 6.4 indicated more biodegradable matter in the soluble phase than in the particulate phase ($S_s > X_S$). This could be attributed to two main factors. First, the higher TSS reduction known to occur in chemically enhanced primary clarifiers contributes to the low fraction of particulates in the primary effluent. Second, hydrolysis could occur prior to the aeration tanks due to the long hydraulic retention time (HRT) of the primary clarifier (3.72 h), which thus permits the conversion of the slowly biodegradable COD (particulate) into readily biodegradable COD (soluble).

Table 6.4 COD composition (%) for the primary effluent wastewater under study, determined following the STOWA protocol.

S_I/COD	S_S/COD	X_S/COD	X_I/COD
6.8	37.7	21.5	34.0

6.5.2. Dissolved oxygen calibration

The average dissolved oxygen over one year was 5 mg/L for the first pass (pass 1) and 7 mg/L for pass 3 of both aeration tanks (i.e. East and West), indicating excessive aeration in the system under study. The oxygen mass transfer coefficient (k_La) was estimated in order to reach a match between the simulated and the measured dissolved oxygen in each aeration tank. An initial guess of the k_La was made by implementing DO controllers that were set to reach the average measured DO by varying the k_La value. The k_La values were estimated to be 8.6 and 11 hr^{-1} for the East and West aeration trains, respectively. Dynamic simulations with these k_La values yielded DO values that are in agreement with the measurements over the whole year, further verifying the validity of the calibrated k_La values. The estimated k_La was higher in the West aeration train than in the East train although the measured DO values were similar in both trains and both trains had the same volume and similar MLSS concentration. This higher k_La in the West train may be attributed to the higher primary effluent and return sludge flows directed towards the West train, which leads to higher oxygen demand and thus a need for a higher oxygen transfer rate to achieve a DO concentration that is similar to that in East train. From both measurements and simulations, it was observed that the DO was higher in pass 3 than in pass 1 for each aeration tank. This spatial variation in the DO throughout the passes could be explained by the fact that while the supplied oxygen is the same in all passes, the incoming load undergoes biodegradation in pass 1 first, leaving less substrate (i.e. less chemical oxygen demand) in pass 3, which results in a lower oxygen consumption rate.

6.5.3. Solids calibration

Reaching a proper solids balance by achieving a match between the measured and simulated TSS in aeration tank (i.e. MLSS) and, more importantly, TSS in the waste sludge (i.e. WAS TSS) is an essential requirement for predicting the fate of micropollutants. This is explained by the fact that the WAS TSS impacts the extent of micropollutant removal by adsorption, as well as the SRT that influences the extent of micropollutant biodegradation (Kreuzinger et al., 2004).

6.5.3.1. Initial simulations

For the purpose of initial simulations, the TSS in the influent was characterized based on particulate COD concentrations according to the typical approach and using typical TSS/COD ratios suggested by Henze et al. (1999). However, the results obtained using this approach showed simulated MLSS concentrations that were higher and contained more seasonal variations throughout the year compared to the measured MLSS that showed no seasonal variation (Figure 6.2(a), Case A). A similar discrepancy between simulation results and measurements was observed for the TSS in the secondary waste sludge (results not shown). This discrepancy in the solids balance was concluded to be caused by the mischaracterization of the incoming solids to the activated sludge system. This was further supported by the fact that manipulating the settler's parameters failed to achieve a correct solids balance and to capture the actual WAS TSS and MLSS concentrations. To remediate the discrepancies in the solids balance and to achieve an agreement between the simulated and the measured MLSS and WAS TSS concentrations, the characterization of incoming solids was analysed in more detail.

6.5.3.2. Characterization of incoming solids

As discussed earlier, the COD-based TSS concentration (i.e. input TSS calculated using TSS/COD conversion factors) showed seasonal variation, similar to the COD, resulting in a seasonally variable MLSS time series, unlike the measured MLSS profile (Figure 6.2(a)). In addition, the average simulated MLSS was observed to be higher than the measured MLSS using the COD-based TSS in the input. In fact, unlike the measured COD, the measured TSS did not show any seasonal variations throughout the year, indicating that the TSS/COD ratio is not constant throughout the year. The presence of the CEPT might cause seasonal alterations to the actual TSS/COD ratio (i.e. caused by changes in the chemical efficiency and solids settling as a result of temperature variation (Tik & Vanrolleghem, 2012)), indicating that the typical ratios used for the calculations of the COD-based TSS in the primary effluent (Henze et al., 1999) may not be valid and that an alternative route for TSS characterization is required. Therefore, simulations were carried out using the measured TSS as an input, which was found to yield a simulated MLSS profile that, as desired, did not show seasonal variations, but that was lower than the measured MLSS and in almost perfect agreement with the measured MLVSS time series, as shown in Figure 6.2(b). The latter indicated that a fraction of the input suspended solids was not properly accounted for in the fractionated influent.

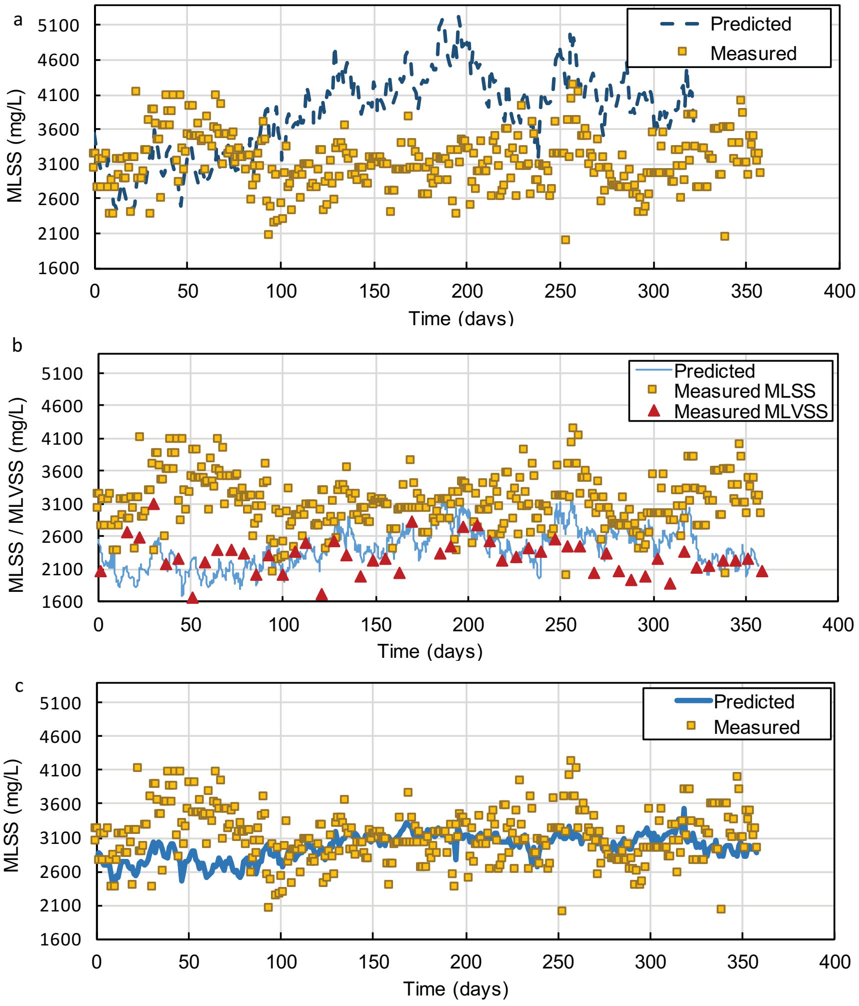

Figure 6.2 Measured and predicted MLSS concentrations. The latter was obtained using the Takács model and standard settling parameters: Case A: COD-based TSS in the influent, Case B: Measured TSS in influent, Case C: 1.35 x measured TSS in the influent. The time axis is from July 2013 to June 2014.

As mentioned earlier in Section 6.4.5, the measured TSS does not account for organic and inorganic colloidal matter, which results in the underestimation of the actual TSS in the influent

(Henze et al., 2000). To remedy this, the TSS at the input was scaled up by a ratio of 1.35, which was shown to lead to satisfactory predictions of the MLSS in the bioreactors and TSS in the secondary waste sludge (Figure 6.2(c) and Figure 6.3 respectively). This obtained ratio between the calibrated and the measured TSS in the input to activated sludge treatment is slightly lower than that reported by Henze et al. (2000) for an ASM2d model (i.e. 1.5). The lower ratio in the present study is suggested to be attributed to the presence of the CEPT that may reduce the colloidal fraction with a higher efficiency than a conventional primary clarifier, requiring a smaller correction for TSS compared to conventional primary clarification. In fact, it was previously shown that a well-operated CEPT is capable of almost completely removing colloidal material (Balmat, 1957; Heukelekian & Balmat, 1959; Ødegaard, 1992; Ødegaard, 2005).

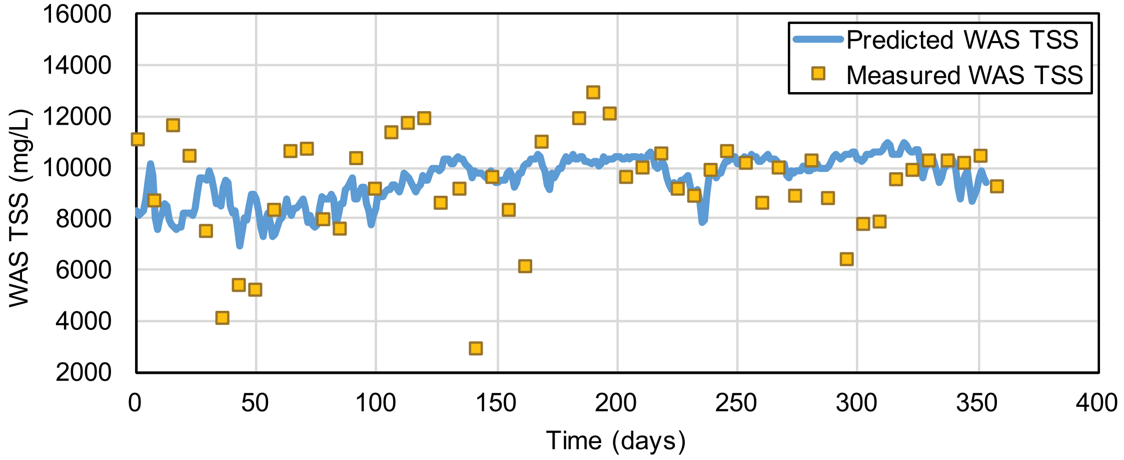

Figure 6.3 Measured and predicted TSS concentrations in waste sludge (WAS TSS) after input solids characterization. The time axis is from July 2013 to June 2014.

6.5.3.3. Calibration with respect to effluent TSS

Once the MLSS and WAS TSS were successfully matched, the settling model was further calibrated to predict the TSS concentration in the effluent. Predicting the TSS in the secondary effluent is essential for the subsequent prediction of adsorbed micropollutants in the secondary effluent stream, as well as to properly calculate the SRT, which determines the micropollutant biodegradation capacity of the sludge. Both the Takács and the Bürger-Diehl settler models were tested as shown in Figure 6.4 with the combination of parameter values summarized in Table 6.5. Although the Bürger-Diehl model allows for including the compression and the dispersion phenomena, the low measured sludge volume index (SVI) values (i.e. average of 58 mL/g) indicated sludge with very good settling and thickening properties, making it reasonable to assume that compression settling does not play a significant role in the system under study. Accounting for dispersion, which is another phenomenon included in the Bürger-Diehl model and expressed as a function of the input flow rate, was deemed unnecessary. This is explained by the absence of significant flow variations in the input to the activated sludge unit, due to the presence of a separate sewer system. Using the Takács model with the default values for the settling parameters, the simulated TSS in the effluent was observed to be much higher than the measured one, as shown in Figure 6.4 (Takács A). Lowering the simulated effluent TSS to match the measured profile was achieved by increasing the r_P parameter (i.e. from 2.86 to 7 L/g) and lowering the f-ns parameter (i.e. from 2.28 to 0), as shown in Figure 6.4 (Takács B). The observed impact of varying these parameters is explained by the fact that lowering the f-ns leads to a lower fraction of non-settleable solids decreasing the effluent TSS, while a higher r_P value increases the settling velocity and, therefore, decreases the TSS in the effluent.

