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**MICROPOLLUTANT REMOVAL AND NUTRIENT
RECOVERY FOR SUSTAINABLE MANAGEMENT OF
URBAN WASTEWATER**

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SCHOOL OF CIVIL AND ENVIRONMENTAL ENGINEERING

2015

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URBAN WASTEWATER**

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School of Civil and Environmental Engineering

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ABSTRACT

The continuous discharge of nutrients and micropollutants such as pharmaceutically active compounds (PhACs) potentially create a threat to urban aquatic environment and water safety. This issue is extremely important for some metropolitans that rely on the renewable/reclaimable water resources such as Singapore. This study explores new approaches to improve the current wastewater management practice particularly in terms of nutrient recovery and micropollutant mitigation from both scientific and engineering perspectives.

In Chapter 2, this study first reviewed the current research on nutrient recovery and micropollutant removal from wastewaters. Cytostatic drugs were selected as target micropollutants, whose environmental occurrence and behaviors, source contributions, and potential ecotoxicity were summarized. Source separation strategy promises to improve the current wastewater management practice, especially in terms of nutrient/energy recovery and separate treatment of pharmaceutical residue. The benefits, limitations, and trends of development of different approaches to address the ‘emerging’ contamination issue are covered for urine source-separation strategy, membrane bio-reactor, reverse/forward osmosis (RO/FO) and advanced oxidation processes. Promising alternatives may essentially lie on the state-of-the-art treatment technologies based on urine source-separation strategy; it deserves further comprehensive evaluation from the technological, social-economical and administerial perspectives.

In Chapter 3, the feasibility and potential limitations of applying urine source separation system in a tropical urban setting was evaluated by systematically investigating urine hydrolysis process (i.e., enzymatic ureolysis, spontaneous mineral precipitation, odor emission issue) and different nutrient recovery approaches (i.e., seawater-induced struvite precipitation and ammonia stripping). The results demonstrated that an effective and automated urine source separation system could be achieved. The entire process can be accurately monitored by measuring conductivity as a timesaving and cost-efficient indicator. Approaches were suggested to cope with pipe clogging and odor emission

issues from an engineering perspective. This study also demonstrated that seawater can be used as a cost-effective magnesium source to recover phosphorus from hydrolyzed urine. Structural characterization of the precipitates confirmed struvite crystals (slow-releasing fertilizer) as the main product with certain magnesium calcite or calcite as co-precipitates. Air stripping process can be further applied to effectively recover ammonia from source-separated urine and the experimental parameters provided certain guidelines for the development of N-recovery process in industrial-scale.

In Chapter 4, the feasibility of applying FO dewatering process for nutrient recovery from source-separated urine was further investigated under different conditions, using seawater or desalination brine as a low-cost draw solution. The filtration process exhibited relatively high water fluxes up to 20 L/m²·h. The process revealed relatively low rejection to neutral organic nitrogen (urea-N) in fresh urine but improved rejection of ammonium (50–80%) in hydrolyzed urine and high rejection (> 90%) of phosphate, potassium in most cases. Compared to simulation, higher water flux and solute flux were obtained using fresh or hydrolyzed urine as the feed, which was attributed to the intensive forward nutrient permeation (i.e. of urea, ammonium and potassium). Membrane fouling could be avoided by prior removal of the spontaneously precipitated crystals in urine. Compared to other urine treatment options, the current process was cost-effective and environmentally-friendly for nutrient recovery from urban wastewater at source, yet a comprehensive life-cycle impact assessment might be needed to evaluate and optimize the overall system performance at pilot and full scale operation. The FO process can be incorporated into a proposed decentralized source-separating sanitation system aiming to recycle the ‘human resource’ in agriculture with minimized environmental discharge and energy demand.

In Chapter 5, a robust analytical method was developed and validated for the trace determination of multi-class cytostatic drugs in environmental matrices based on polymeric solid-phase extraction and liquid chromatography tandem mass spectrometry. The method was intensively optimized for sample pretreatments, instrumental conditions, and quantification in order to enhance the analytes’ recoveries and to mitigate matrix effects. Due to severe SPE loss and matrix effects, only twelve analytes

could be reliably quantified by multiple-reaction monitoring with dual isotope-labelled internal standards. The optimized method was then applied for cytostatic screening in tropical aquatic environment where two hormone antagonists – tamoxifen and bicalutamide – were detected at 11–91 and 32–146 ng/L, respectively, indicating their incomplete removal during wastewater treatment and even trace presence in surface water. Precautionary actions are therefore needed to eliminate these detectable cytostatic drugs from the aquatic environment although their long-term fate in the environment is still beyond the knowledge of current science.

In Chapter 6, the study further investigated the removal of a selected group of 13 cytostatic drugs (including 3 alkylating agents, 2 antimetabolites, 1 plant alkaloid, 3 cytotoxic antibiotics, and 4 hormone antagonists) using FO membrane process. The rejection mechanisms were studied with a thin-film composite (TFC) polyamide membrane and a cellulose triacetate (CTA) membrane under variable conditions. A high proportion of cationic (doxorubicin, epirubicin, and tamoxifen) and zwitterionic (melphalan and methotrexate) compounds were adsorbed to membrane surfaces by electrostatic attraction, resulting in generally higher solute fluxes and lower rejection efficiencies (except tamoxifen). Both membranes exhibited consistently high rejection (> 90%) to anionic cytostatic compounds. The TFC membrane showed generally better rejection to all cytostatics with a greater size-exclusion effect (as indicated by a smaller molecular weight cut-off value). Higher draw solution concentration led to enhanced FO rejection as a result of dilution effect. The presence of effluent matrix in the feed solution generally improved the FO rejection to cationic and zwitterionic compounds. On the other hand, increasing feed pH from 7 to 9 resulted in varied response in rejection as a consequence of complex interplay of size exclusion and electrostatic interaction. The results are practically important when integrating FO technology to strengthen sustainable management of local water resources and wastewater.

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LIST OF ACRONYMS

AOP	:	advanced oxidation process
APCI	:	atmospheric pressure chemical ionization
APIs	:	active pharmaceutical ingredients
ATC	:	anatomical therapeutic classification
Aza	:	azathioprine
BCF	:	bio-concentration factor
Bic	:	bicalutamide
COD	:	chemical oxygen demand
CP	:	cyclophosphamide monohydrate
CP-d ₈	:	deuterium-labelled cyclophosphamide
Cyt	:	cytarabine
Cyt- ¹³ C ¹⁵ N ₂	:	¹³ C- and ¹⁵ N-labelled cytarabine
DDDs	:	defined daily doses
DO	:	dissolved oxygen
Dox	:	doxorubicin hydrochloride
EDX	:	Energy-dispersive X-ray spectroscopy
EMA	:	the European Medicines Agency
Epi	:	epirubicin hydrochloride
ERA	:	environmental risk assessment
ESI	:	electrospray ionization
EU	:	the European Union
Flu	:	flutamide
Flu-d ₇	:	deuterium-labelled flutamide
FO	:	forward osmosis
5-FU	:	5-fluorouracil
FT-IR	:	Fourier transform infrared spectroscopy
GAC	:	granular activated carbon
GC–MS	:	gas chromatography mass spectrometry
Gem	:	gemcitabine hydrochloride

GIS	:	geographic information system
HLB	:	hydrophilic–lipophilic balance
HRT	:	hydraulic retention time
HSDB	:	Hazardous Substances Data Bank (National Library of Medicine)
IARC	:	International Agency for Research on Cancer
IDL	:	instrumental detection limit
IF	:	ifosfamide monohydrate
IQL	:	instrumental quantification limit
K _{oc}	:	organic carbon partition coefficient
K _{sp}	:	Solubility product constant
LC–MS/MS	:	liquid chromatography tandem mass spectrometry
LC–QqQ MS	:	liquid chromatography triple-quadrupole mass spectrometry
logP (logK _{ow}):		octanol-water partition coefficient of an unionized compound
m/z	:	mass-to-charge ratio
MBR	:	membrane bioreactor
MC	:	mitomycin C
MCDA	:	multi-criteria decision analysis
MDL	:	method detection limit
MEC	:	measured environmental concentration
Mel	:	melphalan hydrochloride
Mer	:	6-mercaptopurine monohydrate
Met	:	methotrexate hydrochloride
MF	:	micro-filtration
Mit	:	mitotane
MPs	:	micropollutants
MQL	:	method quantification limit
MRM	:	multiple reaction monitoring
NF	:	nano-filtration
NOM	:	natural organic matter
nZVI	:	nanoscale zero-valent iron
OsMBR	:	osmotic membrane bioreactor
OTC	:	over-the-counter drugs (versus description medicines)

PAC	:	powder activated carbon
Pac	:	paclitaxel
PBT	:	persistence, bioaccumulation, and toxicity
PEC	:	predicted environmental concentration
PhACs	:	pharmaceutically active compounds
PI	:	precursor ion
ppb	:	parts per billion, equal to $\mu\text{g/L}$ in dilute solution
PPCPs	:	pharmaceuticals and personal care products
ppm	:	parts per million, equal to mg/L in dilute solution
ppt	:	parts per trillion, equal to ng/L in dilute solution
PRO	:	pressure retarded osmosis
Pro	:	procarbazine hydrochloride
psi	:	pounds per square inch
PTFE	:	polytetrafluoroethylene
PVDF	:	polyvinylidene fluoride
RO	:	reverse osmosis process
SD	:	standard deviation
SEM	:	scanning electron microscopy
SPE	:	solid-phase extraction
SRT	:	sludge retention time
STP	:	sewage treatment plant or wastewater treatment plant (WWTP)
Tam	:	tamoxifen citrate
U.S. EPA	:	the U.S. Environmental Protection Agency
U.S. FDA	:	the U.S. Food and Drug Administration
UV-vis	:	ultraviolet–visible
WHO	:	the World Health Organization
XRD	:	X-ray powder diffraction

LIST OF SYMBOLS

A	:	water permeability of an FO membrane, $m/s \cdot Pa$
B_s	:	salt permeability of an FO membrane, m/s
C	:	analyte concentration in liquid, g/L
J_n	:	nutrient flux in FO process, g/m^2h
J_s	:	solute flux in FO process, g/m^2h
J_w	:	measured water flux in FO process, L/m^2h
$\tilde{J}_w$	:	predicted water flux in FO process, L/m^2h
$K_{m,s}$	:	mass transfer coefficient of salt in membrane porous layer, m/s
$\ln$	:	natural logarithm
pH	:	$-\lg[H^+]$
pKa	:	acid dissociation constant
R	:	rejection efficiency, %
R^2	:	correlation coefficient (value from 0 to 1)
κ	:	electrical conductivity, S/cm
θ	:	matrix effect (signal enhancement or suppression), %
π_f	:	osmotic pressure of feed solution, bar
π_d	:	osmotic pressure of draw solution, bar
φ	:	matrix effect correction, %

LIST OF PUBLICATIONS

1) Journal articles:

- Zhang, J.**, Wang, K., She, Q., Chang, V.W.C., Tang, C.Y., Webster, R.D. (2015), Chemical structure-dependent removal of cytostatic drugs by thin-film composite and cellulose triacetate forward osmosis membranes, Environmental Science & Technology. In Review.
- Zhang, J.**, Chang, V.W.C., Giannis, A., Webster, R.D., Wang, J.-Y. (2015), Simultaneous determination of multi-class cytostatic drugs in environmental matrices by polymeric solid-phase extraction-liquid chromatography-tandem mass spectrometry, Analytical and Bioanalytical Chemistry. In Review.
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- Liu, B., Giannis, A., **Zhang, J.**, Chang, V.W.C., Wang, J.-Y. (2014), Air stripping process for ammonia recovery from source-separated urine: modeling and optimization, Journal of Chemical Technology and Biotechnology. Vol. 90, No. 12, pp. 2208-2217.
- Liu, B., Giannis, A., Chen, A., **Zhang, J.**, Chang, V.W.C., Wang, J.-Y. (2015), Determination of urine-derived odorous compounds in a source separation sanitation system, Science of the Total Environment. In Review.

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CHAPTER 1 INTRODUCTION

1.1 Background

The continuous discharge of nutrients and organic micropollutants such as pharmaceutically active compounds (PhACs) potentially create a threat to urban aquatic environment and water safety. This issue is extremely important for some metropolitans that rely on the renewable/reclaimable water resources such as Singapore. On the other hand, a paradigm shift for wastewater treatment practice is rising across the world in the shade of global water scarcity and resource depletion. Wastewater has been increasingly considered as a resource rather than ‘nuisance waste’. This study explores new approaches to improve the current wastewater management practice particularly in terms of nutrient recovery and micropollutant mitigation from both scientific and engineering perspectives.

PhACs are produced for bettering human life. However, pharmaceutical residues have been continuously discharged for decades to the aquatic environment mainly via wastewater treatment facilities (Bound and Voulvoulis, 2005). It has been well recognized that conventional STPs carry low removal efficiencies for many PhACs and their metabolites excreted from human body (Verlicchi et al., 2012b). They are continuously introduced into the environment. Acute or chronic toxicity effects to aquatic organisms have been associated with pharmaceutical exposure at environmental relevant concentrations (parts-per-billion/trillion levels) (Fent et al., 2006). Since the mid-1990s, research efforts have been surging to assess PhACs’ potential to harm the ecosystem and human health when entering into the potable water system (Daughton and Ternes, 1999; Heberer, 2002; Khetan and Collins, 2007; Verlicchi et al., 2012b).

To date, great efforts have been made to study PhACs’ environmental occurrence and behaviors, toxicological endpoints, and effective mitigation approaches (Daughton and Ternes, 1999; Heberer, 2002; Khetan and Collins, 2007; Verlicchi et al., 2012b). These efforts have been highlighted by intensive research programs globally and regulations such as ‘Registration, Evaluation, Authorization and Restriction of Chemicals

(REACH)' (European Union, 2006). Several large-scale collaboration programs have been conducted in Europe and the United States, such as 'Poseidon' (EU, 2001-2004), Norman (EU, 2005-2008), PILLS (EU, 2007-2012) and Emerging Contaminants in the Environment (U.S. Geological Survey, 2007-2011). The recent PHARMAS (EU, 2011-2013) and CytoThreat (EU, 2011-2013) are specifically targeting the antibiotic and anti-cancer drugs. PhACs and metabolites were detected worldwide at ng/L to µg/L levels in surface water bodies in different areas (Ashton et al., 2004; Calamari et al., 2003; Kolpin et al., 2002; Mompelat et al., 2009). Furthermore, the consumption of various PhACs will increase as people live longer with higher standards of living (Kümmerer, 2010). Since PhACs are designed to have specific biological interferences on targeted tissues in human bodies, many of them have been determined or suspected for their detrimental effects on aquatic organisms and humans even at trace environmental levels (Fent et al., 2006; Johnson et al., 2008; Khetan and Collins, 2007). The continuous life-long exposure to PhACs and their metabolites as well as their additive and/or synergistic effects may subject the ecosystem to further jeopardy.

1.2 Problem Statement

Among various PhACs, cytostatic drugs (also called antineoplastic drugs) that are widely used in cancer chemotherapy have drawn growing concerns on their adverse effects to the environment in recent years. This is particularly because many cytostatic compounds are identified to be carcinogenic, teratogenic, and mutagenic (Besse et al., 2012; Johnson et al., 2008; Zhang et al., 2013a). There are over one hundred different cytostatic drugs in use today, which are divided into six subgroups according to major therapeutic actions (i.e., alkylating agents, antimetabolites, plant alkaloids, cytotoxic antibiotics, hormones and antagonists, and other miscellaneous agents) with alkylating agents being the largest and biologically active subgroup.

Recent studies have demonstrated the ubiquitous presence of cytostatic compounds in the aquatic environment because of their resistance to biotransformation during wastewater treatment (Buerge et al., 2006; Gómez-Canela et al., 2012; Llewellyn et al., 2011; Mahnik et al., 2007; Martin et al., 2011; Moldovan, 2006; Negreira et al., 2014a;

Valcarcel et al., 2011; Weissbrodt et al., 2009). The biologically active parent cytostatics, metabolites and transformation products can potentially harm the ecosystem and even human health when entering into the water purification system (Parrella et al., 2014a; Parrella et al., 2014b; Rowney et al., 2009; Toolaram et al., 2014). The environmental fate and effects of these genotoxic compounds remain largely unclear. The global consumption of cytostatic drugs is ramping up in the shade of rapidly ascending cancer burdens over past decades (Stewart and Wild, 2014). Precautionary care and action are necessary to prevent these emerging contaminants from threatening human health and the environment.

Various approaches were advocated to prevent PhACs from entering the environment, including ‘green pharmacy’ and return programs for unused and outdated drugs (Daughton, 2003a; Daughton, 2003b; Khetan and Collins, 2007). Another promising option is to adopt source segregation of highly-contaminated wastewater streams (e.g. urine stream, hospital effluent), since many pharmaceutical residues are primarily excreted via urine after metabolism. The source-separated urine distinguishes itself from other wastewater streams by its uniquely high nutrients (N, P) content yet contributing a marginal volume to the total domestic wastewater (Meininger and Oldenburg, 2009). There is a research gap to know whether the application of source separation system promises to significantly decrease the discharges of both nutrients and various micropollutants to the environment (Winker et al., 2008). The combination of smart and cost-effective treatment technologies are also needed to harvest the valuable resources (e.g., nutrients, clean water) from municipal wastewater and essentially prevent various micropollutants from continuously entering the environment. The results from this study would help promote the sustainable management of urban water resources.

1.3 Objectives and Scope

The overall objective of this study was to provide novel approaches to improve the conventional centralized wastewater management practice towards a more sustainable pattern, especially in terms of nutrient recovery and separate treatment of pharmaceutical residue. To date, there are thousands of different PhACs and even more

micropollutants including heavy metals, endocrine-disrupting compounds, plasticizers, phalates, pesticides, etc. that have been produced and readily discharged into the environment after usage. After an intense and extensive scrutiny on literature, this study particularly focuses on cytostatic drugs (a very specific group of PhACs) that are widely used for cancer chemotherapy over the past decades. Many of them have been categorized as carcinogenic, mutagenic and teratogenic compounds, triggering widespread concerns about their occupational exposure and ecotoxicological risks to the environment in recent years. Particularly, this study aims:

- 1) to provide a comprehensive literature review on source contributions, occurrence, transport and transformation, potential ecotoxicity of cytostatic drugs in the environment, as well as different approaches for the control and treatment of cytostatic compounds. The benefits, limitations and trends of development in this domain are covered for urine source-separation strategy, membrane bio-reactor, RO/FO and advanced oxidation processes (AOP).
- 2) to evaluate the adaptability of urine source separation system in a tropical urban setting by systematically investigating enzymatic ureolysis, spontaneous mineral precipitation, and odor emission issue during urine hydrolysis process, as well as different nutrient recovery techniques such as seawater-induced struvite precipitation and ammonia stripping.
- 3) to investigate the feasibility of applying FO dewatering technique for complete nutrient recovery from source-separated urine using seawater or desalination brine as a low-cost draw solution, particularly in terms of water flux, nutrient rejection, membrane fouling, nutrient concentration efficiency, and system design for real-world application.
- 4) to develop a robust analytical method for the trace determination of multi-class cytostatic drugs in different environmental matrices based on polymeric solid-phase extraction and liquid chromatography tandem mass spectrometry (SPE–LC–MS/MS), and apply the optimized method in tropical waters and wastewaters.
- 5) to examine the effectiveness of using FO membrane process (compared to RO) for removing a selected group of cytostatic drugs as a function of their physicochemical properties, and elucidate the chemical structure–dependent rejection mechanisms under variable conditions.

1.4 Structure of the Thesis

Figure 1-1 shows the overall structure of this thesis. Chapter 1 briefly introduces the research background, objectives and scope, and thesis structure. Chapter 2 reviews the current research on nutrient recovery and micropollutant removal from wastewater. This review signified the importance of applying source separation strategy to improve the current wastewater management practice particularly in terms of nutrient recovery and separate treatment of micropollutants (cytostatic drugs as target compounds). Chapters 3 and 4 mainly address nutrient recovery from wastewater streams using different approaches based on source separation concept. Chapters 5 and 6 mainly deal with the PhACs issue, including the development of a robust analytical method for the trace determination of multi-class cytostatic drugs in different environmental matrices, and the application of an FO membrane process for effective removal of target cytostatic drugs. In real-world applications, this study emphasizes the application of novel FO membrane process for two purposes: nutrient recovery and micropollutant treatment. Chapter 7 summarizes the major research findings and scientific contributions in this study, and future research endeavors in this field are also recommended.

Figure 1-1 Structure of the thesis.

CHAPTER 2 LITERATURE REVIEW ON NUTRIENT RECOVERY AND CYTOSTATIC DRUGS REMOVAL

2.1 Introduction

Nowadays, the paradigm of wastewater management is changing and the value of wastewater, for its recoverable water content is increasing. A number of resource-recovery-oriented technologies are in operation and are under development for different recoverable components in wastewater, mainly including nutrients, carbon, and inorganics as well as reclaimable/reusable water and energy. The combination of source separation sanitation strategy with state-of-the-art wastewater treatment technologies promises an environmentally friendly solution for nutrient recovery from wastewater while essentially prevents various micropollutants from entering the environment.

PhACs are characterized by a diverse array of chemical structures and high heterogeneity of properties. Intensive studies have been conducted on a broad spectrum of PhACs regarding their sources (Larsson et al., 2007; Lienert et al., 2007; Thomas et al., 2007; Verlicchi et al., 2010), environmental presence and transport/transformation (Andreozzi et al., 2003; Arpin-Pont et al., 2014; Ashton et al., 2004; Buchberger, 2011; Glassmeyer et al., 2005; Ternes, 1998), human health and ecotoxicological risk assessment (Crane et al., 2006; Emmanuel et al., 2005; Fent et al., 2006; Hernando et al., 2006), as well as various biotic/abiotic treatment (Bolong et al., 2009; Conn et al., 2006; Garcia-Rodríguez et al., 2014; Stieber et al., 2011). However, limited studies have been focused on cytostatic drugs (also called antineoplastic drugs), a broad group of chemotherapy compounds mainly used for tumour treatments with different action modes. Kosjek and Heath (2011) recently provided a first overview on the environmental occurrence of cytostatics but mainly from an analytical chemistry perspective. Based on the French consumption patterns, the nationwide predicted environmental concentrations (PEC) reveals that more research is needed to assess the ecotoxicological risk for anticancer drugs (Besse et al., 2012). Cytostatic drugs and human metabolites are directly discharged into sewage system without any specific control after being administered in the hospitals. Household discharge by out-patients

presents another pathway of cytostatic drugs to the environment (Bound and Voulvoulis, 2005). Due to their highly potent mechanism of action (cytotoxicity, genotoxicity, mutagenicity and teratogenicity), cytostatic drugs could inflict adverse effects on any growing eukaryotic organism (Besse et al., 2012; Johnson et al., 2008).

This review includes two parts. Section 2.2 provides a short literature review on nutrient recovery issue, which summarizes the significance of nutrient recovery from wastewaters, the development of source separation systems, and various technologies available for nutrient recovery from wastewater. The second part of this review (Section 2.3 – 2.6) presents the occurrence, fate and potential ecotoxicological effects of various cytostatic drugs and metabolites in the environment. Source contributions (pathways to the environment) were discussed with a particular emphasis on urinary excretion contribution. In addition, the role of source separation systems on the discharge of human pharmaceuticals to the environment was analyzed. Various state-of-the-art technologies are proposed for effective treatment of wastewater containing cytostatic drugs (e.g. source-separated urine, clinic urine). However, the pharmacokinetics and pharmacodynamics are beyond the scopes of this review.

2.2 Nutrient Recovery from Urban Wastewater

2.2.1 Needs for nutrient recovery from wastewater

During the past decades, wastewater was required to be treated in certain wastewater treatment facilities before being discharged to the environment. As population expansion and water scarcity continue to rise across the world, the paradigm of wastewater management is changing and wastewater is now being considered as a resource rather than ‘nuisance waste’. Both domestic and industrial wastewaters contain a wide range of valuable inorganic components such as nutrients, metals, and water itself, and organic components such as biopolymers and biosolids that can potentially be recovered. Certain wastewater components can be utilized for algal production, animal feed, and fermentation feedstocks to produce raw materials that can be further processed into valuable end-products such as biofuels. In fact, the value of wastewater components has been largely underestimated.

Domestic wastewater contains rather high concentrations of nutrients (N, P, K, S, etc.), carbon, and recoverable/reclaimable water and energy. Water and energy recovery for STPs are providing concentrated waste streams which make recovery of wastewater components much easier than before. Nowadays, a number of resource-recovery-oriented technologies targeting at different wastewater components have been developed. In sync with the increasing world market demand of phosphate, many countries enhance their regulation on discharge of nutrient-containing wastewater and encourage nutrient recovery from wastewaters. Phosphorus recovery from domestic wastewater has been implemented at full-scale operations (e.g., Ostara and Phosnix processes). However, the widespread installations of such resource-recovery facilities across the world are not expected due to market, regulatory, and other local constraints. It is also important to break local market and institutional barriers encountered by the nascent resources recovery entrepreneurs in collaboration with public health officials and not-for-profit groups who concern about the safe usage of resources recovered from wastewaters.