On the other hand, when using the Bürger-Diehl model, the simulated TSS profile is lower than the profile obtained using the Takács model for default parameter values (Bürger-Diehl A vs. Takács A, Figure 6.4). Even though both compression and dispersion of the Bürger-Diehl model were turned off, the better prediction is explained by the existence of two extra top layers in the Bürger-Diehl model that account for the flux over the outlet boundary. Using a larger number of layers in the Bürger-Diehl model (30 layers) as compared to the 10-layer Takács model was also anticipated to result in more representative effluent solids concentrations by diminishing numerical dispersion. Since an agreement between the measured and simulated effluent TSS was not yet achieved using default parameters, the r_P parameter was adjusted to 5 L/g, which resulted in a match between the measurements and simulations (Figure 6.4, Bürger-Diehl B). This indicated that a less extensive calibration was required for the Bürger-Diehl model (i.e. only one parameter) compared to the Takács model in order to match the effluent TSS measurements. This justified the use of this model for the settler rather than the Takács model. The fit of the final model for effluent TSS is shown in Figure 6.4 (Case Bürger-Diehl B).

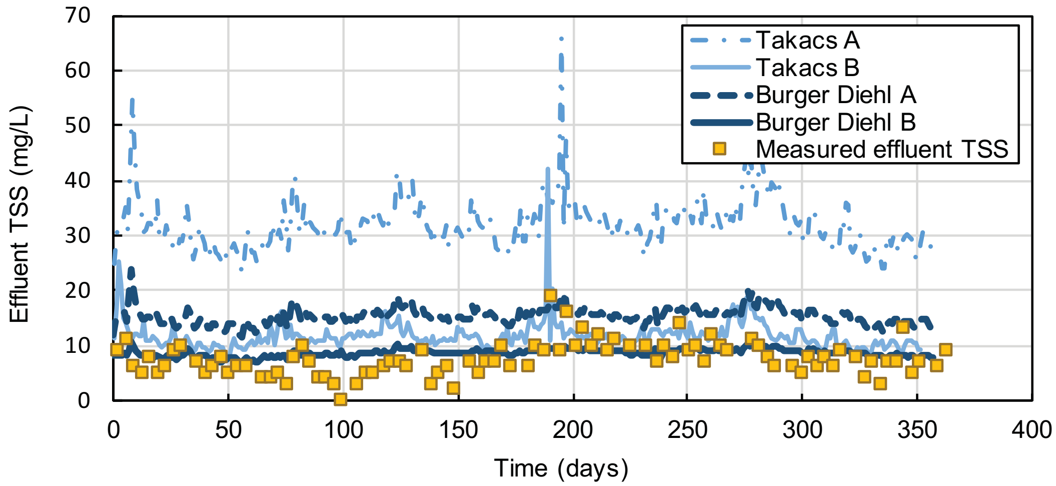

Figure 6.4 Comparison of predicted effluent TSS concentrations obtained using the Takács and Bürger-Diehl settler models with different parameters (Table 6.5). The time axis is from July 2013 to June.

Table 6.5 Parameter values tested for each of the settler models (Takács and Bürger-Diehl). Results corresponding to these cases are shown in Figure 6.4.

Case	r_H (L/g)	r_P (L/g)	f-ns (%)
Takacs A (default)	0.576	2.86	2.28
Takacs B	0.576	7	0
Bürger Diehl A	0.576	2.86	2.28
Bürger Diehl B	0.576	5	2.28

6.5.4. Nutrients calibration

As shown in Figure 6.5, once the model was able to accurately predict the solids balance, the effluent nutrient and COD predictions were on average in good agreement with the measurements, keeping in mind the goal of the modelling, without performing any further calibration of biokinetic parameters. In particular, the slightly reduced nitrification by the end of the winter (i.e. between days 200 and 300 on Figure 6.5) was well captured by the calibrated model. This emphasizes the importance of obtaining the correct hydraulic model and solids balance before modifying the biokinetic parameters. The simulated COD (Figure 6.5) did not show the same fluctuations as the measured values but the general profile matched the collected data. The high nitrate values observed in the secondary effluent of the studied treatment plant (Figure 6.5) can be explained by the high DO in the treatment lines (well above 2 mg/L) throughout the year, rendering it impossible for denitrification to occur. The measured nitrite concentrations in the secondary effluent were <0.5 mg/L, except during the winter where it rose slightly (not shown), indicating that the activated sludge plant under study is a fully nitrifying plant as most of the ammonia is completely converted to nitrate. Since the presence of nitrifying biomass and other slowly growing biomass is expected to enhance the removal of micropollutants through biodegradation (Suarez et al., 2010), capturing the general profile of ammonia removal is essential for the prediction of the fate of micropollutants.

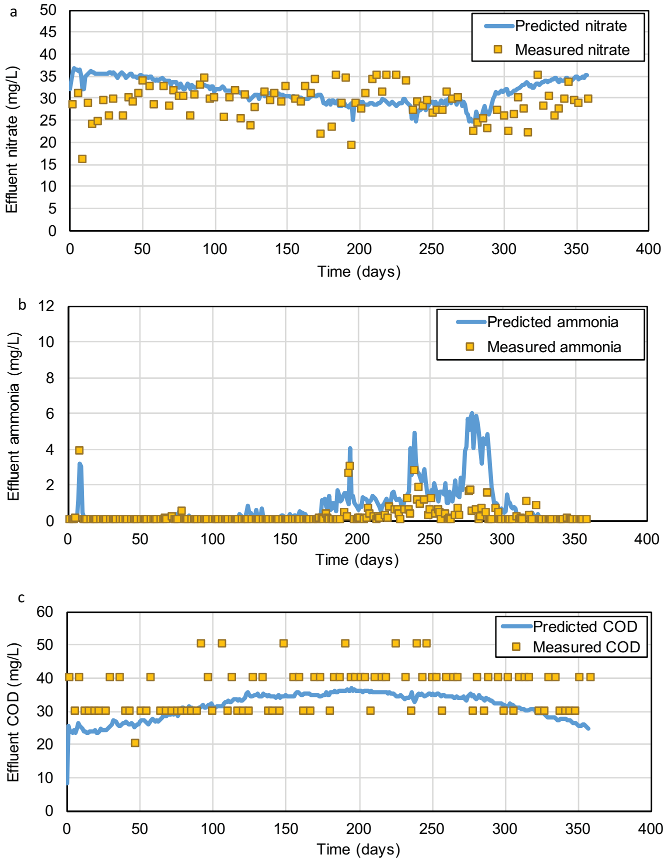

Figure 6.5 Measured and predicted conventional pollutant concentrations: a) nitrate, b) ammonia and c) COD in the secondary effluent using the best-fit model. The time axis is from July 2013 to June.

6.6. Conclusion

In this study, an activated sludge model was calibrated with one-year, full-scale conventional pollutants measurements and a relatively small, dedicated three-day detailed measurement campaign as a preparatory step for the subsequent fate modelling of micropollutants. The following conclusions and insights on the modelling of the fate of conventional pollutants in an activated sludge process were reached:

1- Proper characterization of incoming TSS by accounting for the colloidal matter that is not included in the TSS measurements was found to be one of the main influencing factors on the solids balance.

2- The chemically enhanced primary treatment was considered to seasonally influence the particulate composition of the primary effluent, requiring special considerations during the characterization of the incoming TSS fraction to the activated sludge unit.
3- The Bürger-Diehl model for the secondary settler resulted in improved calibration of the effluent TSS compared to the Takács model, which is related to the inclusion of additional layers to model the outlet boundaries.
4- Careful calibration of the solids balance through detailed influent characterization and appropriate selection of the settler model allowed the measured nutrient profiles in the effluent to be captured by the model using default ASM parameters.

The routine measurements available from the WWTP based on 24-h composite samples were proven to be sufficient to successfully model the general yearly profiles of WAS TSS, effluent TSS, DO and nutrients. Since these variables are linked to the main removal processes of micropollutants (i.e. through SRT, aeration and solids balance), it is shown that the measurement effort and resources required for the conventional modelling that is performed as a preliminary step for calibration of the micropollutant model can remain limited.

6.7. Acknowledgements

The authors thank the staff at the Guelph WWTP for their help in providing the data. We gratefully acknowledge the McGill Engineering Doctoral Award for supporting Zeina Baalbaki. Funding for this study was provided by a research grant to Viviane Yargeau (PI) and colleagues (Chris Metcalfe and Peter Vanrolleghem) from the Natural Sciences and Engineering Research Council (NSERC) of Canada through the Strategic Grants Program (430646-2012). Peter Vanrolleghem holds the Canada Research Chair on Water Quality Modelling.

6.8. Supplementary material:

Table 6.6 Organic nitrogen and phosphorus content in the fractions constituting organic matter (Henze et al., 2000).

Fraction in COD	S_F^a	S_A^a	S_I	X_s	X_I
Nitrogen	0.03	0.01	0	0.04	0.02
Phosphorus	0.01	0	0	0.01	0.01

^aS_F: fermentable organic matter (soluble), S_F: Acetate organic matter and other fermentation products (soluble). $S_F+S_A=S_S$

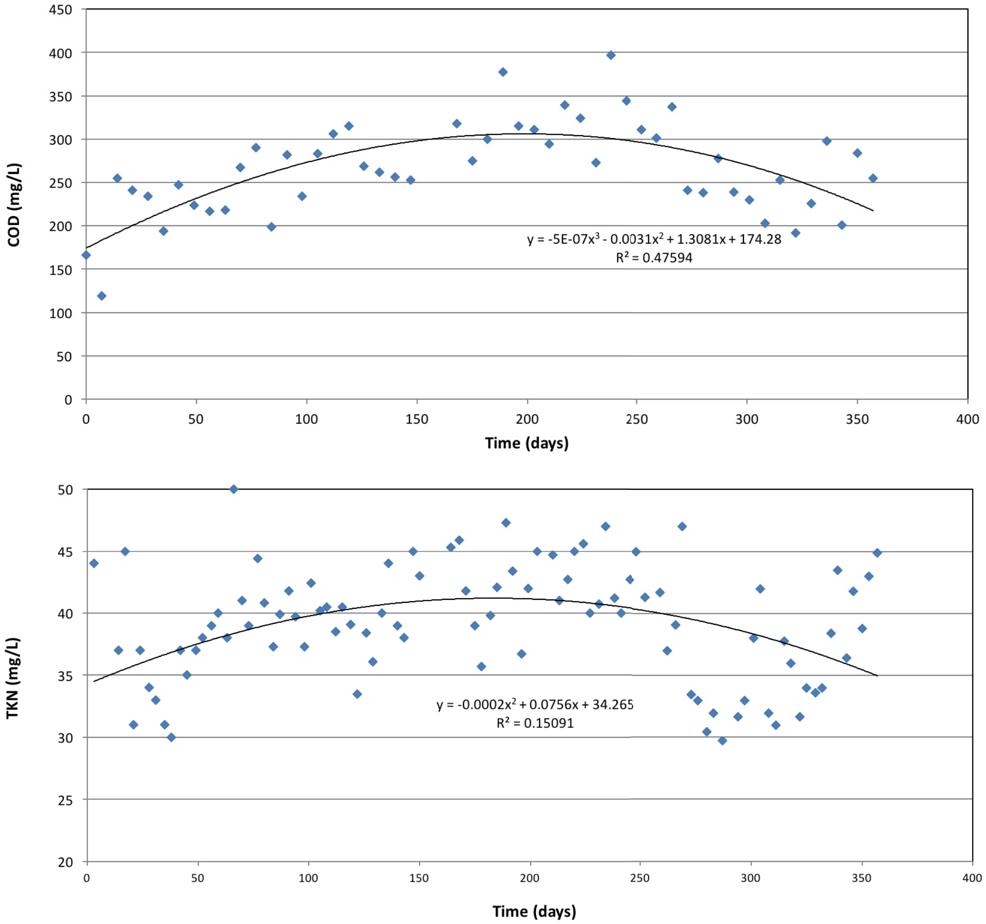

Figure 6.6 Polynomial fit of measured COD (upper) and TKN (lower) in the primary effluent. Time axis is from July 2013 to June 2014.

7. PREDICTING THE FATE OF MICROPOLLUTANTS DURING WASTEWATER TREATMENT: CALIBRATION AND SENSITIVITY ANALYSIS

7.1. Preface

This manuscript addresses the last objective of this PhD project i.e. to predict the fate of CECs in activated sludge treatment and determine the most influential model parameters. Previous dynamic models have been developed to predict the fate of some CECs in WWTPs, but no information is available in the literature on the sensitivity of these micropollutant fate models to the calibrated model parameters or on the uncertainty of the calibrated parameters.

In this manuscript, the focus was on four of the target CECs of Chapter 5 (i.e. caffeine, ibuprofen, androstenedione and triclosan) which belong to different classes of contaminants and are known to have different fate behaviours in activated sludge processes. Full-scale measurements of these CECs in activated sludge treatment obtained in Chapter 5 were used for calibration of the fate model. The model calibrated with respect to the measured dissolved oxygen, solids and nutrients in Chapter 6 was used in the current chapter as a basis for prediction of the fate of CECs. While most studies in the literature ignored the fate processes in the secondary clarifier, in the present manuscript these were included and the effect of ignoring them was investigated. Calibration was performed by minimizing the errors between simulation results and measurements of CEC concentrations in aqueous and particulate output streams fulfilling one aspect of the research objectives i.e. to calibrate the micropollutant fate model.

This manuscript confirmed the basic hypothesis that the fate of the target CECs in activated sludge could be well represented by mechanistic equations describing the biodegradation and sorption fate processes. Calibration yielded output CEC concentrations that are in agreement with measurements in both soluble and particulate phases of the output streams from the activated sludge unit. Ignoring the MP fate processes taking place in the secondary clarifier was observed to bias the concentration of caffeine in the settler's underflow, indicating that some biodegradation of caffeine takes place in the settler. One of the objectives of this work was to determine the most influential parameters on the model output, and this study demonstrated that while the fate model is highly sensitive to some parameters, others had no influence on the model fit implying that calibration of these parameters is not necessary. This study also provided evidence that the set of the most influential parameters is dependent upon the nature of the CEC and the corresponding major fate processes affecting its fate. Further, using dynamic sensitivity analysis, the model's sensitivity to the calibrated parameters was demonstrated to be time-dependent, which was associated to time-dependent input CEC concentration and may help to plan future measurement campaigns.

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Predicting the fate of micropollutants during wastewater treatment: calibration and sensitivity analysis

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7.2. Abstract

The presence of micropollutants in the environment and their toxic impacts on the aquatic environment have raised concern about their inefficient removal in wastewater treatment plants. In this study, the fate of micropollutants of four different classes was simulated in a conventional activated sludge plant using a bioreactor micropollutant fate model coupled to a settler model. The latter was based on the Bürger-Diehl model extended for the first time to include micropollutant fate processes. Calibration of model parameters was completed by matching modelling results with full-scale measurements (i.e. including aqueous and particulate phase concentrations of micropollutants) obtained from a 4-day sampling campaign. Modelling results showed that further biodegradation takes place in the sludge blanket of the settler for the highly biodegradable caffeine, underlining the need for a reactive settler model for accurate fate prediction. The adopted Monte Carlo based calibration approach also provided an overview of the model's global sensitivity to the parameters and showed that for each micropollutant a different set of one or more parameters had a significant impact on the model fit. The set of influential parameters could be related to dominant fate processes of each CEC justifying the selection of parameter subsets for model calibration. A dynamic local sensitivity analysis was also performed with the calibrated parameters. This analysis supported the conclusions from the global sensitivity analysis and linked the dynamic changes in sensitivity to the time-dependent input micropollutant concentrations, providing guidance for future sampling campaigns. This study expands the understanding of micropollutant fate models when applied to different micropollutants, in terms of global and local sensitivity to model parameters, as well as the identifiability of the parameters.

Keywords: Contaminants of emerging concern, Biological treatment, Activated sludge treatment, Dynamic sensitivity, Fate model

7.3. Introduction

The presence of pharmaceuticals and personal care products (PPCPs), as well as hormones, in the environment was proven to cause adverse effects on aquatic life raising concern about their insufficient removal from wastewater (Gay et al., 2016; Kidd et al., 2007; Purdom et al., 1994). The two major processes influencing the fate of micropollutants (MPs) during activated sludge treatment are biodegradation and sorption that act at different degrees on MPs with different characteristics (Joss et al., 2006; Radjenović et al., 2009b; Ternes et al., 2004b). Hence, although most of the emphasis in activated sludge mathematical modelling has been placed on conventional pollutants, some mathematical models were also developed to describe the fate and discharge of MPs in activated sludge systems and proposed as a cost-effective tool for risk assessment (Cowan et al., 1993; Plósz et al., 2012; Urase & Kikuta, 2005; Vezzaro et al., 2014).

Since the fate of MPs is influenced by the fate of conventional pollutants (e.g. solids that affect the sorption and biodegradation of micropollutants), simultaneous modelling of conventional and micropollutants was made possible by extending the well-known Activated Sludge Models (ASMs) developed many years ago (Henze et al., 1987; Henze et al., 1999). The most notable examples of models tackling both conventional pollutants and MPs are the ASM-X model (Plósz et al., 2012; Plósz et al., 2010) and another more recent model based on the ASM2d model (Vezzaro et al., 2014). The majority of MP fate studies in activated sludge focused their efforts on the modelling of the removal in bioreactors and considered the MP fate processes taking place in the secondary clarifier as insignificant (Cloutier et al., 2008; Plósz et al., 2010). However, one study by Vezzaro et al. (2014) extended the Takács settling model (Takács et al., 1991) to include the MP fate processes in the clarifier.

Previous efforts were made to calibrate the model parameters that describe the kinetics and equilibrium of biodegradation and sorption of several MPs (Cloutier et al., 2008; Cowan et al., 1993; Pomiès et al., 2013), with only few studies focusing on PPCPs and hormones (Plósz et al., 2012; Plósz et al., 2010; Urase & Kikuta, 2005). Calibration often focused on the kinetics of biodegradation only, assuming that sorption occurs much more rapidly (Abegglen et al., 2009; Suarez et al., 2010; Urase & Kikuta, 2005). As a result, full-scale micropollutant concentration measurements for model parameter calibration focused mostly on the aqueous phase in previous studies without measuring the MP load partitioned onto sludge (Joss et al., 2004; Plósz et al., 2012; Xue et al., 2010). Moreover, the large uncertainty on the calibrated parameters reported in the literature was identified as a major gap in the field of micropollutant modelling in WWTPs, a factor that is aggravated by the lack of knowledge on the sensitivity of the MP fate models to their parameters (Pomiès et al., 2013). This leaves the model users unsure about the degree of confidence in the parameters values reported in the literature.

In the present study, a MP fate model based on ASM2d (bioreactor) was further modified and coupled to a reactive settler model extended for the first time from the Bürger-Diehl settler model which itself incorporates the latest important advancements in the field of secondary settler modelling (Bürger et al., 2012). The scope of the current paper was to calibrate the MP model's parameters using MP concentration measurements collected from a 4-day sampling campaign at a full-scale WWTP, which was meant to be feasible in terms of efforts and resources supplied by the utility running the plant. Given the chronic nature of the impacts of the studied CECs, sub-daily variations were not deemed of importance for this study, and only the 24-h average load was considered for sampling. Samples were collected under dry weather conditions, since that is when surface water bodies are expected to be the most sensitive to wastewater discharges as a result of the limited dilution. A model that was previously calibrated with respect to conventional pollutants using one-year data was used as a starting point for the current MP calibration. The MPs investigated in the present study included a hormone (androstenedione), a pharmaceutical (ibuprofen), an antibacterial agent (triclosan) and a nervous stimulant (caffeine). Ibuprofen, triclosan and caffeine were selected based on their high detection frequency in wastewater effluents (Dickenson et al., 2011), while little data is available regarding the levels and fate of androstenedione (Baalbaki et al., 2016; Esperanza et al., 2007). The target MPs were also previously observed to be influenced by sorption and biodegradation fate processes to variable extents in WWTPs (Baalbaki et al., 2016), while volatilization is not considered as significant (Struijs et al., 1991; Virkutyte et al., 2010). For sorption, both the kinetics and the equilibrium equations were considered in the bioreactor and settler models. Sensitivity analysis was performed to explore the impact of the MP fate model parameters on each of the fitted variables, in relation to the input dynamics. Prior to calibrating the model for micropollutant fate, the hydraulic model of the WWTP was identified and the activated sludge unit was calibrated with respect to conventional pollutants, as described in our previous studies (Baalbaki et al., 2016; Baalbaki et al., In Press).

7.4. Materials and Methods

7.4.1. Full-scale activated sludge

Concentrations of the target MPs were measured in samples collected over four dry days at the full-scale activated sludge unit of the Guelph WWTP (Guelph, Ontario, Canada). The WWTP contains four activated sludge lines, followed by tertiary treatment by rotating biological contactors (RBCs) and sand filtration, as well as disinfection by chlorine. It serves a population of 135,000 inhabitants and receives an average flow of 50,750 m^3/d. The final de-chlorinated effluent is discharged into a nearby river. The first of the four lines of activated sludge (Line 1) was selected for this study. This line contains two aeration tanks in parallel, and the output of both tanks is combined and sent to a single secondary clarifier. Ferric chloride is added for phosphorus removal to the input to the primary clarifier (i.e. 1 L/min) and at a lower dosage to the return sludge of line 1 (i.e. 0.55 L/min). The main characteristics of line 1, including average hydraulic and solids retention time (HRT and SRT, respectively), as well as the average mixed liquor suspended solids concentration (MLSS), mixed liquor volatile suspended solids concentration (MLVSS) and temperature measurements over the sampling period are summarized in Table 7.1.

Table 7.1 Main characteristics of the Guelph WWTP, including average hydraulic and solids retention times (HRT and SRT), average temperature and mixed liquor suspended solids and volatile suspended solids concentrations (MLSS and MLVSS) during the sampling campaign (i.e. July 2014).

Characteristic		Value
HRT (h)	Primary clarifier (line 1)	3.72
	Aeration tanks (line 1)	6.30
	Secondary clarifier (line 1)	2.90
SRT of line 1 (d)		7.76
Average water T (°C)		20
Average MLSS (mg/L)		3,560
Average MLVSS (mg/L)		2,790

7.4.2. Sampling

Sampling was performed over four days in the period of July 16-20, 2014 in dry conditions. The number of sampling days was selected based on a hydraulic model for this WWTP (Baalbaki et al., 2016). As shown in Figure 7.1, samples were collected from the effluent of the primary clarifier or primary effluent (PE), the effluent of the aeration tanks (AE), the effluent of the secondary clarifier (SE), as well as from the secondary waste sludge at the underflow of the secondary clarifier (WAS) and from the sludge of the primary clarifier (not shown in Figure 7.1). Samples from the PE and SE were collected as 24-h composite samples using onsite Hach Sigma samplers, where the samples were refrigerated at 4°C until retrieved (daily). Due to the absence of an onsite sampler in the AE location, 24-h composite samples from this stream were obtained using ISCO 6712 samplers (Avensys, St-Laurent, QC, Canada) equipped with 24 bottles and packed with ice (replaced daily). Waste activated Sludge samples were collected as grab samples on each of the four days. All collected samples were transferred into 1-L Nalgene certified pre-cleaned amber bottles (Fisher Scientific) at the end of each day of sampling, stored at -20 °C and kept frozen until extracted.