One of the objectives of this work is to explore a sustainable nutrient management pattern applicable in urban environment. It is of importance to safeguard precious water resources by recovering valuable nutrients from brackish wastewater and transforming them into environmentally friendly fertilizers. The high levels of nitrogen and phosphorus in source separated wastewater could be used as nutrient resources for farming, rather than being dumped back into water bodies already overloaded with these nutrients. In this study, efforts were made to develop new strategies and technologies to increase effectiveness of resource recovery for nitrogen and phosphorus in current wastewater management systems. Several nutrient recovery options were demonstrated to assess and better understand recovery technologies.

2.2.2 Development of source separation systems

In the ascent of the global energy crisis and resource scarcity, domestic wastewater has been increasingly considered as a valuable resource rather than ‘nuisance waste’. Innovative sanitation systems based on the source separation concept have been intensively advocated to manage domestic wastewater in a more sustainable way

(Langergraber and Muellegger, 2005; Larsen et al., 2009; Lienert and Larsen, 2010). Relying on the distinct chemical contents and concentrations, yellow water (urine), brown water (feces) and grey water (bathing and laundry wastewater) can be collected and treated separately to seek ‘waste-to-resource’ and ‘waste-to-energy’ purposes (Cordell et al., 2009; Love et al., 2009). For example, brown water mixing with kitchen waste and other organic matters could be used for biogas production in anaerobic digestion (AD) systems; grey water can be largely recovered after certain on-site treatments such as submerged membrane bioreactor and constructed wetland.

In particular, urine stream chemically differentiate itself from the other wastewater streams because of its high nutrients content (about 85% of nitrogen, 50% of phosphorus and 55% of potassium), but only contributes a marginal volume (~1%) to the total domestic wastewater (Larsen and Gujer, 1996; Meinzinger and Oldenburg, 2009). One of the obvious benefits of applying urine source separation is to reduce nutrient loadings to the existing wastewater treatment plants and attenuate the threat of eutrophication in downstream effluent-receiving water bodies. The reduction of nutrient loading may significantly prolong the service life of existing wastewater treatment plants (Wilsenach and Van Loosdrecht, 2004). **Table 2-1** presents the major inorganic compositions of fresh urine (FU) by referring to Griffith et al. (1976)’s recipe.

Table 2-1 Major chemical compositions of fresh urine.

Composition	Concentration (g/L)	Composition	Concentration (g/L)
NH ₂ CONH ₂ (urea)	25.06	C ₄ H ₇ N ₃ O	1.10
NaCl	4.60	CaCl ₂ ·2H ₂ O	0.65
KCl	1.60	MgCl ₂ ·6H ₂ O	0.65
KH ₂ PO ₄	2.79	Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	0.65
NH ₄ Cl	1.00	Na ₂ C ₂ O ₄	0.02
Na ₂ SO ₄	2.30	Others	—

Nutrients, especially phosphorus, present in source-separated wastewaters can be further recovered and utilized in agriculture. This is of great importance as the remaining global phosphorus rock is depleting in 50 – 100 years, suffering from a continuously increasing demand of phosphorus production (Cordell et al., 2009). Nutrient recovery provides an essential driving force for urine source separation. Besides, water-saving purpose can be

also achieved by applying effective source-separating (waterless) toilet technology. This merit has been highlighted in response to the scarcity of pristine water resource and increasing water consumption by industries and agriculture activities. In addition, an interesting study initiated to directly use source-separated urine for electricity production via microbial fuel cells (MFCs) system (Ieropoulos et al., 2012). This has inspired people to think about wastewater management from a sustainable angle.

In view of the listed benefits, an increasing number of ecological sanitation systems (at pilot or full scale) have been implemented all over the world over the last decades based on the urine separation concept (e.g. No-Mix technology) (Hellström and Johansson, 1999; Larsen et al., 2009; Lienert and Larsen, 2010). The goal of modern wastewater management practice is to essentially minimize the discharge of urban wastewater to the environmental, with low energy demand, by fully recycling the ‘human resource’ in agriculture. However, these source separation systems have been largely applied in rural areas rather than in densely-populated cities with shortage of water and resources, like Singapore. They are still subject to numerous issues regarding social acceptance, infrastructure investment, technical availability and local regulations (Lienert and Larsen, 2010). These issues may impede the application of source separation system in urban areas.

2.2.3 Technologies for nutrient recovery

Recirculating urban nutrients such as urine back to agriculture presents an enormous opportunity for the future, since urine is the largest single source of phosphorus emerging from urban areas (Cordell et al., 2009). Nutrient recovery from urine can be energetically more efficient than nutrient removal or new-production from natural resources in traditional ways (Maurer et al., 2003). In view of the favorable properties of a urine separation system, a number of physicochemical and biological processes have been developed for nutrient recovery and/or removal from urine, such as struvite precipitation (e.g., Ostara technology: <http://www.ostara.com/>), ammonia stripping, nitrification–denitrification, and phosphorus removal by phosphate-accumulating organisms (Başakçılardan-Kabakci et al., 2007; Lind et al., 2000; Maurer et al., 2006; Pronk and Kone, 2009; Udert et al., 2003a; Wilsenach et al., 2007). Although

satisfactory outcomes could be achieved, most of these processes require substantial additional cost for energy, chemicals and/or expensive apparatus. In most cases, different nutrients (N, P, K) in urine could not be simultaneously harvested within a process unit. An additional odor control unit is also required when applying ammonia stripping (Zhang et al., 2013b).

Membrane processes have also been utilized for urine treatment, but mainly for micropollutant removal purposes. For examples, both electrodialysis (Pronk et al., 2006a; Pronk et al., 2007) and nanofiltration (Escher et al., 2006; Lazarova and Spendlingwimmer, 2008a; Pronk et al., 2006b) can effectively separate pharmaceuticals and hormones ($MW > 200$ Da) from nutrients in urine that can be consequently used as a safe fertilizer. The former process also resulted in a factor of 3–4 concentration of the nutrient solution. In turn, these membrane processes including pressure-driven NF/RO usually have unsatisfactory performance in nutrient rejection, especially for urea and ammonium (Lazarova and Spendlingwimmer, 2008b; Lee and Lueptow, 2001b; van Voorthuizen et al., 2005). In recent years, osmotically driven membrane processes including both FO and pressure-retarded osmosis (PRO) have received increasing attention for applications in wastewater treatment and seawater desalination. FO involves the water transport across a semipermeable membrane from a low-osmotic-pressure feed solution (FS) to a high osmotic-pressure draw solution (DS) in the absence of an externally applied hydraulic pressure. The osmotically-driven membrane process has been proven to have noticeable advantages over various pressure-driven membrane processes such as RO and NF in terms of lower fouling potential and energy consumption (Zhao et al., 2012).

With purposes of water reclamation, FO needs to be combined with other processes such as RO to further recover water from the salty draw solution. For example, FO combined with osmosis distillation could be installed as a low-cost pre-concentration process for RO reclamation of wastewater in space missions (Cath et al., 2005). Combined FO-RO process was also tested for concentration of the nutrient-rich rejected stream from dewatering anaerobic digester biomass, high nutrient rejection and water recovery were achieved (Holloway et al., 2007). Interestingly, NASA researchers have

developed a forward osmosis bag (FOB) system to convert dirty water (even human urine) into drinking water drawn by concentrated sugar solution. The portable FOB can be used for emergency hydration in military missions. In other applications when salty water is unlimitedly available, stand-alone FO process can be adopted for solute concentration. Inspired by this conception, this study aims to use FO process for nutrient recovery from source-separated urine stream using seawater, desalination brine or salty industrial effluents as low-cost draw solutions. On top of nutrient recovery, engaging FO filtration may facilitate the removal of various excreta-derived micropollutants and pathogens from household wastewater.

2.3 Environmental Occurrence, Behaviors and Ecotoxicity of Cytostatic Drugs

This work was largely based on the original publications by various research groups with some other reliable web-resources. The data regarding specific chemical compounds are collected from various databases, including ChemIDPlus and Hazardous Substances Data Bank (HSDB) on the U.S. National Library of Medicine's Toxicology Data Network (<http://toxnet.nlm.nih.gov/>), ChemSpider (<http://www.chemspider.com/>), Drug Bank (<http://www.drugbank.ca/>), WHO Collaborating Centre for Drug Statistics Methodology (<http://www.whocc.no/>), Drugs@FDA (www.fda.gov/drugsatfda), Material Safety Data Sheet, IARC Monographs, and U.S. EPA's DSSTox database (<http://www.epa.gov/comptox/dsstox/>). Data screen and debugging was performed by consolidating information from different databases. About 50 commonly-used cytostatic drugs were investigated, while only certain human metabolites were covered when the relevant environmental data are available.

2.3.1 Physicochemical properties

Cytostatic drugs are sub-classified under Antineoplastic and Immunomodulating Agents (Class L) in the Anatomical Therapeutic Classification (ATC) scheme by the World Health Organization (WHO). They loosely belong to either cytotoxic drugs (Class L01) or endocrine therapy drugs (Class L02, including hormones and hormone antagonists used specifically in the treatment of neoplastic diseases) according to their intended function (Besse et al., 2012). The large group of L01 is further divided according to the similar therapeutic action into five sub-groups, namely, 1) alkylating agents, 2)

antimetabolites, 3) plant alkaloids, 4) cytotoxic antibiotics and 5) other antineoplastic agents. In particular, the alkylating agents present as the largest group of anticancer drugs, being able to induce alkylation (via covalent bonds) of intracellular nucleophilic targets in DNA, RNA and various enzymes. Several other agents, such as procarbazine, estramustine and mitomycin C, also act at least in part of alkylation. Presently, there are over 100 different cytostatic drugs in use, with more drugs being developed or under clinical evaluation all over the world. A number of 20-30 cytostatic agents are widely used in the treatment of malignant neoplastic disease as recorded in the latest World Cancer Report by WHO (Stewart and Wild, 2014). Based on the intensive literature review, information on the physicochemical properties of 34 cytostatic drugs is summarized in **Table 2-2**.

High consumptions of these drugs have been recently reported in Germany (Nussbaumer et al., 2010), France (Besse et al., 2012), Spain (Martin et al., 2011), Switzerland (Nussbaumer et al., 2010; Tauxe-Wuersch et al., 2006), and China (Yin et al., 2010). As presented, there is a diverse array of chemical structures and physicochemical properties in the family of cytostatic agents. Those important properties include dissociation constant (pK_a) and solubility, octanol-water partition coefficient ($\log P$) and organic carbon partition coefficient (K_{oc}), bio-concentration factor (BCF), atmospheric hydroxyl radical reaction rate and photolysis tendency (i.e. maximum adsorption of UV spectrum). Other parameters such as Henry's coefficient and vapor pressure carry relatively low impacts due to the high polarity of most pharmaceutical compounds (Kosjek and Heath, 2011). It is important to point out that the majority of the available physicochemical parameters are estimated values based on theoretical calculation in relation to their chemical structures. It is therefore accepted to see certain variation among those data in different studies, experimentally or theoretically. Updated information is available in HSDB network (U.S. National Library of Medicine). On top the cytostatic drugs listed in **Table 2-2**, additional attention should be paid to monoclonal antibodies (i.e. FDA-approved bevacizumab (2004), rituximab (1997), trastuzumab (1998) and cetuximab (2004)), which appear as large-molecular-weight proteins and are beyond our investigation in the current work.

Table 2-2 Chemical structures and physicochemical properties of 34 commonly-used cytostatic drugs.

Classification ^a (CAS number)	FDA approval year	Chemical structure	MW (g/mol)	pK _{a1}	Solubility (H ₂ O) (g/L)	LogP	Koc	BCF ^b	Photo-reaction ^c	UV _{max} (nm)
Alkylating Agents										
<i>Cyclophosphamide</i> (50-18-0)	(1959)		260	2.8	40	0.23	59	2.1	7.0	200
<i>Ifosfamide</i> (3778-73-2)	(1988)		260	1.5	3.8	0.23	62	2.2	4.3	290
<i>Melphalan</i> (148-82-3)	(2009)		304	2.5	0.05	1.79	355	0.2	23	260
<i>Pipobroman</i> (54-91-1)	(1966)		356	5.8	2.5	0.42	40	1.2	19	256
<i>Temozolomide</i> (85622-93-1)	(1999)		194	15.3	11.5	-1.27	4.9	1.0	3.1	327
Antimetabolites										
<i>Methotrexate</i> (59-05-2)	(1988)		454	3.8	insol.	-0.24	1.0	3.2	32	290
<i>Pemetrexed</i> (137281-23-3)	(2004)		247	3.6	insol.	0.16	22000	3.2	24	225
<i>Mercaptopurine</i> (50-44-2)	(1953)		152	7.8	68.5	-0.18	7.2	1.0	20	327
<i>5-Fluorouracil</i> (51-21-8)	(1962)		130	8.0	11.1	-0.78	8	3.0	0.58	266
<i>Tegafur</i> (17902-23-7)	unapproved		200	8.7	3.64	-0.27	11	1.0	5.2	270
<i>Capecitabine</i> (154361-50-9)	(1998)		359	1.9	26.0	1.04	46	1.9	8.3	310
<i>Gemcitabine</i> (95058-81-4)	(1996)		263	3.6	51.4	-0.47	1.4	1.0	0.04	234, 268
<i>Cytarabine</i> (147-94-4)	(1969)		243	4.3	17.6	-1.93	2.4	1.0	12	281

Table 2-2 (continued)

Classification ^a (CAS number)	FDA approval year	Chemical structure	MW (g/mol)	pK _{a1}	Solubility (H ₂ O) (g/L)	LogP	Koc	BCF ^b	Photo- reaction ^c	UV _{max} (nm)
Plant Alkaloids										
<i>Etoposide</i> (33419-42-0)	(1983)		589	9.8	insol.	0.60	51	3.0	30	283
<i>Paclitaxel</i> (33069-62-4)	(1994)		954	11.9	insol.	7.83	3355	591	n.a.	227, 273
<i>Docetaxel</i> (114977-28-5)	(1996)		808	12.0	insol.	2.83	517	43	22	230, 275, 283
Cytotoxic Antibiotics										
<i>Doxorubicin</i> (23214-92-8)	(1974)		544	8.2	2.6	2.82	1.0	1.0	14.5	485
<i>Epirubicin</i> (56420-45-2)	(1999)		544	7.7	insol.	2.82	1.0	1.0	14.5	232, 254, 291, 480
<i>Mitomycin C</i> (50-07-7)	(1995)		334	3.2	8.43	-0.49	11.8	1.0	7.2	216, 360, 560
Hormones & Antagonists										
<i>Tamoxifen</i> (10540-29-1)	(1977)		372	8.9	insol.	7.88	664	827	24	275
<i>Flutamide</i> (13311-84-7)	(2001)		276	13.1	insol.	3.72	1962	280	0.27	240
<i>Bicalutamide</i> (#90357-06-5)	(1995)		430	12.0	insol.	4.94	4229	817	3.0	272
<i>Nilutamide</i> (63612-50-0)	(1996)		317	7.6	insol.	1.93	267	17	n.a.	249
<i>Exemestane</i> (107868-30-4)	(1999)		296	n.a.	insol.	2.43	501	42	0.001	247
<i>Letrozole</i> (112809-51-5)	(1997)		285	n.a.	insol.	0.43	40.8	1.3	0.37	240

Table 2-2 (continued)

Classification ^a (CAS number)	FDA approval year	Chemical structure	MW (g/mol)	pKa ₁	Solubility (H ₂ O) (g/L)	LogP	Koc	BCF ^b	Photo- reaction ^c	UV _{max} (nm)
Miscellaneous Agents										
Carboplatin (41575-94-4)	(1989)		371	6.6	14.0	-0.46	n.a.	n.a.	0.12	n.a.
Procarbazine (671-16-9)	(1969)		221	6.6	1.42	0.77	1.0	1.0	15.4	n.a.
Erlotinib (183321-74-6)	(2004)		393	5.4	0.81	3.37	528	59	n.a.	n.a.
Hydroxyurea (127-07-1)	(1967)		76	13.9	1000	-1.80	2.5	1.0	0.20	n.a.
Lapatinib (231277-92-2)	(2007)		581	n.a.	n.a.	6.30	4464	2535	n.a.	n.a.
Nilotinib (641571-10-0)	(1997)		530	n.a.	insol.	0.43	41	1.3	0.37	n.a.
Imatinib (152459-95-5)	(2001)		494	8.1	insol.	2.89	7.9	1.0	n.a.	n.a.
Estramustine (2998-57-4)	(1981)		440	15.1	insol.	5.50	31954	1378 3	3.49	270.7, 276.5
Irinotecan (100286-90-6)	(1996)		587	11.2	n.a.	3.73	2.0	1.0	n.a.	221, 254, 359, 372
Mitotane (53-19-0)	(1970)		320	n.a.	insol.	5.39	20963	7649	0.43	n.a.

Notes: physicochemical data sources: ChemIDPlus and Hazardous Substances Data Bank (<http://toxnet.nlm.nih.gov/>), ChemSpider (<http://www.chemspider.com>). n.a., data not available;

^a classified by WHO Collaborating Centre for Drug Statistics Methodology (<http://www.whocc.no/>);

^b bio-concentration factor indicates biomagnification risk: low BCF < 30; moderate (30-100); high (100-1000); very high (>1000) ((Franke et al., 1994).

^c atmospheric hydroxyl radical (OH·) reaction rate constants, unit is $\times 10^{-11}$ cm³/mol·sec.

2.3.2 Predicted behaviors in aquatic environment

The specific physicochemical properties play critical roles in determining their environmental behaviors and fate. After their excretion from human bodies, both parent compounds and human metabolites undergo a series of physical, chemical and biological transformation in the environment, such as dilution, hydrolysis and photolysis, adsorption to suspended solids and sediments, biodegradation and bioaccumulation and so forth. The various biotic/abiotic processes usually result in new chemical entities (also called transformation products) with new properties. With consideration of their extremely low vapor pressure, most cytostatic compounds will be mainly distributed in liquid and solid phases (e.g. activated sludge, biofilm, suspended solids, soil and sediment). For instance, the low octanol-water partition coefficient suggests that 5-fluorouracil (5-FU) presents low adsorption to suspended solids in water but high mobility in soil/sediment (Koc 8). Meanwhile, 5-FU is unlikely to be susceptible to direct photolysis by natural sunlight ($UV_{\max}$ 266 nm) or accumulated in aquatic organisms (low BCF). Hence, 5-FU seems rather persistent in natural water bodies especially in view of its high consumption rate (Besse et al., 2012).

On the contrary, acidic oxazaphosphorines like cyclophosphamide (CP) and ifosfamide (IF) are readily dissociated into ionic forms under regular environmental conditions. However, the incubation experiments by Buerge et al. (2006) demonstrated that CP and IF could be rather persistent in surface waters and could experience years of slow dark-chemical degradation. Natural photolysis of both compounds ($UV_{\max} < 290$ nm) is minimal and can occur only in shallow and nitrate-rich water bodies. In addition, the ambient conditions of the receiving water bodies also affect the fates of cytostatic compounds in the environment. In cases of source separation systems, the alkaline hydrolyzed urine may significantly affect the dissociation properties of cytostatic compounds, ultimately increase/decrease their aqueous mobility and affect the efficiency of removal processes.

Prediction of environmental behaviors and concentrations of various pharmaceuticals plays important roles in risk assessment and precautionary policy-making (Anderson et al., 2004; Kümmerer and Al-Ahmad, 2010). The *European Medicines Agency* (EMA)

2006 Guideline (EMA, 2006) proposed a modeling approach to estimate PEC values for pharmaceuticals in surface water. The model has been extensively used (with or without adjustment) in academics (Besse et al., 2008; Buerge et al., 2006; Coetsier et al., 2009). The critical parameters regularly involved in these prediction models are summarized in **Figure 2-1**. Buerge et al. (2006) predicted the concentrations of CP and IF in both untreated and treated wastewater using consumption data and typical renal excretion rates, and the results correlated well with their measured concentrations. Coetsier et al. (2009) also observed an acceptable consistency (PEC/MEC: 0.2~4) between PEC and MEC of tamoxifen and IF in both STP-effluents and effluent-receiving surface waters. Since most anti-cancer drugs are prescribed medicine, their consumption data could be more readily estimated compared to over-the-counter (OTC) pharmaceuticals. However, Coetsier et al. (2009) still observed a slightly overestimation of PEC in surface waters for two investigated anti-cancer substances, which was attributable to unknown transformation processes in natural surface waters. It is also important to note that PECs only provide a rough insight of the overall contamination situation at national or regional scale, not accounting for local specificities and hot spots of contamination. In this view, more input information with regard to population and hospitals may provide fine-scale and visualized judgment on local contamination, which calls for further investigation.

Figure 2-1 Information inputs for predicting the environmental concentrations of cytostatic drugs.

2.3.3 Occurrence in the water cycle

Conventionally, cytostatic drugs have relatively low consumption rate, thus the concentrations in the environment are relatively low compared to other groups of pharmaceuticals with different modes of action. The lack of effective methods for multi-residue analysis further impedes environmental monitoring of cytostatic agents (Martin et al., 2011). This explains the proportionately fewer environmental investigations covering a mere fraction of cytostatic drugs over the last two decades. However, due to their highly potent mechanism of action, this specific group of drugs is conceived to be harmful to aquatic organisms and even human health (Besse et al., 2012; Johnson et al., 2008; Kümmerer and Al-Ahmad, 2010). **Table 2-3** provides a summary of cytostatic drugs globally detected in different compartments of the water cycle.

Cytostatic drugs were detected in the hospital effluents at concentrations up to hundred micrograms per liter (ppm-level) (Mahnik et al., 2007; Mahnik et al., 2004). Large hospitals and specialized cancer hospitals typically carry intensive anticancer medication. They are expected to be key emission points of cytostatic drugs to the environment. Despite the complex composition of hospital wastewaters, they are rarely subjected to any specialized treatment before their discharge into the sewer system. Highly diluted by large volume of domestic wastewater (and industrial wastewater), cytostatic drugs could subsequently be detected at ppb-levels in the influent of downstream serving STP.

As summarized in **Table 2-3**, the detection of cytostatic drugs, such as CP, IF, tamoxifen, in STP effluents have been frequently reported, indicating only partial removal by conventional activated sludge STPs globally. Among these, Martin et al. (2011) detected six (i.e. cytarabine, doxorubicin, etoposide, gemcitabine, IF and vinorelbine) of 14 most frequently-used cytostatic drugs at concentrations up to 15 ng/L in influent and effluent of a traditional activated sludge STP without nitrogen removal unit; they were showing insignificant degradation during the wastewater treatment. The antiestrogen tamoxifen used for breast cancer therapy also showed little reduction within STP and exhibited high concentration in STP effluent (Roberts and Thomas, 2006). By and large, the removal efficiencies of various cytostatic drugs are very well

Table 2-3 Detection of cytostatic drugs in hospital effluents, STP influents and effluents, and effluent-receiving surface waters (ng/L).

Cytostatic	Hospital effluent	STP influent	STP effluent	Receiving surface water	Sampling mode	Removal efficiency	STP characteristics	Detection method	Country (Reference)
<i>CP</i>	146	–	–	–	24-h mixture	No	lab-scale STP	SPE, GC-MS (EI)	Germany (Steger-Hartmann et al., 1996)
	19–4486	<6–143	<6–17	–	24-h mixture (1d); hourly (6am–2pm)	No	CAS	SPE, GC-MS (EI)	Germany (Steger-Hartmann et al., 1997)
	–	–	<10–20 (25%)	<10	Random samples & 24-h composite		49 STPs: CAS+P+N	LC-MS/MS (ESI)	Germany (Ternes, 1998)
	–	–	0.6 (md.)	<0.0074	24-h composite (every 20min); composite (×5)		9 STPs	SPE, HPLC-MS/MS (ESI)	Italy (Calamari et al., 2003; Zuccato et al., 2005)
	–	2.0–11	~2–11	0.15–0.17; ~0.05–0.07 (lake)	24 h flow-proportional	No	(24-h AS incubation) 3 STPs: CAS+P+SF	SPE, LC-MS/MS (ESI)	Switzerland (Buerge et al., 2006)
	–	–	–	<30–64.8	Random samples			SPE, GC-MS (EI)	Romania (Moldovan, 2006)
	<2–21 (8%); <2 (0%)	<2	<2	–	24-h composite	N.A.	CAS+N	SPE, LC-TOF MS (ESI)	Norway (Thomas et al., 2007)
	–	–	<125	<100 (Pre-RO)	duplicate composite	N.A.	CAS	SPE, LC-MS/MS (ESI)	Australia (Busetti et al., 2009)
	6–2000 (md.100, 72%)	–	–	–	Grab samples (7d)			SPE, UPLC-MS/MS (ESI)	China (Yin et al., 2010)
	–	<2.1	<2.3	<1.7	24-h composite	N.A.	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
	–	–	0.19–0.37	–	Spot samples		2 STPs: TF, CAS+UV	SPE, LC-MS/MS (ESI)	UK (Llewellyn et al., 2011)
	0.161±0.026 (71%)	–	–	–	24-h flow/time-proportional	<20%	pilot-scale MBR	SPE, HPLC-MS/MS (ESI)	Switzerland (Kovalova et al., 2012)
	5730	<3.1–13100	<3.1	–	24-h composite	N.A.	3 STPs	SPE, LC-Orbitrap-MS (ESI)	Spain (Gómez-Canela et al., 2012)
	24 (LOD 7)	–	–	–	24-h mixture	No	lab-scale STP	SPE, GC-MS (EI)	Germany (Steger-Hartmann et al., 1996)
<i>IF</i>	<6–1914 (md.109)	<6–29	<6–43	–	24-h mixture (7d); hourly (8am–2pm)	No	CAS	SPE, GC-MS (EI)	Germany (Kümmerer et al., 1997)
	–	–	<10–2900(12.5 %)	<10	Random samples & 24-h composite		~49 STPs: CAS+P+N	LC-MS/MS (ESI)	Germany (Ternes, 1998)
	–	<0.3–5	<2–6	~0.08–0.14; <0.05 (lake)	24-h flow-proportional	No	(24-h AS incubation) 3 STPs: CAS +P+SF	SPE, LC-MS/MS (ESI)	Switzerland (Buerge et al., 2006)
	<2–338 (50%); <2-291 (50%)	<2	<2–71 (14%)	–	24-h composite	No	CAS+N	SPE, LC-TOF MS (ESI)	Norway (Thomas et al., 2007)
	–	–	<3.8	<3.8	24-h averaged flow proportional; grab		CAS+N+P	SPE, LC-MS/MS (ESI)	France (Coetsier et al., 2009)
	–	–	<125	<100 (Pre-RO)	duplicate composite	N.A.	CAS	SPE, LC-MS/MS (ESI)	Australia (Busetti et al., 2009)
	–	–	<0.09	–	Spot samples		2 STPs: TF, CAS+UV	SPE, LC-MS/MS (ESI)	UK (Llewellyn et al., 2011)
	4–10647(md.151, 58%)	–	–	–	Grab samples (7d)			SPE, UPLC-MS/MS (ESI)	China (Yin et al., 2010)
	–	3.5 (mean)	1.2 (mean)	<1.3	24-h composite	Partial	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
	0.895±0.293 (12%)	–	–	–	4-h flow/time-proportional	No	Pilot-scale MBR	SPE, HPLC-MS/MS (ESI)	Switzerland (Kovalova et al., 2012)
	<i>Tamoxifen</i>	–	<10–40 (4%)	<10	3-h composite; grab		5 STPs: CAS/BF(+P+SF)	HPLC-MS/MS (ESI)	UK (Ashton et al., 2004)
	–	–	143–740	27–212 (md. 53)	24-h composite, hourly	Negative	CAS+TF+UV (HRT 12.5h, SRT 2.4d)	SPE, HPLC-MS/MS (ESI)	UK (Roberts and Thomas, 2006)
	<1–4 (70%)	1–4 (100%)	<1	–	24-h averaged flow proportional	Partial	2 STPs: CAS, BF	LLE, GC-MS (EI)	Switzerland (Tauxe-Wuersch et al., 2006)
	–	–	<5.8–102±7	<5.8–25±4	24-h averaged flow proportional; grab	N.A.	CAS+N+P	SPE, LC-MS/MS (ESI)	France (Coetsier et al., 2009)
	<1	<1	<1	–	24-hour composite (hourly)	N.A.	CAS+N+ disinfection (HRT 6h, SRT 8d)	SPE, LC-MS/MS (ESI)	Italy (Verlicchi et al., 2012a)

Table 2-3 (continued)

Cytostatic	Hospital effluent	STP influent	STP effluent	Receiving surface water	Sampling mode	Removal efficiency	STP characteristics	Detection method	Country (Reference)
<i>5-FU</i>	20,000–122,000	–	–	–	24-h averaged			SPE, CE	Austria (Mahnik et al., 2004)
	<15	<15	<15	–	24-h averaged flow proportional	N.A.	2 STPs: CAS, BF	SPE, GC-MS (NCI/EI)	Switzerland (Tauxe-Wuersch et al., 2006)
	< 8600–123,500	–	–	–	24-h averaged	Partial	batch test	SPE, CE	Austria (Mahnik et al., 2007)
	<5.0–27 (76%)	–	–	–	3 or 18-h flow-proportional			SPE, HPLC-MS/MS (ESI)	Switzerland (Kovalova et al., 2009)
	–	<38	<21	<34	24-h composite	N.A.	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
<i>Methotrexate</i>	–	–	0.0 (md.)	<0.044	Single sample (5L)		9 STPs	HPLC-MS/MS (ESI)	Italy (Calamari et al., 2003; Zuccato et al., 2005)
	4–4689 (md.17, 22%)	–	–	–	Grab samples (7d)			SPE, UPLC-MS/MS (ESI)	China (Yin et al., 2010)
	–	<0.1	<0.1	<0.1	24-h composite	N.A.	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
<i>Doxorubicin</i>	<260 - 1350	–	–	–	24-h composite	>90% (batch test)		SPE, HPLC	Austria (Mahnik et al., 2007)
	<10 (<10 for doxorubicinol)	–	–	–	Grab samples (7d)			SPE, UPLC-MS/MS (ESI)	China (Yin et al., 2010)
	–	4.5	<4.3	<5.3	24-h composite	Partial	CAS	SPE, HPLC-MS/MS	Spain (Martin et al., 2011)
<i>Gemcitabine</i>	–	9.3	7.0	2.4	24-h composite	Partial	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
	<0.9–38 (65%); <9.0–840 (dFdU**, md.68, 88%)	–	–	–	3 or 18-h flow-proportional			SPE, HPLC-MS/MS (ESI)	Switzerland (Kovalova et al., 2009)
	<260	–	–	–	24-h averaged	>90%	batch test	SPE, HPLC	Austria (Mahnik et al., 2007)
<i>Epirubicin</i>	–	<3.8	<0.7	<3.5	24-h composite	N.A.	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
	<85	<85	<85	–	24-h composite	N.A.	3 STPs	SPE, LC-Orbitrap-MS (ESI)	Spain (Gómez-Canela et al., 2012)
	–	9.2	14	13	24-h composite	No	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
<i>Cytarabine</i>	–	<5.2	9.1	<4	24-h composite	Negative	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
<i>Vinorelbine</i>	–	–	–	–	24-h averaged			SPE, HPLC	Austria (Mahnik et al., 2007)
<i>Daunorubicin</i>	<290	–	–	–	Grab samples (7d)			SPE, UPLC-MS/MS (ESI)	China (Yin et al., 2010)
<i>Etoposide</i>	6–380 (md.42, 23%)	15	3.4	<2.2	24-h composite	Partial	CAS	SPE, HPLC-MS/MS (ESI)	Spain (Martin et al., 2011)
<i>Azathioprine</i>	9–32 (md. 15, 5%)	–	–	–	Grab samples (7d)			SPE, UPLC-MS/MS (ESI)	China (Yin et al., 2010)
<i>6 cytostatics*</i>	n.d.	–	–	–	Grab samples (7d) or 24-h composite		CAS	SPE, LC-MS/MS (ESI)	China, Spain (Martin et al., 2011; Yin et al., 2010)
<i>Cisplatin & Carboplatin</i>	3,000–250,000	–	2–150	–	24-averaged	~50%	pilot-scale MBR	ICP-MS	Austria (Lenz et al., 2007a)

Notes: md., median concentration; %, frequency of being detected; dashed line, no detection; N.A. information non-available; SPE, solid-phase extraction; LLE, liquid-liquid extraction; CE, capillary electrophoresis; CAS, conventional activated sludge process (preliminary settlement + aerator tank + secondary clarification); +N, nitrification/denitrification, +P, phosphate removal, BF, biological filtration, SF, sand filter, TF, trickling filter;

* including vincristine, procarbazine, docetaxel, irinotecan, mitomycin C and paclitaxel;

** dFdU (2',2'-difluorodeoxyuridine), urinary excreted human metabolite of gemcitabine.

dependent on their physicochemical properties and STP operation parameters. However, the mechanisms of their being relatively persist in STPs, as well as the potential negative effects to the biological community due to their greatly potent mechanism of action, remains largely unclear. The use of advanced membrane bioreactor (MBR) system, operated at relatively long sludge retention time (SRT) and highly-diversified microbial community, favors an enhanced biotransformation and mineralization of resistant pharmaceuticals. Of late, the utilization of novel MBR system exhibited a hopeful choice for effective treatment of cytostatic drugs. In any case, direct discharge of STP effluent containing cytostatic residue to surface water (or used for agricultural irrigation) escalate concerns about ecotoxicity risks and human health threats. This situation may be even worse in areas without access to sanitation system, specifically in densely-populated urban areas.

Recent investigations have demonstrated the trace presences of several cytostatic drugs and their metabolites in surface water (Valcarcel et al., 2011). Further information on their distribution, transport and transformation in natural environment are scarce. Rowney et al. (2009) used a geographic information system (GIS) based water quality model and predicted the combined concentration of 13 cytotoxic drugs over 10 ng/L in raw drinking waters abstracted from the Thames River (in case of mean flow). Although they have been seldom detected in drinking waters, it is not appropriate to claim that cytostatic residue can be completely degraded in current water treatment facilities. In fact, occurrences of many pharmaceutical compounds in finished drinking waters have been reported and recently reviewed by Delgado et al. (2012). In the worst scenario, the discharged anticancer drugs and active intermediate products would enter water supply streams and reach the public.

Advanced analytical techniques are required for accurate environmental measurement. Exposure and ecotoxicity risk assessments on pharmaceutical residues are carried out based on the information of their environmental concentrations. To our knowledge, there exist only few studies detailing multi-compound analysis method for cytostatic drugs in aqueous environmental samples, both using liquid chromatography accompanied with triple-quadrupole mass spectrometry (LC–QqQ MS or LC–MS/MS)

(Martin et al., 2011; Nussbaumer et al., 2010; Yin et al., 2010). Most other studies refer to the quantitative determination of only few kinds of cytostatic compounds, either by GC-MS (MS) or LC-MS (MS) techniques as listed in **Table 2-3**. Solid phase extraction is always required for the pre-concentration and purification of environmental samples with different matrices. Oasis HLB (*polystyrenedivinylbenzene-N-vinylpyrrolidone terpolymer*, manufactured by *Waters*) has been widely used as the preferred choice for multi-class extraction of pharmaceuticals in water samples, which presents satisfactory hydrophilic-hydrophobic balance. Instrumental methods for trace determination of pharmaceuticals and personal care products (PPCPs) in the environment have been well reviewed by Buchberger (2011), and by Kosjek and Heath (2011) specifically for anti-cancer drugs.

2.3.4 Human risk and ecotoxicological potency

Cytostatic drugs and/or their human metabolites are chemically very reactive and have a wide-range of action (cytotoxic, cytostatic and endocrine therapy) (Besse et al., 2012). Unfortunately, they also inevitably react with non-target healthy cell in cancerous patients, probably causing uncontrolled cell damage. This raises concern about the occupational exposure and health risk to people involved in the production, marketing and distribution of anticancer drugs (Sessink et al., 2011; Tuerk et al., 2011; Turci et al., 2003). For instance, researchers have recently observed a significant increase of structural chromosomal aberrations among occupationally exposed nurses during the preparation and administration of anticancer drugs, which may result to elevated risk of cancer (Bouraoui et al., 2011; Smerhovsky et al., 2001).

Both *U.S. Food and Drug Administration* (FDA) and *European Medicines Agency* (EMA, under Directive 2001/83/EC) require pre-marketing environmental risk assessment (ERA) before the approval of new drugs. Regrettably, the discharge of cytostatic drugs (and metabolites) to the environment has neither been well regulated nor undergone any specific control once administered at hospitals; this is primarily because of the absence of robust ERA information. Available toxicological data are usually based on short-lived effects of individual pharmaceutical on fish, daphnids, algae, bacteria, earthworms, aquatic plants and dung invertebrates (Ferk et al., 2009).

Although it seems acceptable to conclude that acute toxicity to aquatic organisms is unlikely to occur at measured environmental concentrations (Ashton et al., 2004; Fent et al., 2006), there is widespread debate that current ecotoxicity tests are inappropriate for assessing the impacts of many pharmaceuticals (Boxall, 2004). Several important limitations exist in the current risk assessments strategy based on standard ecotoxicity tests (Boxall, 2004; Crane et al., 2006; Daughton and Ternes, 1999; Toolaram et al., 2014):

- Mainly focus on early-life effects and mortality as the endpoint. Chronic data (e.g. life-cycle or partial life-cycle fish tests) may be more relevant to pharmaceutical ecotoxicity.
- Focus on water compartment in aquatic tests without considering the sediment phase.
- Observe the effects at much higher concentrations than those measured in the real environment.
- Overlook less conspicuous effects (i.e. growth, fertility or behavior) on organisms in the environment, especially the lower animals sharing similar receptors as humans.
- Overlook potential synergism/antagonism of toxic effects resulting from coexisting multiple pharmaceuticals, rather than simple concentration addition.

Only a handful of studies provide ERA information for the most commonly-used cytotoxic drugs today such as CP, IF, 5-FU, mitomycin C and cisplatin, etc. (Ferk et al., 2009; Kümmerer and Al-Ahmad, 2010; Lenz et al., 2007b; Nakano et al., 2003; Parrella et al., 2014b; Pomati et al., 2006; Rowney et al., 2009; Schulman et al., 2002). Limited is known about their ecotoxicological effects on wildlife; even lesser are the potential additive/synergistic effects of pharmaceutical mixture, together with their human metabolites and degradation products as well as other environmental containments (Escher et al., 2011; Mater et al., 2014). Regarding the potential human risk from exposure to environmental pharmaceuticals through the water cycle, Pomati et al. (2006) have experimentally observed that short-term exposure to a mixture of 13 multi-class drugs (including CP) at ng/L levels could significantly inhibit human embryonic cells growth in vitro. The study indeed encouraged a more comprehensive understanding of

the potential adverse effects on both human health and ecosystem resulted from the chronic exposure to environmental pharmaceutical ‘cocktail’.

A recent study in Europe stated that no significant environmental risk results from the current usage of capecitabine and 5-FU, although further evidence on the persistence, bioaccumulation, and toxicity (PBT) properties of the investigated cytostatic drugs is needed to finalize this notion (Straub, 2010). Ferk et al. (2009) compared the genotoxic effects (DNA damage in primary rat hepatocytes) of the hospital wastewater from an oncological ward and three suspected antineoplastic drugs (i.e. cisplatin, carboplatin and 5-FU); the outcome suggests that the associated adverse effect was likely to be attributed to other chemicals present rather than the cytostatic drugs themselves. More recently, Parrella et al. (2014b) observed a high potential of estrogenic activity for imatinib (at 10^{-7} μ M), cisplatin, and 5-FU detected by E-screen on MCF-7 cells. They further tested the eco-genotoxicity of six cytostatic drugs (i.e., 5-FU, capecitabine, cisplatin, doxorubicin, etoposide, and imatinib) by *in vivo* comet assay and showed that all drugs induced DNA damage in two Cladocerans at environmentally relevant concentrations (Parrella et al., 2015). Such information, unfortunately, is still limited at the moment. To conclude, current evidence remains inadequate to support a definitive conclusion on the ecotoxicological risks posed by cytotoxic molecules, especially on their long-term effects on non-target organisms (Besse et al., 2012; Buerge et al., 2006).

Their presence at trace level in the environment makes it a major challenge for their ecotoxicological studies. This can be even tougher for the chemical quantification of their metabolites and degradation by-products, which may contribute to the total ecotoxicity. In terms of the interaction between different compounds, Chen and Lu (2002) demonstrated that concentration addition, followed by antagonism, was likely to occur as the combined effects of organic toxicants, while the synergism was rare. An imperative point to note for the environmental concentration of an individual pharmaceutical is that, despite it being at a concentration where no observable effect is captured, the overall pharmaceutical load may still be competent of evoking harmful ecotoxicological effects to the environment (Bound et al., 2006).

Environmental safety should be considered as a part of today's therapeutic needs and for human safety. In Europe, Laenge et al. (2006) urged that the highly-standardized testing and assessment procedure in the current EMEA Guideline should be developed to be more compound-specific, and, highlighted the needs of cross-disciplinary collaboration between pharmacological and ecotoxicological expertise. By putting together all the information on possible receptors in non-target organism as well as intrinsic action mode to various exogenous pharmaceuticals, a more appropriate ecotoxicity testing procedure can be derived (Daughton and Ternes, 1999). Furthermore, improved characterization of exposure and effects support a solid ERA process for the emerging contaminants and enables the sustainability of aquatic systems. To achieve this goal, both chemical analysis and effects monitoring (i.e. bioanalysis, biosensing, and real-time bioassays) should be included in the current risk characterization strategy (predicted environmental concentration versus predicted no effect concentration, or PEC/PNEC) (Hansen, 2007).

2.4 Source Contributions to Cytostatic Residue in the Environment

2.4.1 Hospital effluent as a source

Figure 2-2 shows the typical route of cytostatic residue entering and transporting in the water cycle. The following sections discuss the four primary sources (hospital effluent, household wastewater, production discharge, and drug waste disposal) of cytostatic residue. Generally, drug residues are generated after complex but usually incomplete metabolism in human bodies, and excreted via urine and/or feces into both hospital and domestic wastewaters. Source control based on urine separation strategy might be a good approach to prevent those drug residues seeping into the environment. In cases of production discharge and waste disposal, the drugs are not subjected to any intro metabolism and directly discharged into the environment as intact compounds. There are increasing concerns about 'hotspot' environmental pollution.

Hospitals produce large quantities of chemically- and microbiologically-loaded effluents (e.g. *Staphylococci*, coliforms, COD 200 mg/L), which carry high potential ecotoxicity, and should not be considered as possessing the same pollutant nature as

urban wastewater (Boillot et al., 2008; Verlicchi et al., 2012a). In general, hospital effluents are rarely pretreated prior to the discharge into public sewer system, although chlorine disinfection may be practiced for pathogen control purpose in some countries (Verlicchi et al., 2010; Yin et al., 2010). This makes hospitals potential hotspots for the discharge of pharmaceutical residues into the sewer networks. Lienert et al. (2011) disclosed that the top-100 most-used pharmaceuticals in a general hospital can contribute as high as 38% to the total load at the local STP, while this number is less than 15 % for 28 investigated pharmaceuticals in another study (Ort et al., 2010). Beier et al. (2011) also demonstrated that at least 34% antibiotics found in municipal wastewaters were originated to the investigated hospital.

Figure 2-2 Enter and transport route of cytostatic residue in the environment.

There was a common impression that cytostatic drugs in the environment almost exclusively originate from hospital applications, therefore, they were detected primarily in samples collected from large hospitals, and especially those specialized in tumour biology. One thing worth noting is that, with the increasing number of out-patients nowadays, the household discharge is becoming an important source as well. Steger-Hartmann et al. (1996) were among the first who investigated cytostatic residue (IF and CP) in hospital effluent in the mid-1990s. Investigations were executed mainly in Europe and North America. Such studies have been extended to the developing countries recently. As summarized in **Table 2-3**, several cytostatic drugs have been frequently detected at ng/L to µg/L levels in hospital effluents, mainly including CP, 5-

FU, IF, etc. In a recent study by Weissbrodt et al. (2009), both 5-FU and gemcitabine reached daily maximum emissions of 8–19 mg/L from a representative Swiss cantonal hospital, while this number was over 200 mg/L for 2',2'-difluorodeoxyuridine (dFdU, the major human metabolite of gemcitabine). The measured cytostatic levels in hospital sewage indeed correlated with the daily consumption and the pharmacokinetic excretion pattern.

Many factors could affect the occurrence and concentrations of detected cytostatic drugs in hospital effluents including number of patients, types of drugs used, dosing, and excretion rates. To a large extent, the dose intake depends on the type and severity of specific neoplastic disease treated without well-established defined daily doses (DDDs). In addition, improper sampling, storage, transport and pretreatment (i.e. extraction, condensation, purification and derivation) can also introduce certain measurement errors. For instance, the measurement of tamoxifen was underestimated (compared to PEC) probably due to improper methods for sample preservation and sampling (adsorption to particles in wastewater was not measured) (Tauxe-Wuersch et al., 2006). Complex matrices in raw wastewater would also cause low recovery and severe signal suppressions of either hydrophilic or hydrophobic cytostatics (Roberts and Thomas, 2006; Steger-Hartmann et al., 1996). Besides, daily water consumption (depending on hospital capacity) can also significantly dilute the effluent and affect the detectability of cytostatic residue (Yin et al., 2010).

2.4.2 Household effluent by out-patients

Another important source for the cytostatic drugs might from the out-patients in the household. For cytostatic drugs with very short elimination half-life in human body, such as 5-FU showed in **Table 2-4**, it is expected that part of their excretion will take place in hospitals. However, out-patient treatment is of tantamount importance when patients ingest cytostatic drugs at home and produce cytostatic-containing domestic wastewater (Tauxe-Wuersch et al., 2006). In a very recent study, Besse et al. (2012) predicted the environmental concentrations of various cytostatic drugs in France and suggested that hospital effluents may not be the major origination of anticancer drugs in the aquatic environment because of the increase of anticancer home treatments.

Weissbrodt et al. (2009) also reported that the measured 5-FU, gemcitabine and its human metabolite dFdU in hospital effluent only accounted for ~30% of the total consumption, as there was a high amount of cytostatics administered to out-patients. For example, most tamoxifen was sold at local pharmacies, while only 10% at hospitals (Weissbrodt et al., 2009), thus the total amount of tamoxifen residue in domestic wastewater should include contribution from local hospitals as well as from the entire catchment area. CP was even detected (~ 4 ng/L) in the influent of a local STP without wastewater input from the in-hospital chemotherapy treatments, thereby indicating a meaningful household contribution (ambulant chemotherapy) (Buerge et al., 2006). Mahnik et al. (2007) also showed that nearly 80% of cancer therapies in a local hospital are administered in the out-patient treatment ward.

There is still no clear evidence if household origination is stepping up to or even overtakes hospital emissions of the environmental cytostatic residue as their distribution patterns depends on various parameters, such as the size of hospitals, size of catchment area, and the population density, to name a few. Further study on this topic deserves more attentions because the knowledge of source contribution has essential implications on relevant technological, social-economic and administrative issues ahead. When medical products are administered in hospitals, the source control over its admission into the environment is much more manageable; urine source separation, sorption pretreatment or membrane bioreactor could be options (Escher et al., 2011; Kovalova et al., 2012). However, the ease of source control is lost when the doctors' prescription is to be taken at home (Laenge et al., 2006).

2.4.3 Effluents from drug manufacturers

An additional source contributing to the environmental pharmaceutical residues is from the drug manufacturers. Special attention should be given to the effluents from STPs receiving wastewater from pharmaceutical manufacturing plants. The discharge of raw materials, intermediates and active pharmaceutical ingredients (APIs) during pharmaceutical production (from raw materials to final products) could give rise to additional environmental burdens. In these cases, pharmaceutical compounds are discharged in their intact forms without human ingestion and metabolism. Generally,

pharmaceutical manufacturing consist of two steps: API production and the final formulation. Driven by lower costs, API production (the first stage of pharmaceutical) is gradually transferred from western countries to the developing countries, such as India and China (Bumpas and Betsch, 2009). These countries, usually possess less satisfactory sewage treatment facilities, might be subjected to the higher risks of pharmaceutical manufacturing pollution.

At present, although the global pharmaceutical market is dominated by USA (28%), EU (15%) and Japan (12%) (IMAP, 2011), China and India jointly contribute nearly 50% of the world's generic API production (Larsson, 2008). This indicates that pharmaceutical productions vary among countries, and environmental contamination derived from pharmaceutical manufacturing sectors should be of particular concern in certain countries. It remains uncertain whether the amount of drugs discharged from manufacturers on the global scale could compete with the amount reaching to the environment through human excretion and improper disposal (Larsson et al., 2007). So far, there is only little data available in this regard. Larsson (2008) further highlighted a potential gap with a common misleading view considering the discharge of various pharmaceuticals from drug production plants relatively unimportant. Larsson et al. (2007) played a pioneering role to monitor various pharmaceutical residues in the effluent of a common STP receiving wastewaters from about 90 bulk drug manufacturers in one of the world's largest drug production centers near Hyderabad, India. Among the 59 detected pharmaceuticals in the effluent, more than one third (21) of them carried concentrations higher than 1 µg/L, while 11 over 100 µg/L (most of them are antibiotic-fluoroquinolone drugs). It was also found that these pharmaceuticals contaminated the nearby ground and drinking water, posing a high concern of the development and migration of antibiotic-resistant microorganisms (Fick et al., 2009). Further toxicological tests showed that even at high dilution level, exposure to the treated effluent might cause various detrimental effects on aquatic vertebrates (tadpole and fish) (Carlsson et al., 2009; Gunnarsson et al., 2009). Rigid environmental regulation plays an essential role in abating current environmental contamination associated with pharmaceutical manufacturing processes. To date, these polar pharmaceutical compounds have not been well regulated by a wastewater management

framework in any country, with no mention about the specific cytostatic group. With that, U.S.EPA has advised new multi-compound detection methods for 76 priority pharmaceuticals (Method 1694) (EPA, 2007) and hormones (Method 539) (Smith et al., 2010).