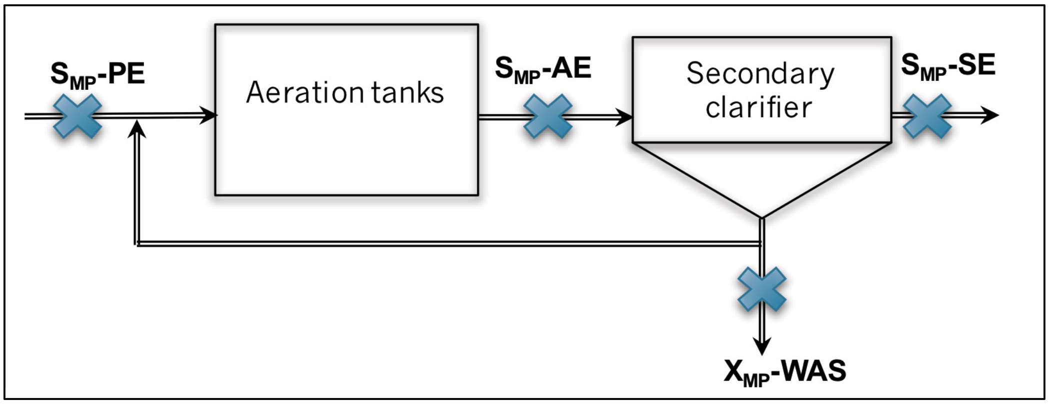

Figure 7.1 Schematic of the investigated full-scale activated sludge unit. Sampling locations are indicated with a cross sign (PE: primary effluent, AE: aeration effluent, SE: secondary effluent, WAS: waste activated sludge) and the corresponding measurements taken (S_{MP} and X_{MP} indicate measurement of the micropollutant concentration in aqueous and particulate phases, respectively).

7.4.3. Sample preparation

The four target micropollutants extracted from the wastewater and biosolids samples, as well as information on their respective internal standards and suppliers, are listed in Table 7.2. The aqueous samples from the PE, AE and SE locations were filtered using 1-µm glass-fiber filters (Fisher Scientific). The filtered samples (100 mL for PE and 200 mL for AE and SE) were spiked with internal standards to account for the extraction recovery and matrix effects and then subjected to solid-phase extraction (SPE). The cartridges used for extraction were the Oasis

MAX anion exchange cartridges (Waters, MA, USA), following the method described by Metcalfe et al. (2014) and summarized in the supplementary material (Table 7.3). The SPE extraction recoveries of target MPs in procedural blanks ranged from 90% to 130%, as reported earlier (Li et al., 2010). Extraction of sludge samples was conducted using a Dionex ASE 350 accelerated solvent extraction (ASE) system (Thermo Fisher Scientific, Waltham, MA, USA) followed by solid-phase extraction. Approximately 1 g of freeze-dried sludge was placed in ASE stainless steel cells and spiked with internal surrogates (Table 7.2, 100 ng/g). The ASE and SPE methods used for the analysis of the biosolids were previously described in the literature (Edwards et al., 2009) and are summarized in Table 7.3 of the supplementary material. The extraction recoveries of target analytes from biosolids was >70%. All aqueous and sludge samples were extracted in triplicates.

7.4.4. Chemical analysis

Chemical analysis was performed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) using an Agilent 1100 HPLC (Mississauga, ON, Canada) coupled to a Q-Trap 5500 instrument (AB Sciex, Concord, ON, Canada) operated with a turbospray ionization source. The method used for chromatographic separation was described by Metcalfe et al. (2014). Ibuprofen and triclosan were measured in negative ionization mode, while caffeine and androstenedione were measured in positive ionization model. Acquisition was performed in multiple-reaction-monitoring (MRM) mode. The parameters used for MRM are listed elsewhere (Metcalfe et al., 2014; Thompson et al., 2011). Results of the recovery of the spiked internal standards were used to determine the concentration of the target MPs.

Table 7.2 Target MPs along with their subtypes, internal standards, limits of detection (LODs) and limits of quantification (LOQs) in aqueous and biosolids samples, as well as suppliers.

Type	Subtype	Compound	Internal standard (Labelled surrogate)	Aqueous samples LOD, LOQ (ng/L)[1]	Biosolids LOD, LOQ (ng/L)[1]	Supplier[2] Compound, surrogate
Pharmaceuticals	Analgesics	Ibuprofen	Ibuprofen-13C3	6, 21	10, 32	S, I
Personal Care Product	Antibacterial	Triclosan	Triclosan-13C12	6, 19	6, 19	K, M
Steroid hormone		Androstenedione	Androstene-3,17-dione-2,3,4-13C3	2, 5	7, 25	S, C
Nervous stimulant		Caffeine	Caffeine-13C3	4, 14	5, 16	S, I

[1]: LODs and LOQs were obtained based on standard deviation of y-intercept of line obtained from measured concentrations of serial dilutions, [2]: Supplier (compound, surrogate): **S:** Sigma-Aldrich Canada (Oakville, ON, Canada), **I:** C/D/N Isotopes (Pointe-Claire, QC, Canada), **C:** Cerilliant Corporation (Round Rock, Tex, USA), **M:** Cambridge isotope Laboratories (Tewksbury, MA, USA), **K:** KICTeam (Langley, BC, Canada)

7.4.5. Modelling and simulations

The residence time and consequent removal of micropollutants in the WWTP is impacted by the hydrodynamics of treatment units (Majewsky et al., 2011; Ort et al., 2010). Hence, prior to modelling the fate of MPs, a hydraulic model of the activated sludge process under study was created using measurements of the wastewater's electrical conductivity as a tracer. The optimum hydraulic model consisted of three aeration tanks in series and one secondary clarifier, which is described in more details elsewhere (Baalbaki et al., 2016). A detailed calibration of the aeration and settler model parameters, as well as characterization of input chemical oxygen demand and suspended solids were performed using one-year measurements of dissolved oxygen, solids and nutrients at different locations of the studied activated sludge unit (Baalbaki et al., In Press). Briefly, the calibration procedure aimed at predicting the concentration of biomass, as well as the solids retention time that is known to affect the diversity of the biomass (Saikaly & Oerther, 2004). The concentration and the diversity of biomass are both expected to have an important influence on the rate of biodegradation of MPs (Kreuzinger et al., 2004; Suarez et al., 2010). The calibration performed by Baalbaki et al. (In Press) also aimed at predicting the solids concentration in the waste stream, which influences the extent of MP removal through adsorption. Compared to the Takács model, the Bürger-Diehl settling model was found to provide a better description of the suspended solids balance of the system that plays a key role in sorption as well as biodegradation of micropollutants. Following proper calibration of the solids balance, the standard ASM kinetic parameters provided a satisfactory fit to the measurements of nutrients. High dissolved oxygen was observed in the aeration tanks, indicating a relatively high oxygen transfer rate parameter, k_La. Further details on the calibration procedure are described elsewhere (Baalbaki et al., In Press).

All model simulations were performed in the software WEST (MIKE by DHI, Denmark). The variable-coefficient ODE (i.e. VODE) integrator available in WEST was used with a tolerance of 10^{-8} for its efficient run time. Processes describing the fate of MPs were included in the bioreactor model based on the extended bioreactor model library that was first developed by Vezzaro et al. (2014). The Bürger-Diehl settling model (Bürger et al., 2012) was extended to include the processes determining the fate of micropollutants (i.e. biodegradation and sorption).

The specific modifications are further elaborated upon in Section 7.5.2 of the discussion. The modified bioreactor and extended settler models were coupled.

All dynamic simulations were preceded by steady-state simulations over 23 days (i.e. 3xSRT) in order to obtain a good estimate of the initial conditions of solids concentration in different locations of the activated sludge unit. Also, to obtain realistic initial conditions of micropollutant concentrations, dynamic simulations were performed over a period of time starting a few days before the first day of sampling and assuming that the micropollutant concentrations were similar to the measured ones. The input to the model (i.e. primary effluent) consisted of the following measured time series: flow rates, measurements of conventional pollutants, such as TSS, ammonia and COD, as well as the incoming concentration of the micropollutants in dissolved and particulate phases. The latter was obtained from analysis of the collected primary sludge.

7.4.6. Model results analysis

The model output variables considered are the soluble micropolutant concentration in the effluent from the aeration tank and secondary clarifier (i.e. S_{MP}-AE and S_{MP}-SE, respectively), as well as the particulate micropollutant concentration in the waste sludge (i.e. X_{MP}-WAS), as shown in Figure 7.1. Calibration of model parameters was carried out using a Monte Carlo based approach with the scenario analysis tool available in WEST, similar to the approach suggested by Sin et al. (2008). For each parameter, a number of discrete values were defined over a broad range of parameter values that were based on literature findings, and simulations were performed for all possible parameter combinations. Subsequently, the goodness of fit for each simulation was determined by the chi-square (χ^2) criterion, as proposed by Dochain and Vanrolleghem (2001) and expressed in Eq. 7.1, where $\hat{y_i}$ and y_i represent the predicted and the measured variable value, respectively at time point i and σ_i is the standard deviation of the measurements at time point i.

$$\chi^2 = \sum_{i=1}^{i=N} \frac{(y_i - \hat{y_i})^2}{\sigma_i{}^2} \qquad (7.1)$$

All measurement values (i.e. soluble and particulate micropollutant concentrations) with their corresponding simulated values were considered for the calculation of χ^2 in Eq. 7.1. The chi-square error was used to quantify the degree of mismatch between the results from simulations and measurements, since it accounts for measurement uncertainty through the standard deviations obtained from lab replicates for each of the four days. In order to compare the simulated profiles with the measured ones for error calculations, the simulated profiles were converted into a similar time scale by calculating the average daily values (flow-average or time-average), since measurements were based on time-proportional and flow-proportional samples at the AE and SE locations, respectively. The optimal parameter set was determined from the simulation with the lowest χ^2 value. Moreover, by studying the evolution of χ^2 values over the tested parameter intervals, conclusions could be drawn on the model fit's global sensitivity to the parameters, as well as the identifiability of each parameter (i.e. the existence of a unique small range of parameter values that minimize the χ^2). In addition to the χ^2 criterion, the two-sample t-test with a confidence level of 95% was performed to verify the capability of the best-fit model to well predict the average micropollutant concentration in the particulate phase in the waste sludge.

Local sensitivity analysis around the optimal parameter values was carried out in the WEST software in order to understand the dynamic effect of small variations in the model parameters on the three model output variables. In theory, the relative sensitivity is determined by Eq. 7.2 in discrete form, where t represents the time, y the variable and θ the input parameter, which is varied systematically by $\Delta\theta$ (perturbation). Therefore, the output of the sensitivity analysis conveys a message about the percentage change in the output variable resulting from a theoretical 100% change in the parameter, even though the actual perturbation is significantly lower than 100%.

$$Relative\ sensitivity = \frac{y(t,\theta+\Delta\theta)-y(t,\theta)}{\Delta\theta}\ \frac{\theta}{y} \tag{7.2}$$

7.5. Results and discussion

7.5.1. Observed concentrations and removals

In the influent to the activated sludge process, triclosan and androstenedione were observed at average concentrations of 132 and 65 ng/L, respectively. The widespread analgesic ibuprofen and the stimulant caffeine present in many beverages entered at higher average concentrations of 1.68 µg/L and 25.3 µg/L, respectively. As observed in the current sampling campaign and reported in previous work (Baalbaki et al., 2016), the removal of ibuprofen and caffeine by the activated sludge unit under study was 99% and 94%, respectively. This high removal efficiency is in good agreement with previous literature (Behera et al., 2011; Clara et al., 2005b; Radjenović et al., 2009b). Triclosan had a removal of 72 %, also consistent with previous observations (Behera et al., 2011), while androstenedione was removed at a lower efficiency of 33%. Ibuprofen and androstenedione had an average particulate concentration in the secondary waste sludge of 39 ng/g and 38 ng/g, respectively. On the other hand, due to its higher input concentration, caffeine was observed at a high concentration (i.e. 776 ng/g) in secondary waste sludge. For triclosan, the measured particulate concentration in waste sludge was 1334 ng/g, which can be attributed to its known hydrophobicity (Petrie et al., 2014). These four MPs were thus selected for the modelling study based on the diversity observed in their biodegradation and sorption tendency, as reported in the literature and indicated by experimental results of the current study. This variability in the fate allows for a more comprehensive exploration of MP fate model calibration and sensitivity analysis for variable combinations of parameter values.

7.5.2. Model structure

The processes accounted for in the model are biological degradation and sorption, since these were suggested to be the most significant processes for the target MPs (Andersen et al., 2005; Joss et al., 2006; Ternes et al., 2004b). For a completely mixed unit with constant volume the micropollutant soluble concentration dynamics are expressed by Eq. 7.3 taking into account the incoming and outgoing MP load, as well as sorption and biodegradation processes. Given the presence of ample oxygen (constantly >2.5 mg/l) in the activated sludge system under study (i.e. above the limit that ensures the absence of anoxic zones), only aerobic biodegradation was considered. The biodegradation pseudo first-order kinetic equation in the model by Vezzaro et al. (2014) where the biomass is assumed to be constant was modified to include the effect of temporal variations in the active biomass concentration, as shown in Eq. 7.4. The active biomass concentration is calculated by the model as the sum of the concentrations of the following particulate ASM2d model components: heterotrophic (X_{OHO}), autotrophic (X_{AOO}) and phosphate accumulating organisms (X_{PAO}). The concentrations of these components composing the biomass are predicted by the calibrated conventional pollutant model (Baalbaki et al., In Press) that is used in the present study. A Monod-type substrate saturation kinetics was not deemed necessary due to the generally low concentrations of micropollutants, as well as the fact that the first-order kinetic equation was proven to yield satisfactory predictions for micropollutants (Namkung &

Rittmann, 1987; Vezzaro et al., 2014). Instantaneous sorption leading to equilibrium was assumed in the majority of studies on the fate of MPs (Kemp et al., 2002; Parker et al., 1994; Plósz et al., 2010; Pomiès et al., 2013). However, in the present study, a more realistic approach was considered by accounting for the kinetics of both sorption and desorption in addition to the equilibrium, as shown in Eq. 7.5 and Eq. 7.6, respectively (Urase & Kikuta, 2005).

$$\frac{dS_{MP}}{dt} = \frac{F_{MP,in}}{V} - \frac{F_{MP,out}}{V} + r_{bio} + r_{sor} \tag{7.3}$$

$$r_{bio} = -k_{bio} * S_{MP} * X_{active} \tag{7.4}$$

$$r_{sor} = -k_{sor} * S_{MP} * X_{TSS} + k_{des} * X_{MP} \tag{7.5}$$

$$K_d' = \frac{1}{K_d} = \frac{k_{des}}{k_{sor} * 1000(\frac{g}{kg})} = \frac{S_{MP} * X_{SS}}{X_{MP} * 1000(\frac{g}{kg})} \tag{7.6}$$

Where:

- S_{MP} & X_{MP}: Concentration of micropollutant in dissolved and particulate phases (ng/L)
- $F_{MP,in}$ & $F_{MP,out}$: Input and output micropollutant load, respectively (ng/d)
- V: Volume of the treatment unit (bioreactor or secondary clarifier) (L, input)
- r_{bio} & r_{sor}: Rate of micropollutant biodegradation and sorption/desorption, respectively (ng/ (L.d), calculated)
- X_{active} & X_{TSS}: Concentration of active biomass and suspended solids, respectively (gSS/L, predicted by the conventional pollutant model)
- k_{bio}: Biodegradation first order rate constant (L/ (gSS.d), input)
- k_{sor}: Sorption first order rate constant (L/ (gSS.d), input)
- k_{des}: Sorption first order rate constant (1/d, calculated)
- K_d': Inverse of the partition coefficient at equilibrium (kgSS/L, input)

To model the behaviour of MPs in the secondary settling tank, two adjustments were made to the Bürger-Diehl secondary settler model: First, instead of tracking the transport of the lumped solids components throughout the clarifier as formulated in the standard Bürger-Diehl model, in this study, the transport of each model component (i.e. soluble and particulate components), including micropollutants, was tracked separately. This allowed accounting for the propagation of micropollutants in the clarifier under the effects of gravity settling (for particulate components), as well as flow rate (for particulate and soluble components). This is important since inaccurate representation of the propagation of the soluble and particulate organics within the clarifier was proven to cause a bias in their concentration in the output sludge that is partially recycled back (Jeppsson & Diehl, 1996).

The second adjustment was the inclusion of the fate processes (i.e. biodegradation and sorption) acting on micropollutants in the secondary clarifier. Almost all of the previous studies on MP fate modelling in activated sludge assumed that removal of micropollutants in the secondary clarifier is negligible. Figure 7.2 demonstrates that removal processes in the secondary clarifier can potentially be of importance. Although ignoring biodegradation in the settler does not impact the MP concentration in the clarifier's supernatant, it increases the average soluble concentration of micropollutants in the waste sludge by more than 100% (Figure 7.2(a)). The target micropollutant caffeine, represented in Figure 7.2, is known to biodegrade extensively, as discussed earlier, indicating that the high biomass concentration at the bottom of the settler allows for significant biodegradation to take place (Eq. 7.4). This result is particularly relevant since, for the system under study, the model predicted only 4% of the total solids mass to be present in the secondary clarifier which can be explained by the good settling characteristics of the sludge (i.e. low SVI with an average of 58 mL/g). Consequently, for other activated sludge systems operating at high sludge blanket and thus containing a higher percentage of solids in the secondary clarifier, the micropollutant biodegradation process in the secondary clarifier could be even more significant. Accurate prediction of the micropollutant load in the underflow of the settler by accounting for the MP biodegradation is particularly important when modelling the overall fate of MPs in the entire WWTP as the underflow is often partially recycled back to the head works after treatment, increasing the load of the MP to the WWTP. In contrast, in the upper region of the settler, the biomass is present at low concentration, which explains the observation that the simulated concentration of caffeine in the supernatant was unaffected by the inclusion or exclusion of the biodegradation reactions (Figure 7.2(b)). This difference in the extent of biodegradation throughout the settler results in spatial variation in the soluble and particulate micropollutant concentrations across the settler that is overlooked in non-reactive settler models.

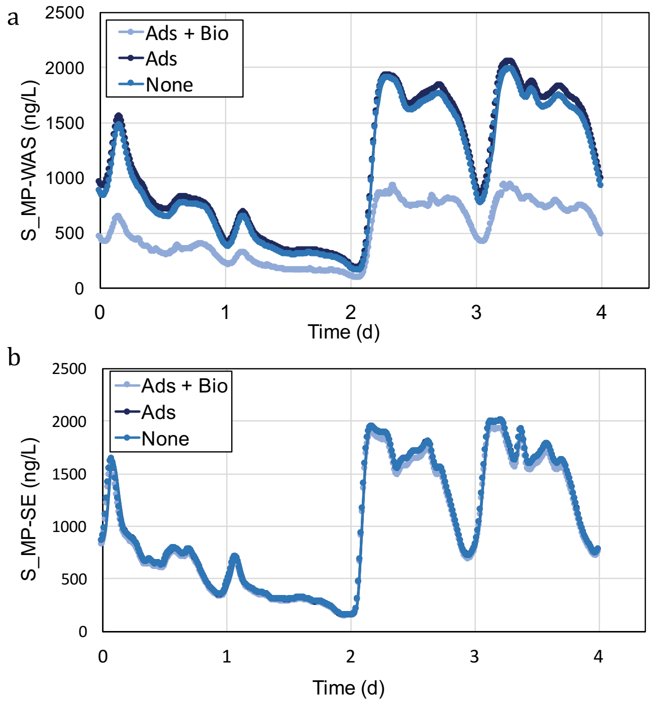

Figure 7.2 Predicted soluble caffeine concentration in the two secondary clarifier outputs: a) secondary waste sludge and b) secondary effluent, accounting for both sorption and biodegradation (Ads+ bio), only sorption (Ads) or none of the fate processes (None).

7.5.3. Impact of individual model parameters

The behaviour of the model and the interaction between the different processes (i.e. biodegradation Eq. 7.4 and adsorption Eq. 7.5) are illustrated in Figure 7.3 by changing process parameters for caffeine, used here as an example. It is important to note that a logarithmic scale was selected for the particulate micropollutant concentration (ng/g) given the high variability in the model output resulting from a change in k_{bio}. The influence of the parameter k_{bio} (present in the biodegradation rate equation (Eq. 7.3) is evident, as with faster biodegradation, both the soluble and particulate micropollutant concentrations decline notably (i.e. increasing k_{bio} from 5 to 10 $L/(gSS.d)$ decreases the S_{MP} and X_{MP} by 75% and 85% on average, respectively). Although the adsorbed portion of the micropollutant is not considered to be biodegraded in this model, the decrease in S_{MP} at higher k_{bio} disturbs the equilibrium and causes subsequent desorption leading to a decrease in X_{MP}.

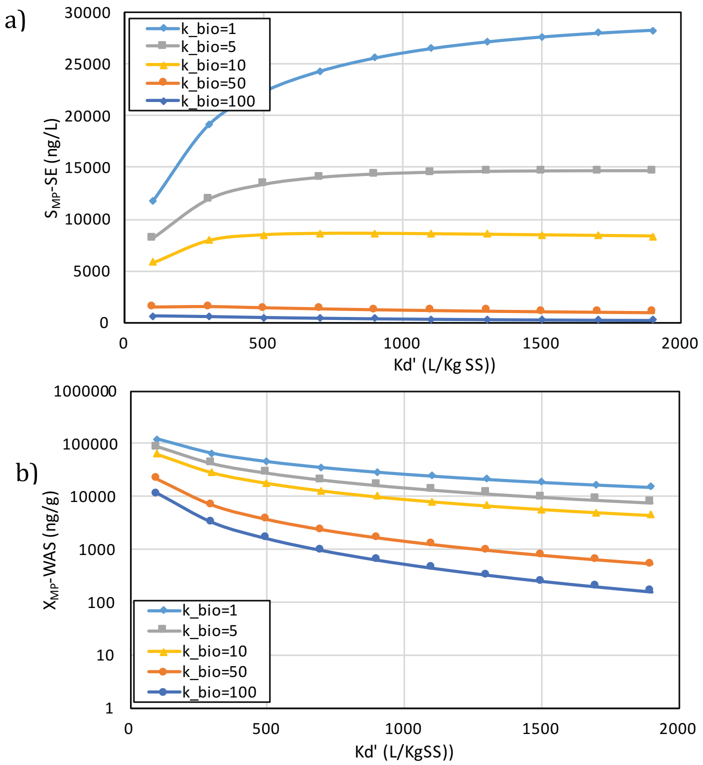

Figure 7.3 Impact of changing $\boldsymbol{K_{d}}'$ on the concentration of caffeine in a) secondary effluent (soluble) and b) WAS (particulate) at different k_{bio} values (L/(gSS.day)) and at fixed k_{sor}=5 L/(gSS.day). X_{MP}-WAS was plotted on a logarithmic axis due to the sharp variations induced by changing k_{bio} and $\boldsymbol{K_{d}}'$.

The influence of $K_d{}'$ that pertains to the equilibrium of adsorption on the micropollutant concentration is less straightforward. As shown in Eq. 7.5, a higher $K_d{}'$ increases the desorption kinetics thus moving the micropollutant sorption equilibrium to the soluble phase. This effect of increasing $K_d{}'$ can be clearly seen in Figure 7.3 through an increase in the S_{MP}, accompanied by a decrease in X_{MP} for slow biodegradation kinetics (defined by low k_{bio} values). When the biodegradation kinetics are fast (i.e. k_{bio}>10 $L/(gSS.d)$), increasing $K_d{}'$ still decreases X_{MP} significantly but the influence on S_{MP} is less pronounced (Figure 7.3(b)). Indeed, when the biodegradation kinetics are much faster than those for desorption, any desorbed MP load will undergo almost instantaneous biodegradation, and hence a change in the desorption rate constant is less effective on the soluble MP concentration. In fact, it was observed that at k_{bio} value that is as high as 50 $L/(gSS.d)$, increasing the $K_d{}'$ (and desorption) induces a slight decrease only in the soluble MP concentration in the secondary effluent. This can be directly associated with the reduced particulate MP concentration due to the high desorption rate, as well as the subsequent immediate biodegradation, resulting in a lower MP load available for desorption and thus lower soluble MP concentrations. Hence, the effects of parameters on the model output are not independent, that is, the effect of one parameter depends on the value of the other parameters. It is thus necessary to evaluate the different combinations of parameters at different ranges for calibration, as explained in the following section. The effect of varying k_{sor} that is linked to $K_d{}'$ by Eq. 7.5 is not shown here, but it is discussed in the following section.