2.4.4 Disposal as solid waste

In most countries, direct disposal as the municipal solid waste is still a common way for the unused and outdated pharmaceuticals. The total concentration of 22 APIs measured within a landfill was 8.1mg per kilogram municipal solid waste, which indicated that at least 11% of all medications were discarded unused (Musson and Townsend, 2009). Pharmaceuticals have also been detected in landfill leachate, posing contamination woes to the territorial groundwater and surface water (Andrews et al., 2012). The disposal of household unconsumed/expired medicines is governed by the prescription practice and personal behavior (Daughton, 2003b). For household drug waste, most people simply throw them into waste bins, while others discard in the toilet or return to the purchase points (Sasu et al., 2011). Another survey conducted in England showed that 63.2% of the interviewee discard their unwanted pharmaceuticals in household waste, while the rest return them to a pharmacist or empty into the sink or toilet (Bound and Voulvoulis, 2005). However, these investigations mainly looked at OTC drugs and did not include cytostatic drugs. It might be needed to further investigate into the landfill disposal pathway for environmental cytostatic residue, especially in view of an increasing number of cancerous out-patients.

Drug-containing waste derived from hospitals is usually considered as hazardous waste and has been included in the frame of hazardous waste management in most countries. Regulation and technology availability (i.e. sanitary landfill, incineration plant, etc.) have great impacts on local hazardous waste management. Recent experience in Brazil and Ghana emphasized the importance of waste segregation and drug-return program in hospital waste management practice to ensure public health and environmental quality (Dorion et al., 2012; Sasu et al., 2012). However, a recent investigation in several local hospitals in Ghana showed that pharmaceutical waste was commonly disposed at

landfills or dumping ground without prior segregation and treatment, creating a high potential for infections spread and contamination of groundwater (Sasu et al., 2011).

The final disposal of pharmaceutical waste, regardless from hospitals or households, primarily relies on the available solid waste management system. For cytostatic drugs, the direct disposal is relatively unlikely and marginal due to their relatively low consumption and at high prices. In any case, precautionary care should be given to environmentally persistent cytostatic compounds since there is a growing demand for it, in view of an increasing number of cancerous patients globally (Besse et al., 2012; Stewart and Wild, 2014). Engaging people in pharmaceutical-return programs and reducing over-prescription by medical professionals could be effective precautions to reduce environmental cytostatic residue (Daughton, 2003b; Doerr-MacEwen and Haight, 2006; Khetan and Collins, 2007).

2.5 Strategy of Urine Source Separation

2.5.1 Urinary excretion of cytostatic drugs and metabolites

Apart from nutrients recovery (and energy recovery) purpose, separate collection and treatment of micropollutants present an additional advantage of urine source separation (Winker et al., 2008). Orally or intravenously injected pharmaceuticals are not completely absorbed/utilized by human bodies, a certain proportion of unchanged parent compounds will be excreted together with various metabolites (commonly in polar water-soluble forms) mainly via renal (urine) and/or biliary excretion (feces). **Table 2-4** summarizes the urinary excretion rates, elimination half-life, accurate toxicity as well as carcinogenicity, teratogenicity and mutagenicity properties for a list of cytostatic drugs frequently detected in the environment.

As summarized in **Table 2-4**, many cytostatic drugs present rather high urinary excretion rates in their unchanged forms (up to 90% for methotrexate and pemetrexed), although with a wide variation for each individual drug and to each other. Some cytostatic drugs show only little excretion rates of the unchanged parent compounds due to confirmed pharmacokinetic facts. For example, CP and IF are enzymatically

activated to their mustard metabolites to exert cytotoxicity in human body. This indicates that not only the parent cytostatic drug, but also their toxic human metabolites should be taken into account for a comprehensive evaluation on their potential adverse effects to the environment. For individual pharmaceutical compound, urinary excretion rate is one of the critical parameters to decide the efficacy of urine separation strategy. A recent simulation study has demonstrated the importance of excretion rates for predicting the concentrations of various cytostatic drugs entering into the aquatic environment (Besse et al., 2008), and assessing the real-world ecotoxicological risks. A large quantity of consumption does not necessarily lead to a high frequency of detection (Ashton et al., 2004). As showed in **Figure 2-1**, many other factors also affect the environmental occurrence and distribution of cytostatic drugs. Although each individual drug is expected to have identical pharmacokinetic and pharmacodynamic processes among treated patients, the excretion rate varies with specific medication (APIs, formulation, duration of infusion), mode of application (oral, intravenous, rectal, dermal) as well as the specific conditions of treated patients. For instance, the excretion rate of most commonly-used CP can vary in a range of 10-40%, indicating distinct metabolizing rates among treated patients. It is also important to note that biliary excretion data are often unavailable and highly variable because of incomplete collections due to fecal retention and inconvenience from patients.

Apart from the intact parent compounds, the various human metabolites of cytostatic drugs attract additional concerns (Kovalova et al., 2009; Weissbrodt et al., 2009). Directive 2004/27/EC also requires environmental risk assessment for human metabolites with excretion rate exceeding 10% of the total dose (Laenge et al., 2006). For instance, the urinary excretion rate of 5-FU after ingestion of capecitabine (oral form of 5-FU) is about 3% (Judson et al., 1999). On the contrary, a high proportion of the administered 5-FU is excreted via urine primarily as R-fluoro-alanine (~80%) and unchanged 5-FU (~15%). In case of gemcitabine, about 5% of the total intake will be recovered as unchanged compound and 60% as its major metabolite (dFdU) in urine within 24 h. Although there are certain inconsistencies, pharmacokinetic data resources are normally available to the public for the determination of metabolism pathways of PhACs and the excretion fraction of the active molecules.

Table 2-4 Urinary excretion rate, elimination half-life in human body, acute toxicity and CTM properties (carcinogenicity, teratogenicity and mutagenicity) of commonly-used cytostatic drugs.

Classification	Urinary intact drug ^a	Elimination half-life	Toxicity ^b (mg/kg) (Carcinogenicity ^c)	Teratogenicity & Mutagenicity ^d	Classification	Urinary intact drug ^a	Elimination half-life	Toxicity ^b (mg/kg) (Carcinogenicity ^c)	Teratogenicity & Mutagenicity ^d
Alkylating Agents					Antibiotics				
<i>Cyclophosphamide</i>	10–40%	6.5 h	94 (Group 1)	<i>T, M</i>	<i>Doxorubicin</i>	4–5%	20–48 h	698* (Group 2A)	<i>M</i>
<i>Ifosfamide</i>	6.6%	6–8 h	143 (Group 3)	<i>T, M</i>	<i>Epirubicin</i>	< 20%	33 h	1350	
<i>Temozolomide</i>	37.7%	1.8 h	1937		<i>Mitomycin C</i>	10–30%	1 h	23* (Group 2B)	<i>M</i>
<i>Chlorambucil</i>	minor	1.5 h	76 (Group 1)	<i>T, M</i>	<i>Daunorubicin</i>	12–29%	18.5 h	290 (Group 2B)	
<i>Melphalan</i>	10–15%	1.5 ± 0.83 h	11.2 (Group 1)	<i>M</i>	<i>Bleomycin</i>	60–70%	9 h	240 i.p. (Group 2B)	<i>T</i>
<i>Carmustine</i>	(60–70%)	15–30 min	20 (Group 2A)		<i>Mitoxantrone</i>	7.3 %	> 38 h	7.1 *i.p. (Group 2B)	
<i>Lomustine</i>	(75%)	94 min	70 (Group 2A)		Hormones & Antagonists				
<i>Mechlorethamine</i>	minor (50%)	< 1 min	76 (Group 2A)	<i>M</i>	<i>Tamoxifen</i>	minor	5–7 d	1190 (Group 1)	
<i>Streptozocin</i>	10–20%	5–15 min	5150 (Group 2B)	<i>M</i>	<i>Flutamide</i>	< 28%	7.8 h	787	<i>T</i>
<i>Dacarbazine</i>	40%	5 h	2147 (Group 2B)	<i>T, M</i>	<i>Bicalutamide</i>	minor	1.2 d	2000	
<i>Busulfan</i>	(~100%)	2.5 h	110* (Group 1)	<i>M</i>	<i>Nilutamide</i>	< 2%	38–59 h	195	
<i>Treosulfan</i>	~15%	1.8 h	n.a. (Group 1)		<i>Exemestane</i>	< 1%	24 h	5050	
<i>ThioTEPA</i>	(63%)	2.4 h	23 (Group 1)	<i>T, M</i>	<i>Letrozole</i>	6% (90%)	2 d	2000	
<i>Methyl-CCNU</i>	(60%)	72 h	49.9* (Group 1)	<i>T, M</i>	<i>Medroxyprogesterone</i>	7.3 %	32–44 h	3000 (Group 2B)	<i>T</i>
Antimetabolites					<i>Diethylstilbestrol</i>	minor (5%)	24 h	3000 (Group 1)	<i>T</i>
<i>Methotrexate</i>	80–90%	3–10 h	135 (Group 3)	<i>T, M</i>	Others				
<i>Pemetrexed</i>	70–90%	3.5 h	500		<i>Carboplatin</i>	n.a.	1.1–2 h	343	
<i>Mercaptopurine</i>	50%	50 min	1250*	<i>T, M</i>	<i>Cisplatin</i>	23.3 ± 8.6%	20–30 min	25.8 (Group 2A)	<i>M</i>
<i>5-Fluorouracil</i>	15%	10–20 min	230 (Group 3)	<i>T</i>	<i>Procarbazine</i>	< 5%	10 min	570 (Group 2A)	<i>T, M</i>
<i>Capecitabine</i>	3%	45 min	2000		<i>Erlotinib</i>	n.a.	36.2 h	1000	
<i>Gemcitabine</i>	< 10%	1.49 h	236 i.p.		<i>Hydroxyurea</i>	50%	3–4 h	5760 (Group 3)	<i>T</i>
<i>Cytarabine</i>	10%	1–3 h	5000		<i>Lapatinib</i>	n.a.	14.2 h	n.a.	
Plant Alkaloids					<i>Nilotinib</i>	minor	17 h	n.a.	
<i>Etoposide</i>	5–22%	4–8 h	1784 (Group 2A)		<i>Imatinib</i>	5%	18 h	n.a.	
<i>Paclitaxel</i>	< 10% (14%)	5.3–17.4 h	32.5 i.p.		<i>Estramustine</i>	< 1%	15–20 h	5400	
<i>Docetaxel</i>	< 5%	48 h	156* i.p.		<i>Irinotecan</i>	11–20%	6–12 h	867	
<i>Methoxsalen (UV)</i>	minor (95%)	0.75–2.4 h	423* (Group 2A)	<i>M</i>	<i>Mitotane</i>	< 10%	18–159 d	5000 (Group 2B)	
<i>Teniposide</i>	4–12% (44%)	5 h	980 (Group 2A)		<i>Azathioprine</i>	< 2% (40–60%)	12–15 min	400 (Group 1)	<i>T, M</i>

Notes: Pharmacokinetic data sources: Hazardous Substances Data Bank (<http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB>), Drug Bank (<http://www.drugbank.ca/>), and FDA-approved labelling information. n.a., data non-available;

^a values in brackets are total renal rates of both parent and metabolites;

^b Acute toxicity data (LD50, rat/mouse*, oral/intraperitoneal (i.p.) administration) collected from the latest material safety data sheets;

^c Agent classified by the IARC Monographs is carcinogenic (Group 1), probably carcinogenic (Group 2A), possibly carcinogenic (Group 2B), or not classifiably carcinogenic (Group 3) to humans; other compounds listed have not been classified due to insufficient investigation. (<http://monographs.iarc.fr/ENG/Classification/index.php>);

^d Agent is teratogenic and/or mutagenic to different species (i.e. bacteria, yeast, *drosophila melanogaster*, rat/mouse, monkey and even human, *in vivo* or *in vitro*) according to IARC Monographs (<http://monographs.iarc.fr/ENG/Monographs/vol26/volume26.pdf>) and EPA's DSSTox database (<http://www.epa.gov/NCCT/dsstox/>).

2.5.2 Treatment of cytostatic drugs in source-separated urine

Overall, parent drugs together with their metabolites contribute high proportion of the total dosage. Source diversion of source-separated urine (public or clinic), therefore, attracts special interest to minimize the inputs of pharmaceutical residues in the environment (Dodd et al., 2008; Khetan and Collins, 2007). However, a recent study by Lienert et al. (2011) testified, based on multi-criteria decision analysis (MCDA) and combined expert predictions, the incompetence of separate collection of urine to decrease pharmaceuticals in hospital wastewater as compared to other technical alternatives (reverse osmosis, ozonation, and activated carbon adsorption). The main reasons cited include the insignificant improvement of wastewater quality and failure to remove pathogens. Further study is required to obtain a fair judgment of the advantages and potential issues/drawbacks with the source separation strategy.

Various MPs found in urine pose additional health risk on top of potential pathogen transmission. For places recycling urine as agricultural fertilizer, source-separated urine should be treated before actual application. The distribution of both pathogens and harmful MPs into soil may give rise to ill effects on human health and the environment. A comprehensive review by Maurer et al. (2006) assessed many available technologies for the treatment of source-separated urine with emphasis on nutrients recovery. After excretion, urine undergoes a series of physicochemical and biological changes, but the fate of those MPs during urine hydrolysis is uncertain (Udert et al., 2006). The elevated ammonia concentration due to urea hydrolysis might strongly interfere with MPs treatment. Only little has been done to remove MPs (e.g. cytostatic drugs) for producing safe urine-derived products. Since protecting the overall environment from potentially harmful substances is essential to human beings, precautionary actions deserve further attention.

Increasingly powerful analytical techniques with better detection limits have opened the door for the treatment of MPs from different environmental matrices (e.g. urine, hospital effluent). The state-of-art technologies can be utilized for the removal of cytostatic drugs in source-separated urine will be discussed. Generally, the available technologies can be divided into the three major categories according to their primary

removal mechanisms: i) advanced biotic treatment, ii) physicochemical separation, and iii) AOPs. Hybrid methods have also been developed to deal with cytostatic pharmaceuticals (Alturki et al., 2010; Dodd et al., 2008; Li et al., 2011). These processes are discussed in next section with regard to their efficacy and effectiveness for the treatment of cytostatic drugs in different matrices. **Table 2-5** presents a summary of recent studies in this area.

2.6 Biotic and Abiotic Treatment of Cytostatic Drugs

2.6.1 Membrane bioreactor

Biodegradation and adsorption to biomass are the main removal mechanisms for various organic contaminants during the passage of conventional activated sludge SPTs and onsite wastewater treatment systems (Conn et al., 2006). As discussed in Section 2.3.3, several cytostatic drugs have been detected in STP effluents and downstream surface water worldwide, demonstrating the unsatisfactory removal by conventional wastewater management facilities. There are commonly two controversial options regarding the approaches to further remove various micropollutants from wastewater, either upgrading the current STPs or applying tertiary treatment (e.g. ozonation). Based on a large-scale demonstration program, researchers in UK raised concerns about the overuse of those advanced tertiary processes nowadays, in view of excessive energy consumption, CO₂ emissions and economic costs (Jones et al., 2007). From the overall sustainability point of view, it might be more environment-friendly and cost-effective to directly improve those problematic STP by prolonging its hydraulic retention time (HRT) and sludge age and combining nutrient removal stages (Jones et al., 2007; Verlicchi et al., 2012b). Further study in terms of life cycle assessment might be needed in this regard.

A foreseeable alternative technique is to engage membrane bioreactor (MBR) system. Operated at highly-intensified biomass and high sludge retention time, MBR favors an enhanced biotransformation and mineralization of resistant pharmaceuticals (Martin Ruel et al., 2011). Mahnik et al. (2007) utilized radio-labelling techniques and demonstrated that 5-FU could be almost completely eliminated within 24 h by a pilot-

Table 2-5 Removal of cytostatic drugs from different environmental matrices using advanced technologies.

Methods	Target Cytostatics	Matrices	Main Conclusions and Implications	Reference
MBR-pilot (HRT 20-24h, SRT n.a.)	5-FU and anthracyclines (doxorubicin, epirubicin, daunorubicin)	oncologic wastewater	5-FU was readily biodegradable and adsorption to sludge was marginal, it could be completely eliminated from the liquid phase; over 90% of anthracyclines were removed mainly due to adsorption to suspended solids.	(Mahnik et al., 2007)
MBR-pilot (HRT n.a., SRT 42, 98, >300d)	cisplatin, carboplatin	oncologic wastewater	Moderate elimination efficiency (51%–63%) of total platinum was achieved; carboplatin showed relatively low adsorption to activated sludge and was mainly present as intact drug in both influent and effluent.	(Lenz et al., 2007a)
MBR-pilot (HRT 48h, SRT 50d)	CP and its human metabolites	domestic wastewater with inoculated activated sludge	CP removal was up to 80%, however, residue cytotoxicity was measured in permeate.	(Delgado et al., 2011)
MBR-pilot (HRT 1.7d, SRT 30-50d)	CP	hospital wastewater	Poor CP elimination (< 20%) was exhibited.	(Kovalova et al., 2012)
NF, NF/GAC	CP	pre-treated surface waters	NF rejection > 90% operated at 10% water recovery, but only ~30% at 80% water recovery. Size exclusion is the major mechanism. NF followed by GAC could highly reject CP.	(Verliefde et al., 2007)
NF/ RO	CP	ultrapure water and MBR effluent	RO performed high rejection (> 90%) of CP under all conditions; NF poorly rejected CP (20–40% in water and 60% in MBR effluent), during which trans-membrane pressure, CP initial concentration or ionic strength of feed solution had no influence.	(Wang et al., 2009)
Electrolysis (anodic oxidation)	epirubicin, irinotecan, vincristine, mitomycin C, paclitaxel, methotrexate, cisplatin	clinic wastewater	Cytotoxicity, mutagenicity and antibacterial activity of epirubicin were ~100% eliminated after electrolysis (6h), 72–100% for other investigated cytostatics. The cost-effective apparatus can be adopted to treat clinical wastewater.	(Hirose et al., 2005)
Electrolysis (anodic oxidation)	methotrexate	urine	Electrolysis generates active chlorine and decomposes/detoxifies (cytotoxicity) methotrexate in the presence of urine.	(Kobayashi et al., 2012)
Indirect photochemical degradation	CP, IF	lake water	Removal efficiencies in 70d were up to 80% and 60% for CP and IF, respectively. Increased OH• concentration by adding nitrate enhanced the photochemical degradation of CP and IF.	(Buerge et al., 2006)
PAC absorption, ozonation, UV/H ₂ O ₂	CP, irinotecan, tamoxifen	deionized water	PAC absorption capacity (tamoxifen > irinotecan > CP) was dosage-dependent. UV/H ₂ O ₂ was more effective than ozonation to remove the three cytostatics. Ozonation alone cannot destroy CP.	(Chen et al., 2008)
UV and UV/H ₂ O ₂	CP	pure water and biologically treated water	UV dose decreased from 5201 to 1695 mJ/cm ² (UV/H ₂ O ₂) for 90% CP-degradation. H ₂ O ₂ addition significantly enhanced CP degradation by UV radiation. H ₂ O ₂ enhancement was more effective to less readily-degraded PPCPs. Dissolved organic matters might act as scavengers of both OH• and UV energy.	(Kim et al., 2009)
Ozonation	CP, methotrexate	drinking water	CP degradation rate with O ₃ was low ($k_{O_3} \sim 3.3 \text{ M}^{-1}\text{s}^{-1}$, pH 8.1) without significant natural water matrix effects; reaction with OH• was much easier ($k \sim 2.0 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$). A high CT (oxidant conc. × contact time) value of ~45 mg min/L was required to remove 96% CP from natural water. By comparison, methotrexate reacted quickly with O ₃ ($k_{O_3} \sim 3.6 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$) at typical dosages applied in drinking water treatment.	(Garcia-Ac et al., 2010)
UV/H ₂ O ₂ /O ₃ and its sub-processes (UV, UV/H ₂ O ₂ , UV/O ₃ , O ₃ , H ₂ O ₂ /O ₃)	CP	deionized water	$k_{O_3} = 2.5 \text{ M}^{-1}\text{s}^{-1}$ (CP + O ₃ in excess); $k_{OH\cdot} = 1.3 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ (CP + OH•). H ₂ O ₂ /O ₃ show highest degradation rate among different AOP conditions, followed by ozone treatment alone. H ₂ O ₂ concentration was found to be an important parameter.	(Lester et al., 2011)
Ozonolysis and sonolysis/ozonolysis	methotrexate, doxorubicin	pure water at different pH	Methotrexate was readily degraded by both methods with degradation efficiencies up to 80% in 12 min at basic pH. By comparison, the second-order reaction rate for doxorubicin was much lower and < 50% was removed in 20 min. Removal efficiency for both compounds was pH-dependent, and could be significantly enhanced by sonolysis.	(Somensi et al., 2012)

Notes: GAC/PAC, Granular/powder activated carbon; HRT, hydraulic retention time; SRT, sludge retention time; 5-FU, 5-fluorouracil; CP, cyclophosphamide; IF, ifosfamide.

scale MBR system mainly due to biodegradation. Complete biodegradation of 5-FU was also observed within a few days in early OECD confirmatory test (Kiffmeyer et al., 1998). In case of anthracyclines, up to 90% were removed from liquid phase primarily because of adsorption to sewage sludge (Mahnik et al., 2007). Only moderate elimination was displayed in the same MBR system for hydrophilic cisplatin and carboplatin (51% and 63%, weekly), also primarily due to their irreversible adsorption to the activated sludge (Lenz et al., 2007a). As such, the overall removal efficiency varied among different cytostatic compounds, highly associated with their physicochemical properties (e.g. hydrophobicity) and biodegradability. In this light, more hydrophilic cytostatic compounds (i.e. $\log P < 2$) with lower biodegradability are most likely to sneak out of MBR system, especially those with relatively small molecular weights. Kovalova et al. (2012) observed a low elimination efficiency ($< 20\%$) for CP through a MRB system fed with hospital's sanitary wastewater. Furthermore, the MRB operation conditions (biomass, retention time, feed concentration, redox, etc.) could also exhibit high influences on biotic treatment processes. For instance, Delgado et al. (2009) demonstrated that lab-scale cross-flow MBR system operated at aerobic/anoxic conditions ($\text{DO } 0\text{--}4.5 \text{ mg O}_2/\text{L}$, $\text{HRT } 32\text{h}$, $\text{SRT } 70\text{d}$) could achieve 80% removals of CP and its metabolite 4-ketocyclophosphamide due to both adsorption and degradation. Unfortunately, to our knowledge, such information is very limited for other cytostatic drugs. Tertiary treatment units might be needed to facilitate the safe use of treated effluent from MBR system.

Although MBR system seems to perform higher removal efficiency against pharmaceutical contaminants compared to conventional wastewater treatment plants, more evidence is required to draw a convincing conclusion. Negative effects of cytostatic compounds on the microbial community have also been observed. Delgado et al. (2010b) reported an enhanced endogenous respiration and reduced sludge production in lab-scale cross-flow membrane bioreactors at the presence of $\mu\text{g/L}$ -level CP and its principal metabolites; this suggests extra carbon/energy was exhausted to respond to the chemical stress caused by CPs. An elevated concentration of extracellular polymeric substances (mainly polysaccharides and proteins) in the biological sludge was further observed in the same MBR system, which probably promoted faster membrane fouling

(Avella et al., 2010). The early study by Kiffmeyer et al. (1998) also demonstrated that the biodegradation of methotrexate could generate toxic and persistent transformation product 7-hydroxymethotrexate. These studies provided in-depth insights into the negative effects of cytostatic compounds on microbial activity during advanced wastewater treatment processes at community-levels. Despite the adverse effects on microbial behaviors in the MBR system, removal efficiencies of COD and total nitrogen were not altered by the anticancer drug toxicity under aerobic/anoxic operating conditions (Delgado et al., 2010b). In order to utilize advanced MBR system for pharmaceutical removal in the presence of urine, special concerns might be brought up regarding the coexisting high concentration of toxic ammonium (due to ureolysis) and urine-derived odor issue (Zhang et al., 2013b).

2.6.2 Physicochemical separation

Evaporation. Most cytostatic drugs are rather polar compounds with multiple hydrophilic functional groups (e.g. $-\text{OH}$, $-\text{COOH}$, $-\text{NH}_2$, etc.), therefore, direct evaporation from wastewater seems infeasible for the majority of them. In terms of urine source separation, however, it is interesting to note that solar thermal evaporation could be potential technology for nutrients recovery from urine, especially in developing countries with insufficient infrastructure and sanitation facilities (Pronk and Kone, 2009; Verlicchi et al., 2012b). Additionally, simultaneous photodegradation of pharmaceuticals may occur during this process due to exposure to ultraviolet radiation and infrared heat (Doll and Frimmel, 2003). Although this process presents certain benefits for generating hygienically-safe fertilizer in rural regimes, it is unacceptable in urban areas because of land scarcity and odor concerns. In addition, solar ultraviolet radiation might not be effective to photodegrade persistent cytostatic residue such as toxic CP (Kim et al., 2008).