7.5.4. Calibration

A scenario analysis was performed by simulating the model for all possible combinations of parameters over pre-defined intervals based on literature values. For each simulation the χ^2 was calculated based on both the model outputs and the observations. In each dot plot in Figure 7.4, the evolution of the normalized χ^2 (i.e. expressed as χ^2 of each simulation divided by the minimum χ^2) is shown as a function of changes in a specific parameter but taking into consideration all combinations of numeric values of the other parameters. These plots allow to assess the global sensitivity of the micropollutant model to changes in each parameter, as well as to analyze the identifiability of the parameter. A clear minimum in the χ^2 value at a specific parameter value indicates that the parameter is identifiable. Large changes in χ^2 over a parameter interval correspond to a high sensitivity of the model output to this parameter. It is important to note that the χ^2 takes into consideration the errors (i.e. standard deviations) in measurements during χ^2 calculation. A logarithmic axis was used for the normalized χ^2, since it was observed to vary greatly at different combinations of the parameters.

7.5.4.1. Caffeine

For caffeine, it was observed that changing k_{bio} value (i.e. from around 20 to 30 $L/gSS.day$) decreases the minimum χ^2 by more than one third, demonstrating the high impact of this parameter on the model fit. Low χ^2 values are clearly found at high biodegradation rates. The optimum fit was observed at k_{bio}= 33 $L/gSS.day$, which is within the wide range of parameter values encountered in the literature 1- 240 $L/gSS.day$ (Bertolini, 2011; Xue et al., 2010). The large variability in the observed k_{bio} values in the literature could be attributed to the variable properties of the biomass and the suspended solids in the systems used for calibration (Ternes & Joss, 2006).

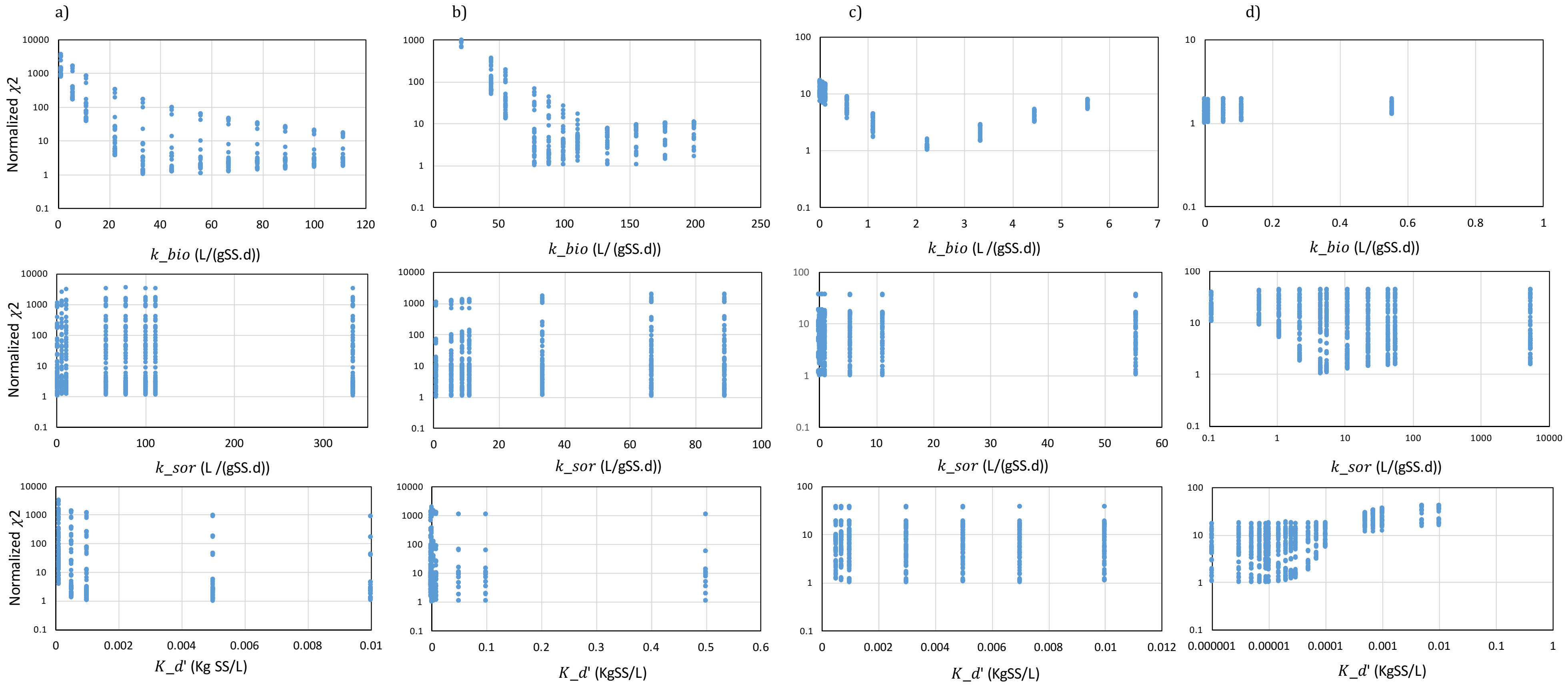

Figure 7.4 Normalized χ^2 represented as ratio of the minimum χ^2 for a) caffeine, b) ibuprofen, c) androstenedione, d) triclosan as a result of varying each model parameter: k_{bio} (first row), k_{sor} (second row) and $K_{d'}$ (third row).

The optimal model fit was observed at $K_d' > 0.001\ kgSS/L$ which is consistent with previous literature (Stevens-Garmon et al., 2011; Xue et al., 2010). Contrary to k_{bio}, varying the value of k_{sor} did not have a pronounced influence on the minimum χ^2, as low χ^2 values can be found over the entire parameter range. This indicates the lack of identifiabiltiy of k_{sor} and K_d' and can be explained by the extensive biodegradation of caffeine at the investigated range of k_{bio}, which reduces the model's sensitivity to the other fate processes. It is also observed that increasing k_{bio} beyond 40 $L/gSS.day$, decreases the degree of variability of the χ^2 value (i.e. the length of the dotted lines in Figure 7.4), indicating a lower sensitivity of the model to the other parameters when biodegradation is more significant. This is reasonably interpreted by the fact that when biodegradation is faster, resulting in a rapid decline in the MP concentration, the effect of changing sorption parameters is less influential.

The best-fit model yielded a predicted profile of caffeine's concentration in the secondary effluent that was in agreement with the measured profile, as shown in Figure 7.5(a) by comparing the simulated and measured MP concentration profiles. In addition, the predicted output concentration time series with a 15-min time step was used to obtain a daily flow-average of the simulated micropollutant's concentration represented by triangles in Figure 7.5. This allows comparison with the measured concentrations obtained from 24-h composite samples (i.e. represented by squares). Similar to the secondary effluent, the best-fit model yielded a good fit between the simulated and the measured caffeine concentrations in the aeration tank outlet (results not shown). In addition, the average (i.e. over the 4 measurement days) simulated concentrations of MPs in the solids of the waste sludge from the best-fit model are visually compared to the measurements in Figure 7.6. The p-value representing a measure of the match between the average measurements and simulations are shown to be >0.05, indicating that the model well predicts the underflow particulate concentration of caffeine, as the difference between these is statistically insignificant. Average particulate MP concentrations were used for assessing the model fit in Eq. 7.1, unlike soluble MP concentrations, since the particulate MP concentrations are less temporally variable throughout the four days of sampling, due to the SRT of almost eight days.

7.5.4.2. *Ibuprofen*

The plots for ibuprofen are similar to those for caffeine, which could be explained by the fact that both are highly biodegradable, with no significant adsorption taking place. The best fit was observed at k_{bio}=77 $L/gSS.day$. This value is higher than the previously observed values in the literature 1.3-38 $L/gSS.day$ (Abegglen et al., 2009; Fernandez-Fontaina et al., 2012; Suarez et al., 2010). The high k_{bio} values obtained in the present study relative to the literature for ibuprofen could be associated with the higher dissolved oxygen concentration (DO) in the aeration tanks of the system under study (i.e. 4-6 mg/L during the majority of the sampling period compared to <4 mg/L in the literature). Xue et al. (2010) demonstrated that at higher DO values, the biodegradation of micropollutants is enhanced, which is represented by a higher k_{bio}. Figure 7.4 shows that both K_{d}' and k_{sor} are non-identifiable. The best-fit model provided good predictions of the soluble micropollutant concentration in secondary effluent and particulate phase in the settler's underflow, as shown in Figure 7.5(b) and Figure 7.6, respectively.

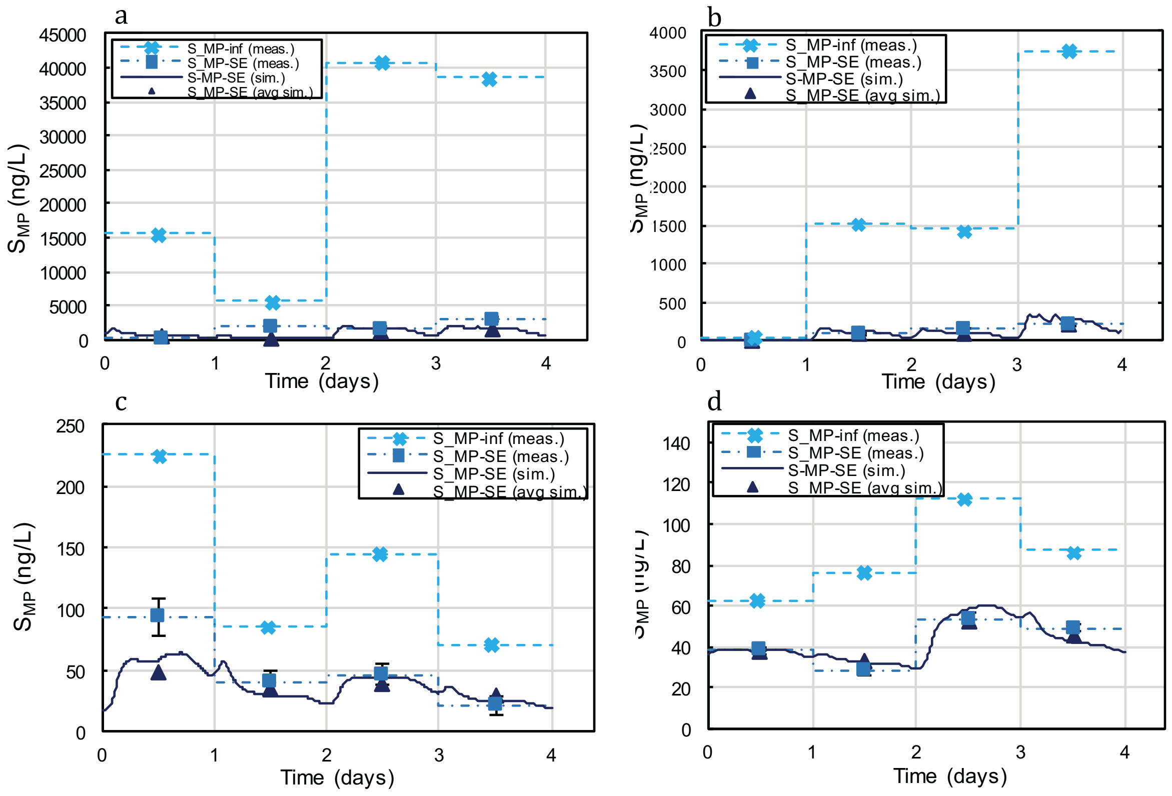

Figure 7.5 Measured (meas.) influent concentrations as well as measured and simulated (sim.) soluble secondary effluent concentrations for a) caffeine, b) ibuprofen, c) triclosan, d) androstenedione. The flow-average of simulated values are shown as (avg sim.). For ibuprofen only, the soluble effluent concentrations are displayed on a separate secondary axis since values are much lower than in the input.