Adsorption. During wastewater treatment, adsorption to activated sludge (biomass) could be important sinks of more hydrophobic pharmaceuticals ($\log P > 2$). Adsorptive capacity depends largely on the physicochemical interactions between target molecules and adsorbing medium, highly associated with the environmental conditions. The removal via powder activated carbon (PAC) adsorption depends largely on PAC dosage

and analytes' octanol-water partition coefficients, especially for those hydrophobic species (Westerhoff et al., 2005). Chen et al. (2008) demonstrated that PAC absorption capacity to tamoxifen (logP 7.88) is better than irinotecan (3.73), followed by CP (0.63), but depending on initial PAC dosage. Granular activated carbon (GAC) filtration also showed nearly complete removal of neutral CP from pretreated surface water (Verliefde et al., 2007). However, since many water-soluble cytostatic drugs are readily protonated or deprotonated in neutral waters (see pKa values in **Table 2-2**), PAC adsorption as a stand-alone unit seems not suitable for satisfactory removal from water or industrial wastewaters. In addition, the adsorption onto organic membrane materials has also been recognized as important mechanism of rejecting trace contaminants by NF/RO processes, especially when the feed concentration reaches to the ng/L-levels (Kimura et al., 2003b).

Similar to other pharmaceuticals, cytostatic residue is excreted as water-soluble conjugated and/or unconjugated (free) forms in urine. They are unlikely to adsorb to solid precipitates during struvite precipitation in urine, as indicated by Ronteltap et al. (2007) who reported that over 98% of the tested pharmaceuticals remained in the solution after nutrient recovery. Therefore, the direct usage of fresh or hydrolyzed urine might introduce toxic cytostatics as well as other pharmaceutical residues into agricultural land, indicating the possible needs for further treatment before application. Similarly, activated carbon adsorption might not be an effective approach for complete removal of polar cytostatic residue, such as CP and IF, from source-separated urine. Since the urine in the liquid form might carry cytostatic residue and pose potential concerns, using urine-derived solid struvite ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) might be a safer alternative that deserves further investigation.

Electrodialysis. Electrodialysis is defined as ion-exchange membrane separation technology with apparent molecular weight cut-off to be around 200 Da. Since the molecular weights of most cytostatic drugs lie around or above this value, the removal potential of these compounds from urine becomes prospective. The concept behind electrodialysis for urine treatment is composed of a concentrated stream containing nutrients (salts) and a dilute stream containing pharmaceuticals. Pronk et al. (2006a)

proposed that the permeation mechanism of pharmaceuticals appeared to depend on the adsorbed amount on the membrane, which indicated that the partitioning and diffusion mechanisms played an important role in the permeation transport. The adsorbed pharmaceuticals might be slowly remobilized by reversing the electrodialysis condition. Escher et al. (2006) evaluated the efficacy of applying electrodialysis for the treatment of pharmaceuticals and hormones in urine. The results indicated a positive toxicity reduction since the substrate estrogenicity was reduced by 99.7% due to efficient separation during the process. Dodd et al. (2008) compared different urine treatment scenarios and found that the combination of electrodialysis with urine ozonation (either electrodialysis with post-ozonation or ozonation with post-electrodialysis) performed better than centralized wastewater treatment strategies in terms of energy efficiency and urine-derived nutrient and pharmaceutical attenuation.

Membrane filtration (NF, RO and FO). The combination of current membrane filtration technology serves both wastewater treatment (e.g. MBR) and water reclamation. In particular, reverse osmosis (RO) has been globally utilized for water reclamation from brackish water and seawater. Theoretically, only dense RO membrane (nonporous) or tight nanofiltration (NF) membrane (porous) are expected to filter out various pharmaceuticals which possess relatively small molecular weights (Snyder et al., 2007). In general, the retention performance of NF/RO membranes is strongly dependent on 1) size-dependent steric hindrance effect; 2) pH-dependent electrostatic repulsion between membrane and pharmaceutical molecules; 3) adsorption of trace pharmaceuticals onto membrane surface; and 4) membrane operating conditions and hydraulic parameters (membrane fouling, membrane defects, trans-membrane pressure, etc.) (Bolong et al., 2009; Nghiem et al., 2005). Hence, the intrinsic mechanisms of retaining trace organic contaminants, such as pharmaceuticals could be rather synergic. Kimura et al. (2003b) demonstrated that thin film composite NF and RO membranes could efficiently reject negatively-charged pharmaceuticals regardless of their other physicochemical properties, while the rejection of neutral compounds mainly depends on their molecular weights. For both neutral and positively charged pharmaceuticals, Verliefde et al. (2007) proved that NF rejection efficiency decreased with increasing solute hydrophobicity due to membrane adsorption, but size exclusion was still the main

mechanism for the rejection of uncharged CP. However, a low CP rejection (~30%) was observed when NF was operated at 80% recovery of water. Wang et al. (2009) also showed that the toxic CP could be effectively rejected (> 90%) by polyamide RO membrane, while poorly rejected by a three-layer thin-film NF membrane. Furthermore, bio-fouling could slightly alter the NF membrane surface be more negative and hydrophilic, lowering to some extents the rejection efficiency for positively-charged pharmaceuticals; no significant effects were observed for neutral and negatively-charged solutes such as CP (Botton et al., 2012). Such evidence should be supplemented to investigate the efficacy of retaining other potentially harmful cytostatic compounds by NF/RO membranes. It is worth noted that related studies for cytostatic drugs are very limited, which indicates the need for further research efforts.

Regardless the noticeable advantages, the pressure-driven NF/RO processes also raise concerns about a hike in energy demand. FO has been attracting increasing interests in water reclamation from salty water/wastewater, in view of lower energy consumption and membrane fouling potential (Zhao et al., 2012). FO process also exhibits satisfactory retention against certain commonly-used pharmaceuticals (Jin et al., 2012a; Xie et al., 2012b), however, such data are still too scarce to justify the argument that FO process carries enough competence to remove other pharmaceuticals, including potentially harmful cytostatic molecules.

It is noteworthy that NF/RO/FO membranes could only physically retain rather than chemically/ biologically destroy the pharmaceuticals from feed water. In other words, the trace contaminants will be concentrated in the retentate, which usually makes up 20–30% of the total influent. The discharge of rejected brine from RO plant directly into river/ocean may cause adverse effects on ecosystems. In cases of RO plants treating secondary effluent for water reclamation, the returning of pharmaceutical-containing retentate as influent into upstream STP might lead to continuously increasing pharmaceutical concentrations in the activated sludge. For instance, 18 PPCPs compounds were identified in the RO retentate with concentrations ranging from 0.1 to 7.9 µg/L; the utilization of AOPs involving hydroxyl radical could effectively remove most of the PPCPs in the presence of complex organic/inorganic matrix (Ben

Abdelmelek et al., 2011). For certain toxic cytostatic drugs, such as CP and its metabolites, special attention should be paid to their potential adverse influences on the physicochemical characteristics of the activated sludge matrix and subsequently on the entire biological process (Delgado et al., 2010a). Additional post-treatments such as AOPs might be needed to eliminate various unwanted pharmaceuticals from the rejected retentate.

In terms of treatment specifically targeting source-separated urine, NF/RO processes also assure effective removal of trace pharmaceuticals. The separation of pharmaceuticals and estrogens from nutrients in urine was investigated by Pronk et al. (2006b). They suggested the use of NF270 membrane to produce a pharmaceutical-free nutrient solution as fertilizer; optimum retention ($> 92\%$) was achieved for target pharmaceuticals in the acidified non-hydrolyzed urine (pH~5). Meanwhile, phosphate and sulfate were almost completely screened out while urea and ammonia were well permeated. Some other studies also demonstrated a high rejection ($\sim 98\%$) of various pharmaceuticals by using NF membrane, while the significant reduction of conductivity in permeate indicated efficient retention against ions (Lazarova and Spendlingwimmer, 2008b). From another aspect, the NF membranes present high adsorption capacity for many pharmaceuticals in the urine because of their relatively high hydrophobicity. The abovementioned study by Pronk et al. (2006b) also indicated that the maximum adsorption corresponds to about 35% of the initial amount in solution.

Considering the small volume of urine stream, it would be very advantageous to utilize membrane technologies (mainly NF/RO) for effective removal of unwanted pharmaceuticals from the 'little' urine. This approach is expected to be cost-effective in view of the huge-volume production of daily wastewater (Larsen et al., 2004). In view of the relatively lower solid concentrations of urine, membrane fouling might not be a serious limitation compared to other wastewater streams. A simple pretreatment (microfiltration or biodegradation) should be applied to further attenuate the potential of membrane fouling. A recent study showed that FO treatment in combination with RO could achieve both high water recovery ($\sim 70\%$) and high nutrients rejection from nutrient-rich anaerobic digester concentrate (Holloway et al., 2007). This inspires the

utilization of FO technique for simultaneous water (80%~90% of urine volume) and nutrients (mainly N, P, K) recovery from source-separated urine. In recent years, the development of osmotic membrane bioreactor (OsMBR) provides a new conception on urine treatment from the perspectives of water reclamation, nutrients recovery and pharmaceutical treatment (Achilli et al., 2009).

2.6.3 Advanced oxidation processes

Electrochemical oxidation. Electrochemical oxidation process has been intensively studied for water and wastewater treatment. Recently, researchers have demonstrated that electrolysis with platinum electrodes could completely eliminate the cytotoxicity, mutagenicity and antibacterial activity of clinic wastewater containing antineoplastics (Hirose et al., 2005; Kobayashi et al., 2012). Lazarova and Spendlingwimmer (2008b) also confirmed that electrochemical oxidation is efficient for the treatment of other urine-derived hormones and OTC pharmaceuticals, and over 80% removal of hormones in the urine could be accomplished without loss of urea. Pérez et al. (2010) also demonstrated that anodic oxidation could effectively remove 10 priority emerging contaminants in RO concentrate generated in tertiary water treatment, all following first order removal rates. However, it is also important to note that trihalomethanes (possible human carcinogens) could be also formed with during the electrochemical process an increased current density (Pérez et al., 2010). Overall, the oxidation process is mainly dependent on the concentration ratio between the target pharmaceuticals and the background organic matters. Since source-separated urine contains higher concentration of target compounds and less organic matter, it seems ideal for the application of electrochemical oxidation from the energy saving point of view.

Ozonation and hydrogen radical attack. Ozonation presents another attractive method for removing a broad spectrum of micropollutants (Broseus et al., 2009; Fitzke and Geissen, 2007). There are two common reaction routes to eliminate the organic contaminants by ozone treatment: direct reaction with molecular ozone and indirect reaction with free radicals, mainly hydroxyl radicals, generated by the decomposition of ozone. H₂O₂ with/without UV radiation is often added to enhance the radical production in the second reaction route. A stand-alone tertiary UV treatment in STP even showed a

capability to remove more than 2/3 tamoxifen in pre-UV effluent samples (Roberts and Thomas, 2006); however, UV treatment showed negligible removal for CP (Llewellyn et al., 2011). In general, molecular ozone reacts selectively with unsaturated bonds, aromatic and amino groups whereas $\text{OH}\cdot$ react much more indiscriminately (von Gunten, 2003). The dominant pathway involved in a specific process largely depends on the specific water matrix (i.e. pH, alkalinity, redox, organic matters). A recent study by Somensi et al. (2012) demonstrated that O_3 oxidation of both methotrexate and doxorubicin were pH-dependent process, with a second-order rate constant at neutral pH of 0.0267/min and 0.3373/min, respectively. Furthermore, the dissolved organic matters might act as $\text{OH}\cdot$ scavengers, which would significantly decrease the efficiency of ozone treatment on target compounds (Kim et al., 2009). In cases of the UV-enhanced AOP, organic substrate might also consume certain UV energy, leading to a higher operation cost. The atmospheric $\text{OH}\cdot$ reaction rate constants (see **Table 2-2**) could provide a rough evaluation on the efficacy of removing cytostatic agents by hydroxyl radical attack.

An increasing number of studies have demonstrated the efficacy of removing cytostatic drugs by various ozone-based AOP, as showed in **Table 2-5**. CP has been often investigated due to its low biodegradability and high toxicity. Garcia-Ac et al. (2010) recently showed that methotrexate can be quickly and effectively removed from drinking water by ozonation, while CP removal needs much higher ozone dosage and longer contact time; however, no further identification of the ozonation by-products was conducted. Until now, there are inadequate data to conclude if current water treatment processes are successful in preventing various polar cytostatic residue from entering the tap water and threatening public health. The by-products generated during ozonation and chlorination might be even more toxic than their parent compounds. Other studies have also demonstrated that ozone can only partially degrade recalcitrant pharmaceutical compounds without complete mineralization in the presence of urine (Dodd et al., 2008; Escher et al., 2006). They also concluded that direct reactions with ozone play an important role in governing oxidation rates during urine ozonation. Compared to the highly diluted influent of wastewater treatment plants, source-separating urine contains relatively low concentrations of organic substrate, but much higher concentrations

(100–500 times) of pharmaceuticals (Larsen et al., 2004). This might assist to achieve more cost-effective removal of various pharmaceutical residues in human urine by ozone treatment.

Catalytic oxidation. Other AOP for the removal of cytostatic compounds using reactive and functional nano-scale materials, such as photo-catalyst titanium dioxide, has been seldom reported. An early study by Low et al. (1991) demonstrated that CP and 5-FU could be completely mineralized to inorganic species (i.e. CO_3^{2-} , NO_3^- , F^- , NH_4^+ , PO_4^{3-} , Cl^-) by UV-illuminated TiO_2 film, with an approximate final NH_4^+ - NO_3^- ratio of 0.5. More recently, nano-scale zero-valent iron (nZVI) has been radically studied as a green environmental technology for the treatment of contaminated sites. Commonly with a core-shell structure, reactive nZVI proves to effectively remove various micropollutants in contaminated water and soil, including halogenated or non-halogenated pharmaceuticals (Ghauch et al., 2009). With no doubt, the versatile nanomaterials indeed promise options to deal with pharmaceutical contamination in different environmental matrices.

2.7 Chapter Conclusions

This review was referred on the environmental presence and transformation, potential ecotoxicity and various source contributions of cytostatic compounds in the environment. Previous research has focused mainly on hospital effluents but few on household discharges. The latter contributes to the cytostatic residue in the environment as an increasingly important source. Limited information is available for predicting the chemodynamics of cytostatic in natural waters, as well as their important human metabolites and environmental transformation products. More urgently, solid ecotoxicity data is needed to further support or exclude their ‘subtle’ yet chronic effects on the ecosystem. In practice, precautionary mitigation approaches such as drug waste management should be adopted to prevent cytostatic residue from continuously admitted into the environment.

Promising alternatives may essentially lie on the state-of-the-art treatment technologies based on urine source-separation strategy; it deserves further comprehensive evaluation from the technological, social-economical and administrative perspectives. Further fundamental understanding is required on the trace contaminants removal by various technical alternatives, such as advanced MBR, RO/FO, and AOPs. Fair judgment cannot be made on these 'end-of-pipe' technologies, regarding their intrinsic advantages, drawbacks and development trends. The combination of urine source separation strategy followed by smart and cost-effective treatment technology promises an alternative solution for preventing cytostatic drugs from continuously entering the environment.

CHAPTER 3 URINE SOURCE SEPARATION FOR NUTRIENT RECOVERY

3.1 Introduction

Human urine undergoes a series of intensive biological, physical, and chemical transformation immediately after its excretion (Hoglund et al., 2002). After sufficient storage, source-separated urine can be essentially stabilized as stale urine with a strong buffering capacity. Ureolysis, one of the most crucial parts, occurs with ease due to the existence of ubiquitous urease-positive bacteria, leading to subsequent mineral precipitation and ammonia volatilization at elevated pH (Larsen et al., 2003; Udert et al., 2006). Mineral precipitation might cause serious blockage in the urinary pipe. In case of a leakage, a strong odor is released during urea hydrolysis, thereby contributing another critical concern to urine separation systems in an urban setting. An enhanced urine stabilization process is expected to not only minimize the storage volume required prior to any further urine treatment, but also shorten the duration of odor emission. Due to strict regulations of odor control in many countries, a systematic odor assessment (recording, characterizing and evaluating) should be done before any sanitation system is applied (Schlegelmilch et al., 2005).

Urine hydrolysis can be affected by many factors such as temperature, mixing ratio of stale to fresh urine and the amount of urease enzyme (Kabdasli et al., 2006; Zhao et al., 2008). The potential effects of other important factors such as fecal contamination have received less attention. One objective of this study is to comprehensively determine the influence of multiple parameters on the urine hydrolysis process, including temperature, dilution ratio, stale/fresh urine ratio, brown water (BW) cross-contamination, and infection of urease-positive bacteria. The characteristics of spontaneous precipitates formed during the hydrolysis process were monitored. In order to provide a detailed description on potential odor emissions, the interaction of urine hydrolysis with the emission of inorganic and organic gases was evaluated as well. The feasibility and potential issues of urine source-separation system applied in a tropical urban setting was investigated.

The presence of excessive nutrients (mainly N and P) in water bodies causes severe eutrophication and impairs the aquatic ecosystem. This study also investigated different nutrient recovery techniques including seawater-induced struvite precipitation and ammonia stripping. Phosphorus recovery from source-separated urine via struvite (a slow-release fertilizer) precipitation has gained increasing interests globally. Spontaneous mineral formation depletes the magnesium in hydrolyzed urine so that additional magnesium source is required to produce more struvite with the remaining phosphorus. In this study, seawater and desalination brine were explored as low-cost magnesium sources to induce struvite precipitation in hydrolyzed urine solution. Air stripping has been widely used to recover ammonia from wastewaters to reduce the discharge of nutrients to water bodies. This study specifically investigated the operational and economic aspects of air stripping for ammonia recovery from source separated urine.

3.2 Materials and Methods

3.2.1 Urine hydrolysis experiments

Lab-scale experiments were performed to investigate the effects of temperature, dilution ratio, volumetric ratio of stale urine to fresh urine (S/F urine ratio), BW cross-contamination and initial infection of urease-positive bacterial on urine hydrolysis process. The investigations on these influencing factors were conducted in several independent runs. Fresh urine was engaged in all experimental runs except for the second one which was meant to investigate the impact of S/F urine ratio. Fresh human urine was collected from a group of 30 healthy adults in a voluntary basis and well mixed in sterile plastic bottles. The ranges of initial pH, conductivity and $\text{NH}_4^+\text{-N}$ concentration of the undiluted fresh urine were between 7.0–7.3, 16.6–22.7 mS/cm and 371–630 mg/L, respectively. In all batches, a quantitative aliquot (200 mL) of urine sample was added into each glass bottle (250 mL), covered tightly to simulate a relatively anoxic environment representing real conditions.

In run 1, the effect of temperature on urine hydrolysis at 4, 18, 23, 28, 34, 40, 50 °C was investigated. The temperatures from 18 to 34°C were carefully selected with an

increment of ~ 5 °C, representing the typical room temperatures with/without air-conditioning, and nocturnal/diurnal/peak ambient temperatures in tropical urban environment. Refrigeration temperature (4 °C) and extremely high temperature (50 °C, with additional heating supply) were also investigated for comparison purpose. Three dilution ratios were also examined (undiluted, dilution 1:2, and dilution 1:5) to simulate the influence of flushing water. The dilution ratios were selected to match the flushing volume of several water-saving urinals, such as waterless system, eco-friendly non-mix toilet, and traditional low-water urinals (Larsen et al., 2009; PUB, 2006).

In run 2, stale urine was mixed with fresh urine at different ratios (v/v) (0%, 10%, 20%, 40%, 60% and 80%) to investigate the effects of S/F urine ratio on urine hydrolysis process at the optimal temperature observed from the first run (34°C). The stale urine (pH, conductivity and $\text{NH}_4^+\text{-N}$ concentration was 9.0, 31.1 mS/cm, 3800 mg/L, respectively) was prepared by storing the fresh urine at room temperature (24 °C) for several weeks. The selection of S/F urine ratios here and BW/fresh urine ratios in run 3 were arbitrarily determined mainly taking into accounts of the potential mixing behaviors from the non-mix toilet system. Furthermore, Runs 3 and 4 were carried out simultaneously with special control on bacterial contamination. Both runs were conducted in a UV sterilization cabinet where all materials and devices, except the fresh urine samples, were disinfected by UV light with a peak wavelength of 254 nm for at least 5 min. In run 3, fresh BW was taken from a conventional toilet and quantitatively introduced into fresh urine since at source-separating toilet cross-contamination with BW tends to occur. In order to further investigate the effects of initial infection of urease-positive bacteria on urine stabilization, the fresh urine sample was centrifuged and the bottles were autoclaved in run 4. The sterilization pretreatment was done before the experiments to rule out any other bacteria source from air or device. T-tests and one-way analysis of variance (ANOVA) was applied at the significance level of 0.05 to analyze the differences between variables. A *p*-value of <0.05 was considered significant.

In addition, a set of experiments were conducted to monitor headspace pressure variation in the urine-loaded glass bottles using Oxitop pressure sensors (WTW,

Germany). Additional experiments were conducted to determine gas emissions, in which the bottle headspaces were first rinsed with N₂ to simulate anaerobic conditions. All the aforementioned experiments including runs 1~4 were carried out in duplicates to verify the reproducibility of the testing procedure. The duplicate experiments showed very slight difference in between.

3.2.2 Nutrient recovery experiments

This study investigated, both experimentally and theoretically, the optimal conditions to induce struvite precipitation in hydrolyzed urine by using seawater and desalination brine as possible low cost magnesium sources. The experimental and modelling methodology was described in our previous publication (Liu et al., 2013). In brief, the chemical equilibrium model Visual Minteq 3.0 (<http://vminteq.lwr.kth.se/>) was used for calculating speciation, solubility and sorption equilibrium of solids in an aqueous solution. Since struvite was not included in the current solid database, the solubility constant $pK_{sp} = 13.15$ was manually incorporated into the model database based on Buchanan's study (Buchanan et al., 1994).

This study further investigated the operational and economic aspects of air stripping for ammonia recovery from source separated urine. Batch air stripping experiments were conducted to investigate the stripping performance under different operational parameters including pH, dilution ratio, air flow rate and temperature. The experimental parts was well described in our previous publication (Liu et al., 2014). For some nutrient recovery experiments, synthetic fresh urine prepared with Griffith et al. (1976)'s recipe was used (see **Table 2-1**); synthetic hydrolyzed stale urine was simply prepared by replacing urea in the recipe with an equivalent amount of ammonium carbonate (Merck).

3.2.3 Analytical methods

The suspension pH-value, conductivity and total ammonium (NH₄⁺-N) concentration were monitored for five consecutive days in each experiment. Electrical conductivity and pH-value were measured using a D-54 pH/conductivity meter (Horiba, Japan). Ammonium concentration was colorimetrically measured based on the salicylate

method (Method 10205) using a DR 2800 spectrophotometer (Hach, USA). All reagents and chemicals used in the experiments were of analytical grade. At least two parallel replicates were engaged throughout the whole study for quality assurance purpose.

Spontaneous precipitation occurred during urine hydrolysis. Therefore, after hydrolysis completed, the stale urine was centrifuged at 10000 rpm for 15 min to separate the precipitates formed. The collected precipitates were rinsed with deionized water and ethanol (99%), and then dried at 60 °C overnight. X-ray diffraction (XRD) analysis of the precipitates was carried out using a model DMS 2000 system (Scintag Inc., USA). Fourier transform infrared spectroscopy (FTIR, Perkin Elmer PE 1600, USA) and scanning electron microscopy (SEM, Zeiss Evo 50, Germany) were also used to further characterize the spontaneous precipitates. The concentrations of VFA in urine were determined using gas chromatography with a flame ionization detector (GC–FID, Agilent). A selective capillary GC column (DB–FFAP) was applied for good separation of nine VFA species including acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, iso-caproic, n-caproic and heptanoic acids. For each analysis, 1.0 mL sample was taken and filtered through a 0.20 µm Whatman glassfibre filter, then mixed with 0.2 ml phosphoric acid (85%) and finally stored at 4 °C until analysis.

The inorganic gas composition in headspace (CO₂, N₂, CH₄, H₂ and H₂S) was determined using gas chromatography with a thermal conductivity detector (GC–TCD, Agilent) and a selective packed column (HP–PLOT Q). The amount of NH₃ generated during the process was directly calculated based on the assumption that the headspace gases mainly consisted of N₂, CO₂ and NH₃, while other compositions can be safely neglected in terms of volume. The measured pressure was also used to correct all the calculations to STP condition (298.15 K and 1.0 atm).

3.3 Factors Influencing Urine Hydrolysis

3.3.1 Effect of temperature

Figure 3-1 presents the pH, conductivity and NH₄⁺-N concentration profiles during urine hydrolysis at different temperatures and dilution ratios. Fresh human urine

contains nitrogen mainly in the form of urea, with relatively low ammonium content. The enzyme urease generated by urea-hydrolyzing bacteria catalyzes the hydrolysis of urea to ammonia and bicarbonate (Larsen et al., 2003). As seen in **Figure 3-1a**, the temperature increment had a dynamic effect on urea hydrolysis process. The optimal temperature for rapid urea hydrolysis process in undiluted urine was between 34–40 °C, at which the pH, conductivity and $\text{NH}_4^+\text{-N}$ concentration of urine solution reached their maximum values in less than two days. The results indicate that at higher temperatures a more rapid urea hydrolysis rate could be achieved. Moreover, the extremely high temperature (50 °C) significantly inhibited the growth of urease-positive bacteria in urine and ultimately the entire hydrolysis process. The variations of conductivity and pH-value followed the same trend as $\text{NH}_4^+\text{-N}$ concentration. These results are in accordance with other similar studies on urine hydrolysis (Larsen et al., 2003; Zhao et al., 2008).

Figure 3-1 Variation of pH-value, conductivity and ammonium concentration during urine hydrolysis at different temperatures and dilution ratios (a. no dilution; b. dilution 1:2; c. dilution 1:5)

Based on the above batch experiments, it is inferred that the relatively higher ambient temperature in tropical or semitropical areas could benefit the urea hydrolysis process. On the contrary, extra energy is needed (e.g. from adjacent anaerobic digestion system) to maintain a favorable hydrolysis temperature when source separation systems are applied in relatively cold areas. Hoglund et al. (2002) also reported that human urine stored at 20 °C for at least six months can be considered safe to use as fertilizer for crops. When source separation systems are introduced in urban setting, such long storage period is impracticable because of land-scare constrains.

3.3.2 Effect of urine dilution

With a dilution ratio of 1:2, the pH-value of urine solution also needed around two days to reach its highest level. However, both conductivity and $\text{NH}_4^+\text{-N}$ concentration reached their highest values within five days (**Figure 3-1b**). The asynchronous increase implied a slower urea hydrolysis process in diluted urine. The major reaction in fresh urine is urea hydrolysis, which enzymatically converts urea into CO_2 (weak acid) and ammonia (weak base). The generated CO_2 and NH_3 will contribute to a strong buffer solution of hydrolyzed urine (pH~9.3). Dilution of the urine led to a lower substrate (urea) concentration, and therefore retarded the ureolysis process and the formation of buffer solution. As showed in **Figure 3-1b**, urine dilution did affect the pH, but to a lower extent than conductivity and ammonium concentration.

The same observation was demonstrated at even higher dilution ratio of 1:5 (**Figure 3-1c**). At this dilution ratio, urea hydrolysis required more than five days to be completed although the initial urea concentration was five times lower. It is remarkable that high water usage for urine flushing may lead to an increase of precipitates with low solubility because of the presence of water hardness (Larsen et al., 2003). This, in turn, increases the risk of pipe blockage issue in source separation systems. The treatment of highly-diluted urine increases the treatment cost, which concurrently may also reduce nutrients recovery efficiency. The water-saving purpose can be achieved based on novel separated toilet design with high urine-diversion efficiency. Furthermore, low ammonia concentrations in highly-diluted urine can result in slow inactivation of pathogenic microbes (Vinneras et al., 2008). Hence, source-separated urine with less or even no

dilution is favorable for a quick ureolysis process and subsequent inactivation of contained microorganisms.

3.3.3 Effect of stale/fresh urine ratio

Figure 3-2 illustrates the variation of ammonium concentration during urine hydrolysis with different stale-fresh urine ratios, fecal contamination, and urine pretreatments. As showed in **Figure 3-2a**, urine hydrolysis at 34 °C could be significantly enhanced by adding stale urine because of the introduction and growth of urease-positive bacteria. The mixture of a higher ratio of stale urine exhibited a more active urea hydrolysis process. The urea hydrolysis took 3-4 days in a controlled experiment with fresh urine. The slightly slower hydrolysis rate compared to run 1 was likely due to less initial infection of urease-positive bacteria in fresh urine. By addition 10–20% of stale urine into fresh urine, the time needed to complete urea hydrolysis can be reduced to less than two days. The optimal mixing ratio of stale-fresh urine was at 40%, beyond which the process could be completed in less than one day. Assuming a constant influent, however, an even higher mixing ratio ($> 40\%$) would probably enlarge the volume of urine hydrolysis tank.

Figure 3-2 Variation of ammonium concentration during urine hydrolysis with different stale-fresh urine ratios (a), fecal contamination (b) and pretreatments (c). (NN: normal bottle, normal urine; NC: normal bottle, centrifuged urine; AC: autoclave bottle, centrifuged urine; AN: autoclave bottle, normal urine; NN + 1% BW: normal bottle, normal urine with 1% brown water)

From an engineering perspective, mechanical stirring can be applied to enhance the mixing of incoming fresh urine with the existing stale/semi-stale urine in the urine stabilization tank, which benefits the reproduction and growth of activated urease-positive bacteria; this leads to rapid urea hydrolysis. The mixture also prevents potential

nitrification and autotrophic denitrification in the anoxic tank. After hydrolysis, nitrogen in the urine (mostly as ammonium) can be easily recovered through various physicochemical technologies, such as ammonia stripping (Gustin and Marinsek-Logar, 2011), struvite precipitation (Kabdasli et al., 2006; Ronteltap et al., 2010), selective ion exchange (Hedstrom and Amofah, 2008), or a combination among these technologies (Lind et al., 2000). The recovered nitrogen, either in the form of struvite or concentrated ammonium solution, can be further used for horticultural activities in cities. On the other hand, mechanical stirring in the urine stabilization tank will also increase stripping of urine-derived VOCs and intensify the potential odor problems. Some preventive measures, such as a well-sealed and/or buried urine tank or odor control devices, might be necessary to prevent odor leakage to neighboring communities.

3.3.4 Effect of cross contamination with brown water

Urine contamination by feces is very possible in source separation systems (Hoglund et al., 1998). As illustrated in **Figure 3-2b**, urine hydrolysis was significantly enhanced by adding merely 1% (v/v) BW into fresh urine due to the introduction of urease-positive bacteria and urease enzymes that exist in BW. Some of these bacteria, like *Proteus*, belong to the family of *Enterobacteraceae*. *Proteus* are non-pathogenic enteric bacteria that lack of the ability to ferment lactose, but characterized by fast urease activity (Stuart et al., 1945). Stuart's urea broth was analyzed to confirm the existence of urease-positive bacteria in fresh BW (data not shown). In addition, other enterobacteria are also detected for their weaker urease-positive activities using Christensen's urea agar (Christensen, 1946). The enhancement of urine hydrolysis by minor cross-contamination from brown water was therefore confirmed. However, urine hydrolysis was observed to slow down significantly by adding higher portion of BW. As the microbial pathogens in urine are mainly derived from fecal cross-contamination (WHO, 2006), the complicated microbial community contains a wide range of species. The urease-positive bacteria might not have the advantage competing with others in the early intense microbial competition stage (Brooks and Keevil, 1997; Sears, 2005), which resulted in a slower hydrolyzing process. However, the enteric pathogens quickly died off due to over exposure to ammonia (Vinneras et al., 2008). As such, the urease-positive bacteria became the dominators in the hydrolyzing urine.

As expected, the urine hydrolysis process in run 3 was much slower compared to those in other runs. The process was lagged due to the unavailability of urease-positive bacteria from the sterile environment. Nevertheless, complete urea hydrolysis was achieved in less than 10 days. Larsen et al. (2003) indicated that 14% of urea hydrolysis activity could be due to free urease that presents in fresh human urine. In run 4, the bacteria cells and large organic molecules in the fresh urine were settled down via centrifugation. **Figure 3-2c** shows that urine hydrolysis in the pre-treated urine was much slower than in the urine contaminated with 1% BW. However, no significant effect ($p > 0.05$ for all conditions at the significance level of 0.05) on urea hydrolysis was observed either by autoclaving pretreatment of the bottles (NN versus AN, NC versus AC) or by centrifuge of the urine sample before experiments (NN versus NC, AN versus AC).

It is also important to note that, although the cross-contamination with BW has certain positive effect on urea hydrolysis process, the recycling of source-separated urine could introduce a new route for pathogens transmission (Vinneras et al., 2008). The fecal indicators total coliforms, *E. coli*, clostridia and fecal streptococci were found in various amount in urine collection tanks and some of them only have a minor decrease in several months (Hoglund et al., 1998). Dilution, pH-value and temperature affect the survivability of the microorganisms in urine. For example, urine storage below 20 °C has a high risk of containing viable viruses. High ammonia concentration (2100 mg/L, pH 8.9) is recommended for rapid inactivation of enteric pathogens (Vinneras et al., 2008). At 20 °C, the time required for 90% inactivation for rotavirus and phage is 35 and 71 days, respectively (Hoglund et al., 2002). Therefore, further risk assessment on pathogens transmission via source separation systems is needed.

3.4 Spontaneous Precipitation during Urine Hydrolysis

3.4.1 Characterization of spontaneous precipitation

Small amount of precipitates appeared at the initial stage of hydrolysis. That was likely due to the insoluble uric acid and urate in slightly acidic fresh urine, but they became

readily soluble and degradable at alkaline conditions in stale urine (Kirchmann and Pettersson, 1995). Significant precipitates were then generated in all experiments when the pH-value of urine increased above 8.0. The production of spontaneously precipitated solids was around 0.53 g (TSS) per litre of undiluted urine. Human urine generally contains abundant soluble phosphate, about 30% of which can naturally precipitate as insoluble minerals (Larsen et al., 2003). Over time, the natural precipitates tend to accumulate in the urinary pipes and may lead to serious blockage.

Figure 3-3 SEM images, X-ray diffraction patterns and FT-IR spectrum of spontaneous precipitates generated in synthetic urine (a) and in human urine (b). The standard XRD lines of pure struvite refer to JCPDS (No: 15-0762).

The spontaneous precipitates produced in synthetic and real human urine were characterized using SEM, XRD and FT-IR techniques as showed in **Figure 3-3**. Although many intermediate precipitates could be produced during urine hydrolysis process, crystalline struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) were estimated as the final dominant minerals (Larsen et al., 2003). The XRD pattern and SEM image confirmed the formation of well-crystallized struvite in urine. The

characteristic XRD peaks of the spontaneous precipitates were well matched with those of pure struvite. However, the high noise peaks and heterogeneity of SEM morphology indicated a low extent of struvite crystallization and simultaneous precipitation of other solids (e.g. hydroxyapatite, magnesium phosphate). This was probably due to the effects of complex matrices. Additionally, the strong characteristic absorption bands at around 570, 1010, and 1430 cm^{-1} of the FT-IR spectrum were corresponding to ν_4 (PO_4^{3-}) P-O bend, ν_3 (PO_4^{3-}) *antisym str* and NH_4^+ (Banks et al., 1975), respectively, further demonstrating the formation of crystalline struvite.

Many source separation systems encounter costly maintenance issues mainly due to serious blockage in the urinary piping system (SWITCH, 2008). The blockage issue was associated with spontaneous precipitation in urine. The installation of extra urine stabilization tank (as pretreatment) beneath densely-populated buildings may prevent further mineral precipitation in the downstream piping system. The struvite generated in the tank can be periodically collected for further use. However, early crystallization may inevitably take place in the inner surfaces of urinary pipes, which serves to connect source-separating toilets to the urine stabilization tank. Consequently, a suitable piping system design is needed to reduce or avoid early crystallization, such as proper pipe diameter, pipe material with smooth inner surface, piping system with less sharp turns, instant flushing after urination and regular clean-up using chemical detergents, etc.

3.4.2 Conductivity as process indicator

From the engineering perspective, it is essential to find out a suitable process indicator, which can be easily installed in an automated urine source-separation system. The indicator should be reliable, simple and inexpensive. From this point of view, multiple physicochemical parameters were studied as potential process indicator. In particular, the correlation among pH-value, conductivity and corresponding ammonium concentration within the urine hydrolysis experiments (Runs 1, 2 and 3) is shown in **Figure 3-4**. The pH-value quickly increased to 9.0 under partial urea hydrolysis while the corresponding ammonium concentration was still low (1500 mg/L). This indicated the formation of a strong ammonium-bicarbonate buffer in the partially-hydrolyzed urine. In contrast, conductivity increased gradually and linearly with the ammonium

concentration in the undiluted urine. They appeared to be proportional to each other with high correlation coefficient ($R^2 = 0.977$, $p < 0.0001$). The total ammonium concentration of the undiluted urine during ureolysis can be estimated by a regression equation, $C = 280.6 \kappa - 4778$, where C is total ammonium concentration (mg/L) and κ is the measured electrical conductivity of urine solution (mS/cm). Hence, with a conductivity value around 40 mS/cm, the final ammonium concentration will be around 6000-7000 mg/L.

Figure 3-4 Correlation of pH and electrical conductivity with corresponding ammonium concentration during urine hydrolysis process.

In case of diluted urine, the conductivity and corresponding ammonium concentration also showed a very good correlation. The precise correlation between these two factors was likely due to the continuous conversion of organic urea into both ammonium and carbonate, leading to a gradual increase of ion strength and ultimately the solution's conductivity. The fluctuation was probably due to the conductivity measurement without temperature adjustment, ammonia volatilization, CO_2 loss, phosphate precipitation and other potential chemical and biological reactions. In overall, the results indicate that conductivity can act as a better indicator for urine hydrolysis process compared to pH-value. Other studies have also reported conductivity as a time-saving and cost-efficient indicator for phosphate monitoring in wastewater treatment (Maurer and Gujer, 1995; Udert et al., 2011). Thus, conductivity seems to be a simple but more reliable indicator to monitor the progress of urine hydrolysis process.

3.5 Odor Emissions during Urine Hydrolysis

3.5.1 Elevated headspace pressure

Figure 3-5 presents the typical changes of relative headspace pressure during regular and enhanced urine hydrolysis processes (at 34 °C). For hydrolysis enhancement, 2 mL stale urine was added. The gases (CO_2 and NH_3) released during the initial step of the process led to an increase in headspace pressure in both experiments. However, during the enhanced hydrolysis process, the pressure suddenly dropped to negative value as a result of complete urea hydrolysis (final pH = 9.3). Both CO_2 and NH_3 could be released from the alkaline urine solution during ureolysis process, supposedly leading to an elevated headspace pressure. In fact, the system arrived at a negative headspace pressure (about -180 hPa) at the end of ureolysis process. This was most likely due to the O_2 consumption by microbial activity rather than the CO_2 absorption by the alkalized urine. Comparing the two experiments, it seems that the regular experiment without enhancement underwent a much slower hydrolysis process (final pH = 7.1) because of the absence of initial bacteria loading, meanwhile, its headspace pressure remained at a level of more than 50 hPa (for 7 days). The positive pressure compared to the ambient pressure indicated a high potential of gas emission from source-separated urine during hydrolysis. It is a fact that a strong unpleasant odor appeared especially during the first few days of urine storage. Apparently, a slower urine hydrolysis process may induce more odor emissions.

Figure 3-5 Typical variation of relative pressure in headspace during regular (with final pH 7.1) and enhanced (with final pH 9.3) urine hydrolysis.

3.5.2 Inorganic gas emissions

Table 3-1 shows the composition of inorganic headspace gases monitored at different stages of urine hydrolysis at 34 °C. Urea gradually decomposes into CO₂ and NH₃ during urine storage. Since the headspace of Oxitop bottles were rinsed with N₂ before experiments and isolated as anaerobic spaces, the data presented in **Table 3-1** showed both CO₂ and NH₃ emissions from urine solution, which led to a noticeable increase of headspace pressure (above 200 hPa). Due to the absence of oxygen in the headspace, the increasing pressure could be only due to the gas emissions from the hydrolyzing urine. After three days, the CO₂ generated together with the existing N₂ contributed about 92% (v/v) of the total headspace gas. The rest was mainly due to NH₃ emission. When the system reached a high relative pressure (~ 200 hPa), the corresponding concentration of NH₃ in the headspace measured was 9520 mg/m³, which was much higher than the theoretical equilibrium concentration of 2300 mg/m³ (at standard temperature and pressure), exceeding the odor threshold (Udert et al., 2006).

Table 3-1 Variation of volumetric proportion of headspace gases during urine hydrolysis at 34 °C.

	Day 1	Day 3	Day 5	Day 7	Day 9	Control ^a (pH 9.3)
Pressure ^b (hPa)	0	114	238	197	135	-180
N ₂	100%	81.6 %	77.1 %	76.4 %	81.0 %	85.0 %
CO ₂	0	10.8 %	15.3 %	16.5 %	10.4 %	1.0 %
NH ₃ ^c	0	7.5 %	7.6 %	7.1 %	8.6 %	14.0 %

Notes: ^a control experiment conducted in bottle without N₂ purging, the final relative pressure in headspace was negative primarily due to microbial oxygen consumption; ^b relative pressure in headspace; ^c assume that the headspace gases mainly consist of N₂, CO₂ and NH₃.

Theoretically, the volatilization of CO₂ and NH₃ (as well as other trace volatile compounds) from urine is mainly dependent on two thermodynamic equilibriums: species dissociation equilibrium in aqueous solution and gas/liquid equilibrium at the water surface (Ni, 1999; Srinath and Loehr, 1974). Both equilibriums are significantly affected by temperature and pH conditions. At an elevated pH-value, the accumulated CO₂ in the headspace will be absorbed by the alkaline solution as bicarbonate and carbonate (HCO₃⁻, $\alpha_1=0.939$ and $\alpha_2=0.059$ at pH = 9.1 (Benjamin, 2002)), resulting in a decreasing amount of CO₂ in the headspace (Day 9 in Table 3-1). Contrarily, the

equilibrium NH_3 concentration in headspace will increase due to a higher distribution coefficient of free ammonia in stale urine ($\alpha_1=0.420$ at $\text{pH}=9.1$).

The gas composition analysis also shows that there was no H_2S , H_2 or CH_4 emission from source-separated urine during nine-day's storage, although urine contains high concentration of sulfate. It is also important to note that humidity and other volatile organic compounds (VOCs) generated can also contribute to the elevated headspace pressure to a certain amount. Even though NH_3 was the major odorous compound released during urine storage, its concentration calculated in headspace might be slightly overestimated. Other trace organic compounds can be of critical importance in view of their low odor thresholds, such as methyl mercaptan, ethyl mercaptan, methylamine and butyric acid (Vincent and Hobson, 1998).

3.5.3 Organic odor emissions

The production of VFA during urine hydrolysis is presented in **Figure 3-6**. The total amount of VFA gradually increased until day 7 reflecting intense microbial activity during urine hydrolysis process. Acetic, propanoic and butyric acids were the three most abundant VFA fractions in urine at highest concentrations 54.3, 6.0 and 2.3 mmol/L, respectively. The valeric acid was consumed during the entire process, while other compounds with longer carbon chain remained around 0.2 mmol/L. However, it required more than one month to complete the urine hydrolysis process since this experiment was done in the absence of oxygen which led to the unavailability of initial urease-positive bacteria. Also, it was noticeable that most VFA produced during the ureolysis process were substantially degraded in a longer period (2~4 months).

The acid dissociation constants of VFA compounds are generally low. For instance, the pK_a of acetic acid is 4.75 (Benjamin, 2002). The VFA compounds produced during ureolysis process were mainly remained in their ionized forms. Nevertheless, the volatilization of VFA, especially with short carbon chains, was inevitable because of their high concentrations in urine and extremely low odor thresholds. The persistent and strong odor generated during the slow ureolysis process was also attributed to other odorous organic compounds at trace levels. Moreover, a significant reduction of COD

(about 30%) was observed along with the ureolysis process. Since urea or its decomposed products (i.e. CO₂ and NH₃) does not contribute to COD, the COD reduction was likely due to the degradation of organic compounds by the microbes growing in urine. Fresh urine contains high concentration of creatinine, amino acids, uric acid and other organic compounds (Udert et al., 2006). In anoxic environment, these compounds can be degraded into soluble organic substances (e.g. amino acids and fatty acids) (Madsen et al., 2011). However, the main drawback is the high ammonium concentration simultaneously produced during urine hydrolysis, which can significantly inhibit the anaerobic digestion.

Figure 3-6 Production and degradation of volatile fatty acids during urine hydrolysis.

Generally, odor is generated from different stages of conventional end-of-pipe wastewater treatment plants during the treatment of domestic wastewater (Capelli et al., 2009). The source separation systems produce highly-concentrated wastewater streams (i.e. yellow water, brown water and grey water) posing higher potential of odor emissions. This becomes a critical concern and even constrains the application of source separation systems in urban areas. It is, therefore, vital to systematically monitor and control the odor emissions from a source separation system. Additional attention should be paid to other trace organic odorants generated during the downstream treatment of source-separated urine.

A further investigation on these odorous compounds was conducted regarding to their occurrence, composition, concentration and emission properties during urine hydrolysis based on thermal-desorption cold-trap and gas chromatography-mass spectrometry (TCT–GC/MS) (article in preparation). A total of 60 volatile organic compounds were identified in the urine headspace, including 17 ketones, 13 sulfur-containing compounds, and 11 aromatic hydrocarbons. Six out of the gaseous compounds were quantified as the main odorous compounds generated from human urine in consideration of their relatively high concentration in urine headspace and extremely low odor thresholds, including dimethyl disulfide, allyl methyl sulfide, 4-heptanone, allyl methyl disulfide, methyl propyl disulphide, and menthol. The headspace concentrations during urine hydrolysis reached to hundreds of times higher than their respective odor thresholds.

3.6 Nutrient Recovery from Source-Separated Urine

3.6.1 Seawater-induced struvite production

Figure 3-7 presents the effect of Mg/P molar ratio on nutrient recovery from hydrolyzed urine and magnesium consumption using MgCl_2 and seawater as magnesium sources. The nitrogen recovery efficiency in both cases was only about 7% due to the high ammonium concentration in hydrolyzed urine. Mg/P molar ratio critically affected the struvite precipitation process. The modeling result shows that the P-recovery efficiency increased from 50% to 98% when Mg/P molar ratio increased from 0.5:1 to 1.1:1. Complete P-recovery could be achieved only when the Mg/P molar ratio ascended beyond 1.1:1. At the same time, the consumption of magnesium was nearly 100%, but became over-dosed when Mg/P molar ratio was more than 1.1:1. This signifies that at low Mg/P molar ratio, the added magnesium was completely utilized for struvite precipitation under alkaline condition, resulting in a fast decrease in phosphorus concentration in liquid phase. The specific Mg/P molar ratio at 1.1:1 was considered as the optimal parameter for complete P-recovery from hydrolyzed urine.

There was a high agreement between experimental results and modeling in terms of N, P-recovery efficiencies. The demand of magnesium for complete P-recovery was similar using both magnesium sources. As such, seawater could serve as an ideal

alternative for commercial MgCl_2 for nutrient recovery from urine. When dosing seawater at excessive Mg/P molar ratios (i.e. $> 1.1:1$), however, the experimental Mg consumption was relatively higher than the modeling results although they fitted well at the low range of Mg/P ratio. This indicated that more magnesium was settled down in the experiments probably due to the co-precipitation of other Mg-containing minerals that were not included in the modeling. However, such experiment-modeling disagreement was not observed when real hydrolyzed urine was tested.

Figure 3-7 Effect of Mg/P molar ratio on recovery efficiencies of phosphate, ammonium, calcium and magnesium from synthetic urine (pH 9.3) by adding MgCl_2 (a) and seawater (b) as magnesium sources.

Calcium co-precipitation as calcite is likely to occur during urine nutrient recovery since seawater contains high concentration of calcium, and would affect the quality of final struvite product. The calcium recovery efficiency were almost 100% at any Mg/P molar ratios based on modeling, whereas around 80% for synthetic urine in the experiments. This difference of can be explained: a) In the model, all reactions were in the equilibrium state, whereas the actual state might not be at equilibrium; b) the reactions rate of calcium might not be fast enough due to the low calcium concentration and the presence of magnesium, which retarded the precipitation rate of calcite as the main calcium precipitate.

Figure 3-8 Effect of pH on recovery efficiencies of phosphate, ammonium, and magnesium from synthetic urine (pH 9.3) by adding MgCl_2 (a) and seawater (b) as magnesium sources.

Figure 3-8 shows the effect of pH on the nutrient recovery efficiency by adding MgCl_2 and seawater to synthetic urine at the optimal Mg/P molar ratio of 1.1. The modeling shows that the N, P-recovery and magnesium consumption increase quickly when pH increases from 6 to 8, and remained stable at pH 8–10.5. When pH continues increasing, the N, P-recovery decrease quickly but with more magnesium consumption. The result indicated that lower pH-values were unfavorable for struvite precipitation, while higher pH-values lead to the precipitation of brucite which was simulated as a major competitor of struvite for magnesium in the urine solution. This explains the high magnesium consumption but low nutrient recovery at high pH values. For both magnesium sources, nutrient recovery and magnesium consumption in the experiment matched well with the predicted results. The results indicated that the optimum pH for struvite precipitation should be in the range of 8~10.5 in order to achieve high nutrient recovery and struvite purity. Since the hydrolyzed urine possesses a strong buffering capacity with a pH-value around 9.3, complete P-recovery can be effectively achieved without pH adjustment.

As predicted by the chemical modeling, about 1/3 of the initial phosphorous would be settled down mainly as CaHPO_4 in the fresh urine (pH ~6.5) dosing with seawater. Neither struvite nor other precipitates would be generated under acidic-neutral conditions. Partial or complete urea hydrolysis is required to achieve effective P-

recovery from urine. In case of hydrolyzed urine without dilution with flushing water, nearly all phosphorus (with 90.4% Mg and 3.6% N) could be harvested with a final struvite purity of about 91.8%. Meanwhile, nearly all calcium derived from seawater was also settled down as CaCO_3 impurity in the solid obtained. Theoretically, about 2.184 kg struvite could be recovered per cubic meters of undiluted and hydrolyzed urine using 0.24 m³ seawater. In case of diluted urine, the productivity of struvite was predicted to be inversely proportional to the dilution ratio. However, it is worth noting that the recovery of all elements (P, N, Mg, Ca) decreased with an elevated dilution ratio, which probably due to the less tendency of various ions toward supersaturation in the diluted solutions. Nevertheless, the purity of struvite in the final solid products kept above 91% at all dilution ratios.