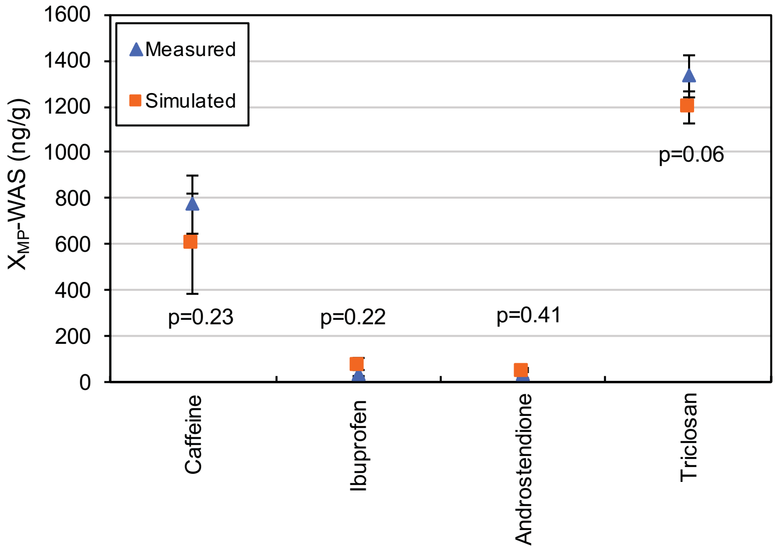

Figure 7.6 Measured and simulated concentrations of each micropollutant in secondary waste sludge (ng/g). The error bars represent the standard deviations obtained from measurements on the four days of sampling for both simulations and measurements. P-values from the two-sample t-test represent the match between the measured and simulated concentration of the micropollutant.

7.5.4.3. Androstenedione

As shown in Figure 7.4, androstenedione exhibits an evident minimum in χ^2 at an optimum value of k_{bio} 2.2 $L/gSS.day$. Higher k_{bio} values lead to higher errors (i.e. increasing k_{bio} from 2 to 4 $L/(gSS.day)$ was shown to triple the minimum χ^2). The clear minimum in the χ^2 values for the partially biodegradable androstenedione also indicates that varying k_{bio} has a profound influence on the model fit. Hence, the biodegradation rate constant for androstenedione can be estimated with high confidence since the model is highly sensitive to this parameter. From the available literature, no data were found to model the fate of androstenedione in the activated sludge process, and only one previous explored its removal in activated sludge treatment (Esperanza et al., 2007). Similar to caffeine and ibuprofen, K_d' and k_{sor} did not have a notable influence on the model fit.

7.5.4.4. Triclosan

For triclosan, a clear optimum in χ^2 values can be found for both k_{sor} and K_d'. The minimum χ^2 was observed at $k_{sor} = 4.4\, L/gSS.day$ and $K_d' = 7x10^{-6}\ kgSS/L$ or lower. The K_d' value that leads to the best-fit model is within the ranges observed in the literature (Nakada et al., 2010), although other literature reported higher values (Hyland et al., 2012). One explanation for the observed K_d' being at the lower end of the literature values is the addition of ferric chloride in the unit under study, which alters the properties of the sludge, potentially increasing the sorption potential of MPs onto it. In fact, it was previously observed that chemical additions to activated sludge decreases the value of K_d' for triclosan (Zhu, 2014). Using the optimum K_d' and k_{sor} resulted in predicted particulate underflow concentrations that correspond to the measurements with p-value >0.05, and the concentration in the secondary effluent also showed a fairly good match with measurements (Figure 7.6 and Figure 7.5, respectively). Unlike sorption parameters, wide variations in the k_{bio} value did not lead to changes in the model fit, indicating low sensitivity of the model to this parameter.

7.5.5. Dynamic local sensitivity analysis

After obtaining the optimal values of the most effective parameters for each of the four micropollutants, a local sensitivity analysis was performed to investigate the dynamic sensitivity of the different model variables throughout the four-day simulations, as shown in Figure 7.7. This analysis provides information to link the model's sensitivity to parameters with the dynamics of input MP concentration and process conditions. Such analysis can provide valuable information for the design of future measurement campaigns based on expected influent dynamic conditions. For illustration, this analysis was performed for caffeine and triclosan only, since their behaviour in activated sludge treatment was shown to be influenced by different fate processes and their concentration in the influent is highly dynamic. Using WEST's default perturbation factor (i.e. the factor change in the parameter) of 10^{-6}, inconclusive sensitivity profiles were obtained, due to interference of numerical errors with the small effects on output variable caused by the applied perturbations, due to the very low MP concentrations (De Pauw & Vanrolleghem, 2006). Hence, the perturbation factor was set to a higher value of 10^{-2}, and the assumption of linearity of the sensitivity was verified to be still valid by the equality of the backward and forward relative sensitivities (i.e. by both increasing and decreasing the parameter with the same perturbation) (De Pauw & Vanrolleghem, 2006).

As shown in Figure 7.7, for caffeine at the best-fit parameters, the most influential parameter on the soluble micropollutant concentrations is k_{bio}, which is in agreement with the observations made in the previous section. This is supported by numerical sensitivity values indicating that a

100% theoretical change in the parameter results in >100% change in soluble micropollutant concentrations at all monitored locations. On the contrary, the sensitivity to k_{sor} was low (<50% change in X_{MP} and S_{MP} / 100% change in parameter), while the sensitivity to K_d' that describes sorption equilibrium was high for the micropollutant concentration in the waste sludge only. A notable reduction in the sensitivity of S_{MP} to k_{bio} is observed to take place on the third day. The specific time where the sensitivity to k_{bio} is lowest corresponds to a drastic increase in the concentration of caffeine at the input to the activated sludge unit (i.e. from, 5,920 ng/L to 40,700 ng/L) and also at the headworks (i.e. from 4,813 ng/L to 50,800 ng/L). The effect of the increase in input concentration on the sensitivity of S_{MP} to k_{bio} is mathematically reasonable, since the expression of the time-dependent S_{MP} obtained from the numerical solution of Eq. 7.3 contains all the following: the parameter k_{bio}, the incoming MP concentration and the initial MP concentration (affected by previous incoming concentrations). A lower sensitivity to k_{bio}, as observed on the third day, indicates that parameter k_{bio} value is not significantly affecting the simulation due to other influences on the system. This is expected to increase the uncertainty of the calibrated k_{bio} value if data were only available in such circumstances. Hence, avoiding sampling during expected sharp dynamics in input caffeine (i.e. such as over weekdays and weekends or around peak periods during the day for high-frequency samples) improves the calibration of k_{bio} by eliminating data in low sensitivity conditions.

For triclosan, hypothetically increasing K_d' by 100% increased the soluble MP concentration by 10-40% and decreased the particulate concentration (in ng/L) by <5%, which is reasonably explained by the higher calculated k_{des} associated with the larger value of input K_d'. Increasing k_{sor} by 100% was continuously shown to decrease the soluble MP concentration by 40-100% at all locations of the activated sludge. Considering that in the input, the soluble to particulate MP fraction (i.e. average of $3\text{x}10^{-4}$ $kgSS/L$) is higher than that for secondary sludge at equilibrium determined by K_d' ($1\text{x}10^{-5}$ $kgSS/L$), faster kinetics towards equilibrium will continuously reduce the soluble MP concentration to achieve K_d'. The amount of reduction in the soluble MP concentration due to perturbations in k_{sor} exhibited a time-dependent profile with higher reductions in S_{MP} (i.e. higher sensitivity to k_{sor}) observed on the first and the third days where the soluble load is the highest. This further emphasizes the impact of the input MP concentration profile on the model's sensitivity. The sensitivity of the particulate MP concentration to all model parameters was lower than that of the soluble MP concentration (i.e. <20% change in X_{MP} for 100% theoretical change in parameter). This indicates that in the case of triclosan, measuring the micropollutant concentration in the aqueous phase might be sufficient for calibration, due to the higher sensitivity of the aqueous phase to the model parameters compared to the particulate phase

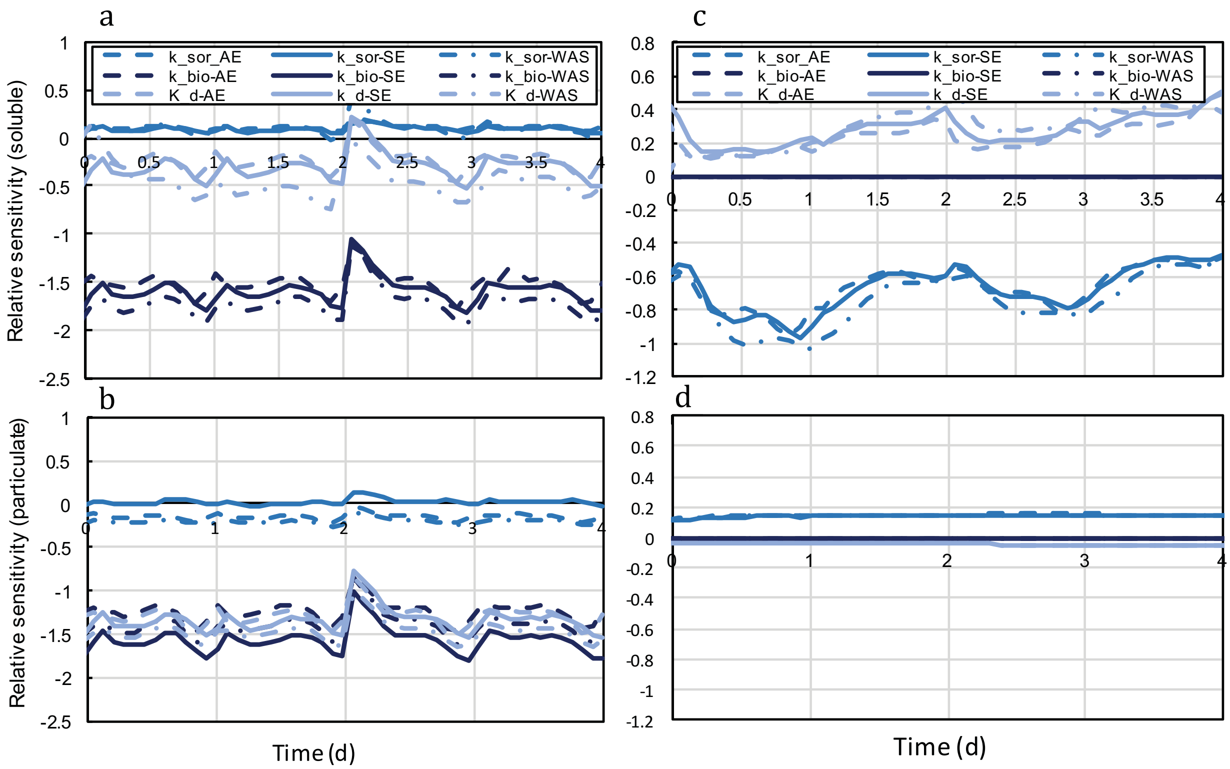

Figure 7.7 Dynamic local sensitivity of the MP soluble concentrations (upper graphs) and particulate concentrations (lower graphs) in different locations: AE, SE and WAS to small perturbations in the three parameters for caffeine (a & b) and triclosan (c & d).