Figure 3-9 shows the SEM images of the precipitates obtained by adding MgCl_2 and seawater to synthetic stale urine. Clear feather-shaped struvite was formed in the solution when using MgCl_2 . Further EDX analysis showed that the main compositions were Mg (10.6%), P (11.3%), O (59.1%) and C (18.5%) tagged with a relative calcium concentration of 0.4%. By adding seawater, particulate impurities was also formed on the surface of the feather-shaped struvite crystals as showed in **Figure 3-9b**. Based on modeling, these particles could be $(\text{Mg,Ca})\text{CO}_3$ and their formation was related with the Mg/Ca ratio and supersaturation situation in solution. Further EDX analysis on the particulate impurities showed that the compositions were Mg 5.0%, P 3.5%, O 65.1% and C 20.4% with a relatively higher calcium concentration at 5.5%. This difference in composition confirms the presence of two distinct entities. In combination with FT-IR and XRD analysis, the feather-shaped crystal was confirmed as struvite and the small pariticle was $(\text{Mg,Ca})\text{CO}_3$. By comparison, the morphology of the precipitates formed in real urine was more coffin-shaped, and the precipitates with addition of seawater also had some fine crystals (mainly as calcite) on their surfaces (Liu et al., 2013). In fact, struvite crystals can grow in in different morphologies such as prismatic type, pyramid type, coffin shaped, feather shaped, and needle type, which are strongly dependent on growth conditions (Chauhan and Joshi, 2013; Le Corre et al., 2005).

Figure 3-9 SEM images of precipitate harvested from synthetic urine (pH 9.3) by adding MgCl_2 (a) and seawater (b) as magnesium source.

This study confirmed that the initial calcium concentration affected struvite purity. When using seawater or desalination brine (with Mg/Ca ratio of about 3:1) as additional magnesium sources, calcium is inevitably brought into the struvite reactor and reacts with carbonate in urine solution to form calcite or Mg-calcite. Overall, struvite comprised more than 95% of the total solids formed under all conditions, which still make the precipitate mixture beneficial in the actual fertilizer application. Although the impurities, mainly the calcite or Mg-calcite, might affect the fertilizing efficiency of the precipitates comparing to the struvite harvested by adding MgCl_2 , the final product is still considered economical and beneficial given the low cost of raw materials. In fact, the calcite or Mg-calcite impurities can be used as soil conditioner in acidic soils.

The struvite formed in the process was readily settled down in the reactor. The supernatant turned out to be clear after one-hour sedimentation and the total solids remained unchanged. The addition of either cationic or anionic flocculants was unlikely to improve struvite recovery during this process (Udert et al., 2011). After struvite precipitation, additional liquid-solid separation process is needed to harvest the struvite from the remaining urine solution. Filtration through nylon bag might be an economic way to effectively separate struvite crystals from urine effluent, depending on the average struvite particle size ($\sim 20 \times 100 \mu\text{m}$) and the pore size of the nylon filter (Udert et al., 2011). The continuous build-up of a filter cake on nylon surface could significantly enhance the struvite recovery efficiency. After liquid-solid separation, the struvite slurry can be further dried out (naturally or mechanically) to remove the surplus

moisture. The final struvite product can be made in powder or granular form for further use in agriculture/horticulture as slow-releasing fertilizer.

3.6.2 Ammonia stripping

Figure 3-10 illustrated the effects of pH, air flow rate, temperature, and dilution ratio on ammonia removal efficiency. The removal efficiency was significantly improved when pH increased from 9.3 to 10, but no further improvement was observed above pH 10. Higher pH facilitates the transition of ammonium ion to free ammonia and improve ammonia removal from urine. Only 58.6% of total ammonium existed as free ammonia in hydrolyzed urine with no pH adjustment (pH ~9.3), while free ammonia became the dominant fraction when pH was adjusted above 10. Besides, increasing air flow rate remarkably enhanced the ammonia removal efficiency and shortened the time required for complete ammonia removal. For example, the time needed to remove ~95% of ammonia was reduced from 24 to 4 h when increasing the air flow rate from 2 to 10 L/min·L. This was likely because a higher air flow rate could reduce the thickness of the boundary layer that introduces the resistance of ammonia mass transfer in the urine and therefore reduces the mass transfer resistance.

As showed in **Figure 3-10c**, temperature had great influence on ammonia stripping. More than 95 % of ammonia could be removed within 6 h at 323 K, but only 33% at room temperature for the same time. This was because higher temperatures increased the molecular diffusion coefficient of ammonia in liquid and its Henry's law constant, resulting in the increase of total mass transfer coefficient. Higher temperatures also promoted affected the dissociation of water and ammonia, and further increased the fraction of free ammonia in liquid. However, urine dilution has no significant influence on ammonia removal efficiency as showed in **Figure 3-10d**. Therefore, when the major operational conditions (pH, air flow rate and temperature) were determined, the total mass transfer coefficient was almost independent of ammonia concentration and dilution ratio. An amended two film model was developed to theoretically explain the effects of multiple operational parameters on the ammonia removal efficiency.

Figure 3-10 (a) Effect of pH on ammonia removal efficiency (Experimental conditions: no dilution, air flow rate = 2 L/min·L, temperature = 323 K). (b) Effect of air flow rate on ammonia removal efficiency (no dilution, pH = 10, temperature = 323 K). (c) Effect of temperature on ammonia removal efficiency (no dilution, pH = 10, air flow rate = 4 L/min·L). (d) Effect of urine dilution ratio on ammonia removal efficiency (pH = 10, air flow rate = 2 L/min·L, temperature = 323 K).

Unit operating cost for 80% ammonia removal efficiency was evaluated under various operation conditions. It was determined that the minimum unit operating cost was achieved at air flow rate 14 L/min·L, temperature 323 K and operation time 2.2 h without pH adjustment. Operating the stripping process at higher air flow rates and temperatures could dramatically reduce the operation time and decrease the unit operating cost. Although high pH could shorten the operation time, the additional chemical cost input compromised this advantage. This study demonstrated that air stripping is effective for ammonia recovery from source-separated urine and the experimental parameters provided certain guidelines for the development of N-recovery process in industrial-scale. In combination with struvite recovery, the majority of

nutrients in source-separated urine could be recovered as valuable fertilizers (struvite and ammonia sulfate). The treated effluent can be safely discharged to sewage system, or further utilized for agricultural purposes (e.g. wetlands, hydroponic plant cultivation) in consideration of the remaining ammonium, potassium, COD and other components.

3.7 Chapter Conclusions

In the ascent of global energy crisis, water shortage and resource scarcity, innovative sanitation system based on the source separation concept has been intensively advocated to manage domestic wastewater in a more sustainable way. The present study evaluated the feasibility and potential limitations of applying urine source separation in a densely-populated tropical urban setting. A significantly enhanced ureolysis process could be achieved under optimized conditions (34~40°C, stale/fresh urine ratios >40%, slight fecal contamination and low dilution), and the entire process can be accurately monitored by measuring conductivity as a timesaving and cost-efficient indicator. The results demonstrated that an effective and automated urine source separation system could be achieved. Urine-stabilization tank beneath high-rise buildings could be adopted as a collector of spontaneous mineral precipitation. This design prevents pipe clogging for an integrated source-separating sanitation system. On the other hand, observations on the initial release of CO₂ and NH₃ indicated a strong potential of odor emission during urine storage. The production of high concentrations of volatile fatty acids (mainly acetic, propanoic and butyric acids) and various odorous organic compounds also contributed to the odor issues. To conclude, an enhanced ureolysis process could attenuate the odor emissions.

Seawater can be used as a cost-effective magnesium source to recover phosphorus from hydrolyzed urine. Feather and coffin shape struvite crystals were obtained from synthetic and real urine, respectively. Structural characterization of the precipitates confirmed that crystallized struvite was the main product. However, co-precipitates magnesium calcite and calcite were observed when seawater was added into synthetic and real urine, respectively. The presence of calcium in the magnesium sources could compromise struvite purity. Higher struvite purity could be obtained with higher Mg/Ca

ratio in the magnesium source. Besides, air stripping process can be used to effectively recover ammonia from source-separated urine and the experimental parameters provided certain guidelines for the development of N-recovery process in industrial-scale. In combination with struvite recovery, the majority of nutrients in source-separated urine could be recovered as valuable fertilizers (struvite and ammonia sulfate).

CHAPTER 4 MINING NUTRIENTS FROM URBAN SOURCE- SEPARATED URINE BY FORWARD OSMOSIS DEWATERING

4.1 Introduction

In recent years, osmotically driven membrane processes, including both forward osmosis (FO) and pressure-retarded osmosis (PRO), have received increasing attention for applications in wastewater treatment and seawater desalination (Cath et al., 2006; Zhao et al., 2012). FO involves the water transport across a semipermeable membrane from a low-osmotic-pressure feed solution (FS) to a high osmotic-pressure draw solution (DS) in the absence of an externally applied hydraulic pressure. The osmotically driven membrane process has been proven to have appealing advantages over various pressure-driven membrane processes such as NF and RO in terms of lower fouling potential and energy consumption (Zhao et al., 2012). FO is especially attractive for applications when the DS is readily available and needs not to be regenerated. Seawater, as an abundant resource for coastal regions, as well as the rejected brine from RO desalination plants (both in inland and coastal areas) can be potentially used as low-cost draw solutions.

As sketched out in **Figure 4-1**, an FO dewatering process can be incorporated into a decentralized source-separating sanitation system. The integral system promises to control domestic nutrients and various excreta-derived micropollutants at source rather than the conventional end-of-pipe practice (Elorri et al., 2012; Zhang et al., 2013a). In particular, the urine stream is collected for complete nutrient harvesting, while brown water is anaerobically digested for biogas production. Inspired by this conception, this study aims to use the osmotically driven FO process for both urine volume reduction and complete nutrient recovery, using seawater, desalination brine or salty industrial effluents as low-cost draw solutions.

Figure 4-1 Schematic diagram of a decentralized source-separating sanitation system designed to covert domestic waste into resources (nutrients and biogas) and to minimize any environmental discharge. YW and BW represent source-separated yellow water and brown water, respectively. The final urine product can be concentrated urine or N, P-enriched bio-fertilizers (i.e. struvite and ammonium sulfate). The AD effluent can also be treated by FO process for extra nutrient recovery with the COD residue fed back to the AD system as carbon sources.

The overall objective of the present study is to investigate the technical feasibility and cost-effectiveness of using a FO dewatering process for nutrient recovery from source-separated urine. In particular, there are three targets: 1) to compare FO with RO in terms of water flux and nutrient rejection under different operation conditions; 2) to investigate the longer-duration FO performance in terms of membrane fouling and nutrient concentration efficiency; 3) to propose an novel FO-based integral wastewater management system based on source separation strategies with the pros and cons clearly evaluated. This work explores new applications of osmotically driven FO processes, and the results demonstrate novel technical viability for recycling domestic nutrients and its underlying socio-economic and environmental benefits for the sustainable development of urban environments.

4.2 Materials and methods

4.2.1 Bench-scale FO experiments

Three types of FS were tested in the bench-scale FO process, namely, synthetic fresh urine (FU), hydrolyzed stale urine (SU) and treated stale urine (TSU). After excretion, FU undergoes rapid urea hydrolysis, enzymatically converting urea into ammonia and CO₂. The hydrolysis results in a higher ionic strength with an elevated pH of the SU, which triggers spontaneous struvite precipitation (Lee et al., 2013). FU was prepared with Griffith et al. (1976)'s recipe (see **Table 2-1**), while SU was simply prepared by replacing urea in the recipe with an equivalent amount of ammonium carbonate (Merck). Both urine solutions were kept overnight for sufficient ageing and natural precipitation. TSU was the residual urine solution in which the majority of phosphate (~100%) and ammonia-nitrogen (~80%) had been harvested by engaging seawater-induced struvite crystallization with subsequent ammonia stripping (Liu et al., 2013; Liu et al., 2014). Feed solution with dilution factors of 1, 2, and 5 were also investigated in consideration of different designs of urine-diverting toilets (or no-mix toilets, e.g. Roediger vacuum, Dubbletten, and Wostman-DS toilets, etc.) giving rise to non-diluted or low-diluted urine. The major characteristics of synthetic feed solutions are presented in **Tables 4-1**. Draw solutions were prepared by dissolving reagent-grade sodium chloride (Merck) into deionized water at different concentrations: 0.5 M (as seawater), 1 M (as seawater RO desalination brine) and 2 M (as brine or salty industrial effluent).

Table 4-1 Main characteristics of synthetic urine as FO feed solution (g/L).

	pH	Osmotic pressure ^a	Urea-N	NH ₃ -N	PO ₄ -P	K ⁺	Mg ²⁺	Ca ²⁺	Na ⁺	Cl ⁻	SO ₄ ²⁻
FU	7.0	18.1 bar	11.7	0.26	0.64	1.64	0.08	0.18	2.72	4.75	1.55
SU	9.3	30.7 bar	0	11.9	0.45	1.64	0	0	2.72	4.75	1.55
TSU	9.0	14.1 bar	0	1.34	0	1.37	0	0	4.59	7.92	1.81

Note: ^a predictive osmotic pressure based on the synthetic urine compositions.

The commercial flat-sheet FO membrane was obtained from Hydration Technology Innovations (HTI, Albany, OR). It is made of cellulose triacetate (CTA) embedded with a polyester woven mesh. The CTA layer is relatively hydrophilic and negatively charged under neutral conditions (pH~7.0) (Jin et al., 2012a; Linares et al., 2011). All membrane coupons were soaked with deionized water prior to batch experiments. Furthermore, both virgin and used membranes were characterized by field emission scanning electron microscopy (FE-SEM) coupled with energy dispersive x-ray detection (JSM-7600F, JEOL). Before SEM characterization, the FO membrane was

dried at room temperature and sputter coated with a thin layer of platinum using an Emitech SC7620 sputter coater (Quorum, UK).

Bench-scale FO experiments were carried out to evaluate the viability of employing FO process for urine treatment. The cross-flow FO setup used in this study was similar to the one reported previously (Jin et al., 2011; Tang et al., 2010). The FO membrane coupon was placed in the middle of a cross-flow FO membrane cell with symmetric flow channels on both half cells. The effective membrane filtration area was 42 cm² (10.1 cm × 4.15 cm). Mesh spacers were inserted in both flow channels for the purpose of supporting the membrane as well as enhancing the mixing and turbulence of the solutions near the membrane surface. Two digital gear pumps (Cole-Parmer, US) were applied to recirculate the FS and DS independently at a cross flow velocity of 20 cm/s. A 4-L FS reservoir was placed on an analytical balance (UX6200H, Shimadzu) interfaced with a computer-based data logging system (LabVIEW). The weight decrease of the FS with time was recorded to calculate water flux through the semipermeable membrane. During each experiment, 5 mL of samples were collected from the DS at predetermined time intervals to determine the nutrient flux and rejection (refer to subsequent sections for more details). Both short-term (~ 3 hr) and longer-duration (~ 70–100 hr) FO experiments were performed to investigate the water flux and the nutrient rejection by FO membranes. All the FO experiments were conducted at room temperature (~ 23±1°C).

The FO tests were conducted in the active-layer-facing-feed-solution (AL-FS) orientation due to better flux stability with lower fouling tendency in this orientation (Tang et al., 2010; Zhao et al., 2011). Additional FO tests were conducted to verify the inferior FO performance in the alternative active-layer-facing-draw-solution (AL-DS) configuration. As illustrated in **Figure 4-2**, the initial fluxes were similar for both orientations, which can be roughly predicted by $\tilde{J}_w \approx K_{m,s} \ln(\pi_d / \pi_f)$ as simplified from eq 4-2. However, the water flux obtained in AL-FS was more stable over time, while the flux in AL-DS was significantly reduced over the testing period. In fact, this observation was opposite to earlier studies (Gray et al., 2006; Xie et al., 2012b), where higher fluxes were measured in AL-DS. The inferior flux in AL-DS observed here was

probably associated to membrane scaling. The porous support layer facing urine solution is also prone to internal pore clogging (Wang et al., 2010). A recent study also observed a more stable and higher water flux in AL-FS than in the alternative orientation when dealing with feed solutions that have high fouling or scaling propensity (Zhao et al., 2011). Therefore, the FO process was operated in AL-FS for urine treatment throughout all experiments.

Figure 4-2 Effect of membrane orientation on water flux performance using fresh urine with no dilution or 1:2 dilution as the feed solution. FO conditions: cross-flow velocity 20 cm/s, 2M NaCl as draw solution, temperature $23 \pm 1^\circ\text{C}$.

4.2.2 Analytical methods

Urea concentration was determined with Fearon's reaction adjusted from Coulombe and Favreau (1963)'s and Marsh et al. (1965)'s methods. The color reagent used for urea determination was composed of 30% (v/v) concentrated sulfuric acid, 0.1% (w/v) diacetylmonoxime, 0.005% (w/v) thiosemicarbazide, and small amount of iron (III) chloride. 3-mL aliquot of color reagent was mixed with 0.2 mL of urea sample, heated in boiling water for 20 min and then cooled down in tap water in 5 min. The red color complex formed was then measured at 520 nm with a Cary 50 UV-Vis spectrophotometer (Agilent, US). As showed in **Figure 4-3**, linear calibration curve was made over a wide range of urea concentrations from 1 – 100 mg/L ($R^2=0.999$).

Figure 4-3 Calibration curve for spectrophotometric determination of urea in water samples.

Furthermore, concentrations of total ammonium ($\text{NH}_3\text{-N}$) and ortho-phosphate (PO_4) were colorimetrically measured with the salicylate method (Hach method 10205) and the ascorbic acid method (Hach method 10209), respectively, using a DR 2800 spectrophotometer and corresponding Hach's reagents (Hach, USA). Potassium, magnesium and calcium were measured with an inductively coupled plasma optical emission spectrometer (Spectro Analytical Instruments, Germany). Proper sample dilution was made prior to analysis to minimize the interference from high salinity. At least duplicate measurements were engaged throughout the whole study for quality control purposes. In addition, electrical conductivity (EC) and pH-value were measured with a D-54 pH/conductivity meter (Horiba, Japan).

4.2.3 Calculations of water flux and nutrient rejection

The experimental water flux through the FO membrane was determined based on the weight decrease of the FS over time. Meanwhile, the actual nutrient concentration in permeate is given by the ratio of nutrient flux over the water flux, taking into account the permeate dilution by the DS. Thus, the experimental nutrient rejection was determined based on eq 4-1 (Jin et al., 2011).

$$R = 1 - \frac{J_n}{J_w \cdot C_{f,n}} \quad \dots\dots\dots (4-1)$$

where J_n and J_w are the nutrient flux and water flux obtained from FO experiments, respectively; $C_{f,n}$ is the nutrient concentration in bulk FS. Aliquots (5 mL) of samples

were collected from DS at predetermined time intervals to measure the nutrient concentrations, then J_n was determined from the regression slope of the total nutrient permeate across unit membrane (g/m^2) plotted versus time.

The FO membrane process operated in AL-FS usually suffers from severe dilutive internal concentration polarization (ICP) within its porous support layer. Consequently, the effective driving force is much smaller than the apparent osmotic pressure differential between feed and draw solutions (Jin et al., 2011; Tang et al., 2010). Assuming a pseudo-linear osmotic pressure–concentration (π – C) relationship, the water flux across the FO membrane in AL-FS can be simulated by eq 4-2 based on the classical solution-diffusion theory (Loeb et al., 1997; Tang et al., 2010).

$$\tilde{J}_w = K_{m,s} \ln \left(\frac{A\pi_d + B_s}{A\pi_f + \tilde{J}_w + B_s} \right) \dots\dots\dots (4-2)$$

where A and B_s are the water permeability and salt permeability of the membrane, respectively; π_f and π_d are the osmotic pressure of FS and DS, respectively. The latter terms were calculated by OLI Stream Analyzer software (Morris Plains, NJ) based on synthetic urine compositions, while A and B_s values were determined in RO mode (refer to subsequent section). $K_{m,s}$ represents the mass transfer coefficient of salt within the membrane porous layer, which is the ratio of the salt diffusion coefficient over the membrane structural parameter. In the current study, $K_{m,s}$ was determined by fitting eq 4-2 with the measured water flux obtained from FO experiments using TSU as feed.

On the other hand, nutrient transport through the FO membrane in AL-FS does not experience significant ICP in the porous layer since nutrients passing through the active layer can be readily carried away from the support layer by the water flux. The nutrient concentration at the interface of membrane active layer and porous layer can be expressed as, $c_i = J_n/J_w$. As such, the nutrient flux across the FO membrane in the AL-FS orientation could be predicted as eq 4-3 (Jin et al., 2011).

$$\tilde{J}_n = B_n(c_{f,n} - c_i) = \frac{B_n}{1 + \frac{B_n}{J_w}} c_{f,n} \dots\dots\dots (4-3)$$

Integrating eq 4-1 with eq 4-3, the nutrient rejection in FO process can be simulated by

$$\tilde{R} = \frac{J_w}{J_w + B_n} \dots\dots\dots (4-4)$$

where B_n is the nutrient permeability determined by separate RO experiments; J_w is the above-mentioned experimental water flux.

The intrinsic membrane separation properties (water permeability A , and solute permeability B) were determined in two independent cross-flow RO units in parallel. The applied hydraulic pressure was fixed at five levels ranging from 50–250 psi. The cross-flow velocity was fixed at 20 cm/s. After filtration through a 0.45 μm membrane, both FS and SU were diluted by a factor of 20 and tested as RO feed waters (20 L). Feed water samples and permeate were collected at specified intervals for water permeability calculations and further chemical analysis. The nutrient rejection in the RO process was readily calculated as the ratio of nutrient concentration in permeate and that in feed water. Furthermore, additional RO experiments were run to determine the salt rejection using a 10 mmol/L NaCl feed solution based on EC measurements. With negligible external CP effects in RO experiments (polarization modulus < 1.06 for all cases according to She et al. (2013)’s method), both salt permeability (B_s) and nutrient permeability (B_n) were determined by eq 4-5 based on the corresponding water flux and RO rejection data (Tang et al., 2010).

$$B = \frac{J_w(1-R)}{R} \quad (\text{in RO mode}) \dots\dots\dots (4-5)$$

4.3 Membrane Characterization

Cross-sectional structure and membrane surfaces were observed by FE–SEM and are shown in Section 6.3 of Chapter 6. In brief, the commercial FO membrane possesses a thin CTA selective layer ($\sim 20 \mu\text{m}$) supported by embedded polyester woven mesh. The selective layer consists of an ultrathin dense rejection layer and a relatively thin porous layer for lower internal CP. The active surface of the FO membrane is relatively smooth without observable pores at low accelerating voltages (5 keV). Other characteristics of the FO membrane have been reported elsewhere (Cath et al., 2006; Tang et al., 2010; Wei et al., 2011).

Figure 4-4 Water flux and nutrient rejection as a function of effective trans-membrane pressure in RO experiments using diluted FU (a) and SU (b) by a factor of 20 as feed waters. The applied hydraulic pressure was changed from 50–250 psi in two parallel RO cells. Other operational conditions: cross-flow velocity 20 cm/s, temperature $23 \pm 1^\circ\text{C}$, membrane orientation AL-FS.

Figure 4-4 illustrates the water flux and nutrient rejection in RO experiments as a function of trans-membrane pressure. The water permeability (A value) was then determined from the regression slope of water flux against hydraulic pressure. As shown in **Table 4-2**, similar water permeability coefficients were obtained for both FU and SU used as the feed water. These A values, however, were approximately 30% higher than those obtained from RO experiments using 10mM NaCl feed water. The distinction may result from the complicated ionic compositions and pH present in urine (Xie et al., 2012b). Future work may be devoted to understand the influences of ionic strength and pH of feed water on water flux performance in the RO process.

The nutrient permeability (B_n value) was also determined by eq 4-5 on the basis of rejection data. **Table 4-2** summarizes the B_n values for various nutrient elements, which follows the order: $B_{urea} > B_{NH3-N} > B_K > B_s > B_{PO4}$. The salt permeability B_s was determined to be 1.70×10^{-7} m/s, and was identical to our previous study (Tang et al., 2010). The relatively high urea permeability indicated inferior rejection to neutral urea molecules by the CTA-FO membrane. Yong et al. studied the reverse solute flux of several neutral draw solutes across similar CTA-based FO membranes and also observed a comparably high permeability coefficient for urea (Yong et al., 2012a). Lee and Lueptow observed lower urea rejection than other ionic nitrogen compounds by typical low-pressure reverse osmosis membranes (Lee and Lueptow, 2001a). By contrast, the charged components had much lower permeability coefficients by one to

two orders of magnitude. The distinction indicates the electrostatic interaction (also called as *Gibbs–Donnan* effect) as an important rejection mechanism on top of the size-dependent steric hindrance effect. The synergic effects of different rejection mechanisms can essentially influence the solute permeability properties.