7.6. Conclusion

In this study, a model calibrated for conventional pollutants was extended to include MP fate processes and was subsequently calibrated for a number of specific MPs of different classes using full-scale measurements. The micropollutants' fate processes were included in the bioreactor model through an available extension of the ASM2d model and in the secondary clarifier model by extending the Bürger-Diehl settler model for the first time to include the propagation and the fate processes of MPs. Comparing results from the reactive and non-reactive settler models indicated that a mismatch of 100% in the simulated micropollutant soluble concentration at the underflow of the settler would occur as a result of ignoring biodegradation taking place in the settler for the case of the highly biodegradable caffeine. A Monte Carlo based scenario analysis was used to find optimal parameter values, as well as to provide information on parameter's identifiability and the model's global sensitivity. Depending on the process that plays the major role in the fate of a specific micropollutant, different model parameters could affect the fit of the model to different extents, which eventually determines the identifiability of the calibrated parameters. While for the highly biodegradable caffeine and ibuprofen, k_{bio} was found to be the most significant parameter influencing the fit of the model, for triclosan, both sorption parameters k_{sor} and K_d' were more significant. For androstenedione, which is mildly degradable, the k_{bio} showed a clearer optimum compared to caffeine and ibuprofen. Dynamic local sensitivity analysis was performed and proposed to serve as a tool in the design of sampling campaigns for improved calibration of model parameters, since it provides insights on preferable input dynamics during sampling, as well as on necessary types of measurements (i.e. particulate or soluble MP concentration). These analyses provided novel insights on the sensitivity of a micropollutant activated sludge model to the different model parameters, as well as on the temporal variability in the sensitivity in relation to input dynamics. The sampling campaign used, limited in resource and time requirements, indicated a potential for future applications of this fate model by WWTP operators for the purpose of developing risk assessment tools to evaluate the discharge of micropollutants into the environment.

7.7. Acknowledgements

The authors acknowledge the staff at the Guelph WWTPs for their support during the sampling campaign. The authors thank Prof. Chris Metcalfe and Dr. Tamanna Sultana for the chemical analysis of the target compounds. We gratefully acknowledge the McGill Engineering doctoral award for supporting Zeina Baalbaki. Funding for this study was provided by a research grant to Viviane Yargeau (PI) and colleagues (Chris Metcalfe and Peter Vanrolleghem) from the Natural Sciences and Engineering Research Council (NSERC) of Canada through the Strategic Grants

Program (430646-2012). Peter Vanrolleghem holds the Canada Research Chair on Water Quality Modelling.

7.8. Supplementary material

Table 7.3 Extraction methods of the target compounds from collected wastewater and sludge samples

Sample	Method	Extraction step	
Aqueous	SPE[1]	Instrument	Manual manifold
		Cartridge	6 mL-500 mg Oasis MAX cartridges
		Sample pH	pH 8.0 using sodium hydroxide
		Cartridge conditioning	Methanol, 0.1 M ammonium hydroxide and water (pH 8.0)
		Elution	2 mL methanol and then 3x3 mL of 2% formic acid in methanol, 1 mL/min
		Reconstitution	50% methanol/50% water to a total volume of 0.4 mL
Biosolids	ASE[2]	Instrument	Dionex ASE 350
		Conditions	Temperature =80 °C Static cycle= 3
		Elution solvent	Acetone: water (3:7)
	SPE	Instrument	Manual manifold
		Cartridge	6 mL-500 mg Oasis HLB cartridges
		Sample pH	pH 7.5 using sodium hydroxide
		Cartridge conditioning	Acetone, Methanol, and Milli-Q water (pH 7.5).
		Elution	3x3 mL of methanol, 1 mL/min
		Reconstitution	50% methanol/50% water to a total volume of 0.1 mL

[1] Solid phase extraction

[2] Accelerated solvent extraction

8. ORIGINAL COUNTRIBUTIONS

The original contributions of this PhD project are:

I. **The demonstration of the ability of the "fractionated approach" to account for differences between treatment plants and the implementation of the method for the first time as an improved sampling and removal calculation strategy for the investigation of CEC removal in primary and tertiary treatment steps.** Although the fractionated approach was previously applied twice for CEC removal calculation, the application was limited for a number of CECs and within the process of activated sludge treatment only, but no data reported removal in primary and tertiary treatment steps determined using this approach. The removal of some target CECs was examined for the first time in rotating biological contactors and sand filters. In addition to that, the fractionated approach was used in the present study to compare the effect of hydraulic differences on the CEC removal calculations between two full-scale WWTPs and between various treatment units within a WWTP.

II. **Coupling mass balance analysis of CECs in a full-scale WWTP with the fractionated approach providing improved data of fate of CECs and for the first time data were provided on the fate of some CECs (few drugs of abuse and androstenedione) in primary sludge and activated sludge**. Furthermore, a biosolids extraction method previously suggested for beta-blockers was proven to yield satisfactory recoveries for the studied drugs of abuse.

III. **Calibration of an ASM2d-based CEC fate model for several CECs of different classes after coupling to a reactive settler.** The reactive settler model was developed in this project as an extension of the Bürger-Diehl model, and in addition to fate processes, it was also extended to account for the propagation of soluble and particulate CECs in the settler due to both flow and gravity settling. Calibrated parameter values were reported for the first time for some CECs. Prior calibration of solids in the activated sludge provided novel insights on the impact of the chemically-enhanced primary treatment on the solids balance that is expected to impact the fate of CECs.

IV. **Exploration of the sensitivity of the fit and output of the fate model to the model parameters for CECs with different fate characteristics.** To date, no published studies had performed analysis of the global and local sensitivity of micropollutant activated sludge models to the model parameters.

9. CONCLUSIONS

The thesis examined the removal of 26 target contaminants of emerging concern in different steps of wastewater treatment plants. One novelty of the work performed is the consideration of the hydrodynamics of the treatment units for the CEC removal calculations by comparing the hydraulic characteristics of two WWTPs and the resulting removal calculations. Another major contribution is the use of a mass balance of the CEC load performed by measuring the CEC load sorbed to solids in primary and secondary treatment steps, which allowed the quantification of the contribution of different fate pathways for each target CEC. Lastly, a model was developed and calibrated to predict the fate of four target CECs in the activated sludge process accounting for fate processes in the secondary clarifier for the first time.

The results showed that:

I. Activated sludge processes with different configurations showed different hydraulic characteristics and, hence, the calculation of removal of CECs by these units had to be adjusted accordingly. Obtaining reliable removal data using the fractionated approach involves determining the proper load fractions representing the output CEC load in terms of incoming loads of previous days, which were shown to be different for different activated sludge units, depending on the hydraulic retention time and the extent of mixing of each unit.

II. The best-fit hydraulic models obtained for the primary and tertiary wastewater treatment units suggested a low extent of mixing compared to the secondary treatment step. Hence, for these units, most of the CEC load discharged on a specific day corresponded to the load entering on the same day with lower fractions coming from previous days compared to the activated sludge process.

III. A mass balance analysis demonstrated that in the activated sludge unit, biodegradation was the major removal process for most of the investigated CECs, while sorption did not play a significant role in the removal of most target CECs (i.e. <5%), except for estrone, triclosan and trimethoprim (i.e. 29-47%). The mass balance analysis in the primary clarifier indicated poor removal for most of the target CECs, with degradation contributing to the removal of some CECs, such as ibuprofen, androstenedione, sulfamethoxazole and triclosan.

IV. The studied tertiary treatment steps, rotating biological contactors and sand filtration, were generally less efficient at removing target CECs than secondary treatment by activated sludge, although these tertiary steps were found to play a role in the removal of conventional pollutants such as ammonia and solids.

V. Measuring the concentrations of the major metabolites of carbamazepine indicated potential inter-transformation between the metabolites and the parent compounds during wastewater treatment. This provided an explanation for the persistence or negative elimination of carbamazepine, which were frequently reported in the literature and theoretically linked to the hydraulics effect on the removal calculations and potential deconjugation of metabolites. Since the hydraulics were properly accounted for in the current study, the observations must be linked to the transformations discussed above.

VI. The solids modelling performed for the studied activated sludge unit, as a prerequisite to modelling the fate of CECs, indicated that the particulate to soluble organics ratio in the effluent of CEPT obtained from characterization (i.e. representation in terms of model components) differed from the ratio observed for effluents from conventional primary clarifiers. Results also showed that special considerations were required to characterize the solids in the primary effluent, which was linked to the effect of CEPT on the composition of the primary effluent.

VII. From the CEC fate model describing the CEC removal by activated sludge treatment, it was observed that a model that ignores the biodegradation in the secondary clarifier, which is general practice, potentially overestimates the settler's underflow concentration of caffeine that is known to be highly biodegradable. Hence, this study showed that to model the fate of CECs in activated sludge units, it is essential to account for the fate processes not only in the aeration tanks but also in the secondary clarifier. The state-of-the-art Bürger-Diehl model for secondary clarifiers was thus extended for the first time with CEC fate submodels.

VIII. The fit of the secondary treatment fate model was shown to be sensitive to different parameters depending on the compound of interest (i.e. $\mathrm{k_{bio}}$ was more significant for the prediction of androstenedione followed by ibuprofen and caffeine, while $\mathrm{k_{sor}}$ and $K_d{}'$ were more important to predict the fate of triclosan). The most influential parameter set was observed to depend on the target CEC and its corresponding predominant fate process (i.e. biodegradation or sorption), and indicated the need to identify specific parameters to focus on during calibration.

IX. From the dynamic sensitivity analysis, it was shown that the profile of the incoming CEC concentration measured during sampling campaign and used in the model input can potentially cause temporal variability in the model's sensitivity to the parameters. Considering that calibrating a model parameter based on instants of low sensitivity to the parameter reduces the confidence in the obtained calibrated parameter, it is important to enhance the sensitivity of the model or to measure at those instances where sensitivity is high. Hence, it is preferable to perform sampling campaigns at specific conditions of CEC levels in the influent. For caffeine, the absence of sharp changes in the input concentrations was found to enhance the model's sensitivity to k_{bio}, while for triclosan a higher soluble to particulate CEC concentration increased the sensitivity to k_{sor}.

10. RECOMMENDATIONS

Based on the literature, results and conclusions of this study, the following areas were identified to be in need for further consideration:

i. All the sampling campaigns in the present study were carried out to collect 24-h composite samples on weekdays. Due to the previously proven variations in wastewater composition throughout the week, it is recommended that future sampling campaigns include both weekdays and weekends and more frequent sampling to account for diurnal variations of CEC load. This would help to capture higher temporal variability of the input CEC load and examine its effect on the removals and calibrated model parameters.

ii. Collection of higher volumes of aqueous samples enables the measurement of CEC concentration in the suspended solids at each sampling location, including the settled wastewater at the secondary effluent. This would further enhance the reliability of the mass balance results and provide more data for a more detailed calibration of the CEC fate model.

iii. In this project, the concentration of the main metabolites of carbamazepine were measured and inter-transformations between these and their parent compound was suggested. Being able to integrate these transformations into the CEC fate model in activated sludge would improve the study of the fate of persistent CECs but is currently beyond the capabilities of the existing models. Hence, further modifications of the CEC fate model are recommended to introduce extra CEC components to the model base, as well as simplified equations for their inter-transformations.

iv. In the present study, the CEC fate model was calibrated using data from a full-scale WWTP. Testing the model with data from other WWTPs would provide further validation of the CEC fate models and the calibrated parameters. Sensitivity analysis of the CEC fate model with respect to the conventional pollutants would also provide insights on the effect of varying characteristics (i.e. solids concentrations and removals) between the different WWTPs.

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APPENDIX I: SOLIDS SETTLING MODELS

Table A.1 Main differences between the Takács and the Bürger-Diehl settling models (Bürger et al., 2012; Bürger et al., 2011; Takács et al., 1991; Torfs et al., 2015).

Aspect	**Takács**	**Bürger-Diehl**
Model	1-D (i.e. only vertical flow)	1-D (i.e. only vertical flow)
Number of layers	10 layers	The number of layers can be set by the user, with higher number resulting in a physically-correct representation
Boundary layers	No boundary layers; i.e. the concentration in the underflow of the clarifier is assumed equal to the concentration in the bottom layer	Two extra layers at the top and at the bottom to better describe the outlet concentration taking into account the effect of the outlet pipe
Settling	Accounts for gravity settling	Accounts for gravity settling and compression settling that is a phenomenon observed in high-concentration sludge present in the sludge blanket
Inlet dispersion	The incoming solids are assumed to be distributed instantaneously and homogeneously over the feed layer	Accounts for dispersion by taking into account the mixing phenomenon in the feed layer as a function of the input flow rate