Table 4-2 Intrinsic separation parameters of the FO membrane tested in RO mode.

Feed solution	Fresh urine ^a	Stale urine ^a	NaCl (10 mmol/L)
pH	7.0~7.5	9.0~9.4	7.0
Pure water (A), m/s·Pa	$2.80 \pm 0.27 \times 10^{-12}$	$2.62 \pm 0.10 \times 10^{-12}$	$2.09 \pm 0.07 \times 10^{-12}$
NaCl (B_s), m/s	– ^b	–	$1.70 \pm 0.52 \times 10^{-7}$
Urea (B_{urea}), m/s	$1.12 \pm 0.24 \times 10^{-5}$	–	–
NH ₃ -N (B_{NH3-N}), m/s	$0.93 \pm 0.34 \times 10^{-6}$	$1.21 \pm 0.55 \times 10^{-6}$	–
K (B_K), m/s	$2.10 \pm 0.36 \times 10^{-7}$	$2.34 \pm 0.47 \times 10^{-7}$	–
PO ₄ (B_{PO4}), m/s	$4.42 \pm 0.74 \times 10^{-8}$	$4.45 \pm 1.02 \times 10^{-8}$	–

Notes: ^a both diluted by a factor of 20; ^b non-applicable or non-determined.

Compared to dilute FU, the RO experiments with dilute SU as feed water indicated 30% and 11% higher ammonium permeability and potassium permeability, respectively. Relatively lower rejection rates were observed for these two positively charged ions (**Figure 4-4b**). The inverse relationship between solute permeability and rejection efficiency was also predictable by eq 4-5. A possible reason for this disparity is that the active surface of the CTA-FO membrane becomes more negative at higher pH (Jin et al., 2012a), resulting in more positive ions attracted to the membrane surface (higher partitioning). The higher pH of SU is also favorable to the conversion of ammonium ions into liberated ammonia molecules. Similar to urea, the CTA-FO membrane cannot successfully retain neutral ammonia molecules. Comparable pH influences on ammonium rejection by RO membranes and on reverse draw solute flux of weak electrolytes (propanoic acid/propanoate) in FO processes have been observed (Lee and Lueptow, 2001b; Yong et al., 2012b).

4.4 Water Flux and Nutrient Rejection in FO Process

4.4.1 Measured water flux and modeling

Bench-scale FO experiments were performed to investigate the effect of feed and draw concentrations on water flux. The results are presented in **Figure 4-5**. Generally, relatively high water fluxes of 9–18 L/m²h were obtained when treating FU or TSU using seawater desalination brine (1~2 M NaCl) as the draw solution. Similar water fluxes were obtained when using FS and TSU as the FO feed solution, due to similar osmotic pressure in FS. It is also worthwhile noting that seawater could not draw pure water from undiluted stale urine due to reverse osmotic pressure.

Figure 4-5 Experimental water flux of the FO process with various feed and draw conditions

Figure 4-6 shows the measured and predictive water fluxes as a function of DS concentration for SU at different dilution ratios. Overall, the measured water flux showed good agreement with flux simulation. Similar results were obtained when using FS and TSU as feed solutions. To a certain extent, the agreement was weakened when the FO process was running with the FU feed solution, especially at lower water flux levels. The flux disparity was probably derived from the fitting parameter $K_{m,s}$, since water flux can be simply simulated by $\tilde{J}_w \approx K_{m,s} \ln(\pi_d/\pi_f)$ when $A\pi_f \gg J_w$ and B_s . In the AL-FS configuration, salt transport at the DS side suffers from severe dilutive ICP within the porous support layer (facing DS). Thus, it is crucial to obtain a ‘real’ mass transfer coefficient of salt to better predict the FO water flux. Compared to FU and SU, TSU has low potential of membrane scaling/fouling and mainly contains sodium chloride as its dominant composition. For this reason, the $K_{m,s}$ value in the current study was calculated by fitting eq 4-2 with the measured water flux from FO experiments using TSU as FS. The obtained $K_{m,s}$ ($= 4.62 \times 10^{-6}$ m/s) was considered representative for

other FO experiments using SU or FU as the feed solutions. The $K_{m,s}$ value complies with our previous study by Tang et al. (2010), where $K_{m,s} = 4.2 \times 10^{-6}$ m/s was reported for FO experiments running with 10 mmol/L NaCl feed solution.

Figure 4-6 Experimental and predictive water fluxes in short-term FO process as a function of draw concentration when using FU (a), SU (b), and TSU (c) with different dilution factors as the feed. Eq 4-2 was used to simulate the water flux. Water permeability was 2.80×10^{-12} , 2.62×10^{-12} , and 2.75×10^{-12} m/s·Pa for FU, SU, and TSU, respectively. Salt permeability was 1.70×10^{-7} m/s and its mass transfer coefficient was 4.62×10^{-6} m/s for all cases. FO operational conditions: cross-flow velocity 20 cm/s; temperature $23 \pm 1^\circ\text{C}$; membrane orientation: AL-FS.

4.4.2 Measured nutrient rejection and modeling

Figure 4-7 illustrates the experimental and predicted nutrient rejection in the FO process as a function of water flux. Generally, higher nutrient rejection was achieved with increasing water flux, which was predictable by eq 4-4. According to the classical solution-diffusion theory, solute diffusion through the dense selective layer was mainly

driven by the differential of solute concentration at the two sides of the membrane layer. The nutrient transport through the dense layer is therefore diffusion-limited and uncoupled with concurrent water transport. In the AL-FS orientation, a higher water flux would dilute the permeate concentration of nutrients at the interface of the dense layer and porous support layer as $c_i = J_n/J_w$, resulting in a higher nutrient rejection.

Figure 4-7 Simulation and experimental nutrient rejection as a function of water flux in both FO and RO experiments: (a) urea, (b) ammonium, (c) potassium, and (d) ortho-phosphate. Eq 4-4 was used for nutrient rejection simulation based on experimental water flux and nutrient permeability coefficients in Table 4-2. FO conditions: cross-flow velocity 20 cm/s; temperature $23 \pm 1^\circ\text{C}$; membrane orientation AL-FS; FS dilution factors 1~5; DS concentrations 0.5~4 M NaCl. RO experiments were conducted by altering the applied hydraulic pressure (50–250 psi) using diluted FS and SU by a factor of 20 as the feed water.

Overall, the FO process revealed fairly low rejection ($< 50\%$) against urea molecules, which limits the utilization of the CTA-FO membrane for concentrating FU due to urea loss. By comparison, a relatively higher rejection (31–91%) was obtained against

charged ammonium. Urea hydrolysis is therefore favorable to improve nitrogen recovery in the filtration process. In addition, the FO process revealed high rejection of phosphate (97–99%) and potassium (79–97%), and no significant difference was observed for different feed solutions. The observed nutrient rejections are comparable to those reported for other applications. For instance, favorable rejections of ammonium (83–92%) and phosphate (> 99.5%) were observed in the batch FO process used for concentrating the rejected stream from dewatering AD biomass (Holloway et al., 2007). The combination of FO with pressure-driven NF and RO (van Voorthuizen et al., 2005), osmosis distillation (Cath et al., 2005), and membrane bioreactor (Achilli et al., 2009) can further improve the overall nutrient rejection efficiency, though this is beyond the scope of the current study.

Figure 4-7 also compares the nutrient rejection performance between FO and RO, as the solute retention mechanisms are considered similar in the two processes. For neutral urea molecules, both processes showed comparable rejection rates within the operating water flux range (0~24 L/m²·h). However, the FO rejection of ionic nutrients (i.e. NH₄⁺, K⁺) was relatively lower than in the RO process, and cannot be accurately predicted by the existing model (eq 4-4) on the basis of B_s values obtained in RO tests. That is, the FO process revealed higher permeability coefficients for ionic solutes than the model predictions. This disparity was probably attributed to the coupled solute diffusion phenomena in the FO process, since reverse salt flux of sodium chloride usually occurs and jeopardizes the FO process (Hancock and Cath, 2009; Irvine et al., 2013). Coupling with the reverse sodium flux, ammonium and potassium ions in the feed are likely to permeate rapidly into DS due to their higher permeability than the sodium ion (**Table 4-1**). In comparison, the reverse chloride flux might be slightly retarded by the less permeable anions (e.g. phosphate, sulfate, and citrate) existing in the urine feed solution. This would lead to a charge imbalance in DS, and was corrected by rapid permeation of positively-charged ammonium and potassium ions. For neutral solutes (e.g. urea) and those with less permeability coefficients (e.g. ortho-phosphate), the ion exchange activity could be ignored and there was strong agreement between the experimental results and simulation.

The above-mentioned ion exchange mechanism (solute-solute interaction) highlights the difference in rejecting specific ions by the osmotically driven FO process and by the pressure-driven RO process. However, the coupled solute diffusion was not considered in the existing prediction models (eqs. 4-2 – 4-4) which are built on the classical solution-diffusion mechanism (Irvine et al., 2013). Systematic experimental investigation and modeling might be needed in this regard to further explore these phenomena. It is also notable that the various nutrient permeability coefficients determined in RO tests slightly increased with increasing applied hydraulic pressure, which was probably due to the compression of the membrane porous layer (Coday et al., 2013; Persson et al., 1995). Consequently, the average B_s values obtained might be slightly underestimated for the osmotically driven FO process.

4.4.3 Longer-duration operation and membrane fouling

The variation of water flux and potential membrane fouling were also investigated through longer-duration FO operations. The concentration factor (CF) was employed to evaluate the FO dewatering efficiency and is defined by eq 4-6 as the volumetric ratio of the FS before and after the filtration process.

$$CF = \frac{V_{f,0}}{V_{f,0} - A_m \int J_w dt} \dots\dots\dots (4-6)$$

where A_m is the working membrane area and $V_{f,0}$ is the initial feed volume for batch sequential treatment. The integration of water flux over time represents the accumulative permeate of water across the FO membrane. CF is related eqto water recovery efficiency (θ), i.e. $CF = 1/(1-\theta)$.

Figure 4-8 illustrates the water flux lines for three different feed solutions. In all cases, water fluxes remained rather stable at the initial 3-5 hours, and then decreased gradually mainly due to continuous water migration from feed to draw solution. About 60-85% of water could be effectively removed from the urine solution ($CF = 2.5 \sim 6$) within a 70-hr consecutive filtration using seawater brine as initial DS (2M NaCl). From a system-level point of view, such concentration factors are still considered significant, especially in view of the energy-saving advantages of the FO membrane process. By increasing the

membrane area, maximum volume reduction can be achieved within a much shorter period (e.g. in the scale of hours). A concentration factor of up to 15 was achievable when urine diluted by factor of 2-5 was treated. Consequently, the total energy need for the entire system (Figure 4-1) can be significantly reduced in terms of system operation, transportation, and infrastructure construction. With reduced water volume, better treatment efficiency is also expected for ozonation/AOP, anaerobic digestion, and N, P-recovery processes.

Figure 4-8 Variation of experimental water flux and simulation (based on concentration factor) in 70-hr FO process using non-diluted FU (a), SU (b), and TSU diluted by a factor of 2 (c) as feed solution. Initial DS was 2 M NaCl for all batches. Eq 4-2 was adopted for water flux simulation.

It is worth noting that the decreasing rate of experimental flux line was slowed down when using non-diluted FS and SU as feed. One possible reason is that, the continuous transport of urea (from FU) and ammonia (from SU) to the DS side (due to relatively low rejection but high concentration in feed) retarded the increase of FS osmotic pressure and the decrease of DS osmotic pressure. Consequently, the decline trend of actual water flux was slowed down when the process reached high concentration factors

as the diminishing rate of driving force was decreasing. In addition, ammonia evaporation from SU (pH ~9.4) might inevitably occur during the process and further retard the expected flux decreasing rate (**Figure 4-8b**).

The variation of water flux was also simulated by eq 4-2, where the osmotic pressure of FS and DS was corrected by the experimentally determined CF . In general, the simulation results show declining water fluxes with respect to time, reflecting the effects of the increased osmotic pressure in the FS and the decreased osmotic pressure in the DS as water permeates from the FS to the DS. The experimental water flux for TSU agreed well with the simulated results (**Figure 4-8c**). However, when either FU or SU was used as the feed, the simulation underestimates the experimental water flux, especially at higher CF values ($CF > 2$). As discussed above, this discrepancy was probably due to the intense forward solute flux of urea/ammonia migration from FS to DS, which lowered the osmotic pressure in the FS and thus improved the experimental water flux.

Figure 4-9 further illustrates the measured EC of FS (and DS) as a function of concentration factor (and dilution factor); they are supposed to be linearly correlated. By using SU as the feed, however, the measured EC was much lower than the CF -corrected EC, which clearly implied the intense forward salt flux (larger than inverse salt flux) across the membrane. For FU, the discrepancy was minor and could not fully explain the experimental water flux declination in **Figure 4-8a**; this is because neutral urea does not contribute to EC, but the intensive urea flux to DS (due to high permeability) significantly lowered the feed osmotic pressure. In addition, the discrepancy was enhanced by the bulk crystallization in both FU and SU under such conditions, which also lowered the actual osmotic pressure in the feed. In case of diluted TSU without membrane fouling issues, the changes of osmotic pressure in both feed and draw solutions showed a linear correlation with the volumetric changes, indicating the effectiveness of flux prediction by eq 4-2 during the long-term FO process.

Figure 4-9 Electrical conductivity of FS as a function of concentration factor (a) and electrical conductivity of DS as a function of dilution factor (b). The lines represent theoretically linear correlation relation between EC of FS and its concentration factor, or between EC of DS and its dilution factor. Data points represent conductivity measurements for different FS including FU, SU, and TSU (diluted at 1:1), and NaCl DS. FO operational conditions: 2 M NaCl as initial DS; cross-flow velocity 20 cm/s; temperature $23 \pm 1^\circ\text{C}$; membrane orientation: AL-FS.

Figure 4-10 showed the mineral precipitates deposited on the membrane active surfaces facing FU and SU. EDX spectroscopy in **Figure 4-11** further confirmed calcium and phosphate as two major elements in both cases although with different morphology and crystallization properties. The spontaneous precipitation in FU was predicted to be dicalcium phosphate ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) (Lee and Lueptow, 2001a), while in SU it was mainly a mixture of struvite crystals ($\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$) and hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) (Udert et al., 2003b). These precipitates, however, can be readily removed from the membrane surfaces using weak acids (e.g. 5% acetate acid) and might not impose restrictions on the FO process in real-world applications. By comparison, membrane scaling was not observable when TSU was used as the feed. This highlights the necessity of removing the naturally precipitated crystals from urine prior to FO dewatering. Furthermore, the CTA-FO membrane showed its stability after longer-duration exposure of urine solutions without significant morphologic transformation on active surfaces.

Figure 4-10 SEM images of scaled FO membrane with active surface facing FU (a) and SU (b), respectively.

Figure 4-11 EDX images of the scaled FO membrane surface when using FU (a) and SU (b) as feed solution.

4.5 Cost-Benefit Evaluation and Implications

Conventionally, the FO process requires additional energy input to recover and maintain the synthetic draw solution at a relatively high osmotic pressure. The use of expensive and non-environmental-friendly draw solutions could compromise the virtues of the FO process. A simulation study indicated that an ammonia–carbon dioxide FO process

coupled with a low-temperature single vacuum column (for DS recovery) required 0.84 kWh/m³ of equivalent work for seawater desalination purpose (at a recovery efficiency of 75%) (McGinnis and Elimelech, 2007). The simulation is indicative of a commensurable energy need for urine treatment in a similar FO system, since the urine solution treated in this study has comparable osmotic pressure with seawater (~25 bar). As a rough comparison, this energy level is much lower than that of the conventional nutrient management practices (~798 MJ/m³, industrial fertilizer production + centralized nutrient removal in STPs) (Maurer et al., 2003). This highlights, from both holistic and prospective viewpoints, the probable significance of applying source separation strategy in an urban context, and, essentially recycling urban nutrients to the adjacent suburban/rural areas for agricultural purposes. Clearly, the overall energy demand for the FO system could be significantly reduced by using locally available low-cost draw solutions. For example, about 10% of the total water supply (~136,000 m³ per day) in Singapore is currently provided by seawater RO plants, and this number will be ramping up to at least 30% by 2060 (<http://www.pub.gov.sg/>). This means ~136,000 m³ of brine is generated daily, considering an average water recovery efficiency of 50%. The rejected brine should be sufficient for the FO-based urine treatment, considering a total urine production of 8100 m³ per day (i.e. ~1.5 L/d·cap × 5,400,000 of population in 2013) in the entire city.

On the other hand, there are still uncertainties to scale up the proposed FO-based system. For example, the use of treated urine (with prior N, P-recovery) as feed water facilitated the FO performance with higher water flux. However, it is not absolutely conclusive in the present work whether an additional N, P-recovery process is necessary and where it would be placed (i.e. before or after FO process). In other words, this configuration might not be optimum from the energy footprint perspective. Furthermore, only medium rejection of ammonia was observed in the current FO process. In this regard, the development of a novel FO membrane that has higher nutrient rejection can compensate this weakness. Hancock and Cath have showed the possibility to use a more selective, less permeable FO membrane to substantially reduce the forward solute flux of ammonium (Hancock and Cath, 2009). From a life-cycle viewpoint, the potential environmental impacts of the FO process can be further reduced by improving the

membrane separation properties and packing density and system optimization (Hancock et al., 2012). In addition, the FO process might suffer from severe membrane bio-fouling when AD effluent is also treated, and further systematic investigation is needed to better understand this configuration.

As programmed in **Figure 4-1**, cost-effective FO process can be incorporated to significantly reduce the volume of urine stream. The final products can be concentrated urine or N, P-enriched bio-fertilizers (i.e. struvite and ammonium sulfate). Meanwhile, the rejected effluents from AD system can also be introduced into the FO process for extra nutrient recovery, since AD effluents are the second largest nutrient source from households (Meininger and Oldenburg, 2009; Siegrist, 1996). Essentially, the integral system aims to minimize any environmental discharge, with low energy demand, by fully recycling the ‘human resource’ in agriculture. On top of nutrient recovery, engaging FO filtration facilitates the removal of various excreta-derived micropollutants and pathogens from household wastewater (see Chapter 6). Recent studies reveal that the CTA-FO and other FO-designed membranes exhibit high retention to natural hormones (Cartinella et al., 2006), pharmaceuticals and personal care products (Hancock et al., 2011; Jin et al., 2012a; Linares et al., 2011). In this view, it is necessary to minimize any possible micropollutants and pathogens in the final urine products by oxidative treatment prior to agricultural/horticultural applications.

Despite the attractive virtues of the osmotically driven FO process, there are still limitations and challenges in practices. A critical challenge is related to the development of a high-performance FO membrane (i.e. with high water permeability, low solute permeability, anti-biological property, etc.), which calls for intensive interdisciplinary collaborations between material scientists and environmental engineers. The present study demonstrates the technical feasibility of using seawater and desalination brine as powerful draw solution, yet a comprehensive life-cycle assessment is still needed to evaluate the performance of the proposed integral source separation sanitation system, especially in terms of environmental impacts and carbon footprint. Further investigations on pilot-/full-scale source-separating sanitation system would facilitate an integrated optimization at the system level.

4.6 Chapter Conclusions

Separating urine from domestic wastewater promotes a more sustainable municipal wastewater treatment system. This study investigated the feasibility of applying FO dewatering process for nutrient recovery from source-separated urine under different conditions, using seawater or desalination brine as a low-cost draw solution. The filtration process with the active layer facing feed solution exhibited relatively high water fluxes up to 20 L/m²·h. The process also revealed relatively low rejection to neutral organic nitrogen (urea-N) in fresh urine but improved rejection of ammonium (50–80%) in hydrolyzed urine and high rejection (> 90%) of phosphate, potassium in most cases. Compared to simulation based on the solution–diffusion mechanism, higher water flux and solute flux were obtained using fresh or hydrolyzed urine as the feed, which was attributed to the intensive forward nutrient permeation (i.e. of urea, ammonium and potassium). Membrane fouling could be avoided by prior removal of the spontaneously precipitated crystals in urine. Compared to other urine treatment options, the current process was cost-effective and environmentally-friendly for nutrient recovery from urban wastewater at source, yet a comprehensive life-cycle impact assessment might be needed to evaluate and optimize the overall system performance at pilot and full scale operation.

CHAPTER 5 SPE–LC–MS/MS ANALYSIS OF MULTI-CLASS CYTOSTATIC DRUGS IN ENVIRONMENTAL MATRICES

5.1 Introduction

The simultaneous detection of multi-group cytostatic compounds is a practical prerequisite for environmental screening and routine laboratory analysis. The development of liquid chromatography coupled with tandem mass spectrometer (LC–MS/MS, also called LC–QqQ MS) during the past decade has greatly strengthened the analytical capability for accurate identification and qualification of environmental trace organic contaminants. Sample treatments are usually needed to remove matrix components present in environmental samples and to provide up to a 1000-fold concentration of the analytes in order to extend detection limits down to ng/L-level. Off-line or online solid-phase extraction (SPE) is intensively used for this purpose (Ferrando-Clement et al., 2013; Martin et al., 2011; Negreira et al., 2013). The SPE packing sorbents can be octyl/octadecylsilane or polar-functionalized polymers. The latter has gained rapidly growing applications in recent years mainly because of: 1) the capability of retaining both polar and non-polar pharmaceutical residues in water samples; and 2) the larger loading capacity and higher resilience to become dried out between stages. It is therefore interesting to know whether such polymeric sorbents, either co-polymers with a polar monomer or hydrophobic polymer functionalized with a polar moiety, can consistently enhance the SPE recoveries for various cytostatic compounds in environmental samples.

Additional procedures are required to further avoid erroneous quantification due to signal suppression or enhancement, since both electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources are sensitive to co-extract and co-elute matrix components such as natural organic matter (NOM). Sample dilution or extract dilution is a simple approach to reduce matrix effects, but it can compromise the detection limits (Gros et al., 2006). Decreasing the flow delivered to the ESI-interface can also facilitate reducing the signal suppression, but it prolongs the chromatographic elution time (Kloepfer et al., 2005). Extra sample clean-up procedures such as tandem

SPE can be used to further purify the sample extracts, but it can introduce analyte losses due to excessive sample manipulation.

Another approach to overcome matrix effects is to engage correct calibration methods such as standard addition calibration, matrix-matched calibration, and more importantly, isotope dilution calibration (Comerton et al., 2009). In general, standard addition calibration is rather labor-consuming and might not accurately quantify analytes at ng/L-level due to improper data interpolation, while it is usually difficult to obtain analyte-free matrix blanks to conduct matrix-matched calibration (Hewavitharana, 2011; Stüber and Reemtsma, 2004). More promisingly, internal calibration with one or more standards can be used to satisfactorily solve the matrix issue (Gros et al., 2006). Currently, no standardized analytical methods are available for the analysis of cytostatic drugs in environmental matrices, with few methods applicable to multi-class compound analysis. Isotope-labelled analogues are ideal internal standards (ISs) but they are usually rather expensive and might not be commercially available for target contaminants. Particular effort should be also made to ensure the chosen ISs have high extraction recovery and low signal suppression in MS detectors. It is therefore interesting to explore whether there are specific isotope-labelled analogues that can be used as common IS to effectively correct matrix effects for a group of cytostatic compounds with a variety of physicochemical properties.

The major objective of this study is to develop a reliable and versatile SPE–LC–MS/MS method for the determination of multi-class cytostatic drugs in different environmental matrices including surface water, sewage treatment plant (STP) influent and effluent. The method was systematically optimized in terms of sample filtration, SPE methodology, mobile phase, LC column, multiple reaction monitoring (MRM) transitions, and calibration methods (extract dilution versus calibration calibration). Special effort was induced to minimize matrix effects by engaging enhanced SPE and proper internal standard calibration. To the best of our knowledge, this is the first instance that a polished LC–MS/MS method was applied for environmental screening of cytostatic residue in tropical aquatic environment.

5.2 Materials and Methods

5.2.1 Chemicals and standards

High purity ($\geq 98\%$) of 18 multi-group cytostatic standards: azathioprine (Aza), bicalutamide (Bic), cyclophosphamide monohydrate (CP), cytarabine (Cyt), doxorubicin hydrochloride (Dox), epirubicin hydrochloride (Epi), 5-fluorouracil (5-FU), flutamide (Flu), gemcitabine hydrochloride (Gem), ifosfamide (IF), melphalan (Mel), 6-mercaptopurine hydrate (Mer), methotrexate hydrochloride (Met), mitomycin-C (MC), mitotane (Mit), and procarbazine hydrochloride (Pro) were supplied by Sigma–Aldrich (Singapore), and paclitaxel (Pac) and tamoxifen citrate (Tam) by Merck (Singapore). Three isotopically labelled analogues with distinct hydrophobicity, i.e., cyclophosphamide- d_8 (CP- d_8), flutamide- d_7 (Flu- d_7) and cytarabine- $^{13}C^{15}N_2$ (Cyt- $^{13}C^{15}N_2$) delivered by TLC PharmaChem (Ontario, Canada) were used as internal standards (IS). Formic acid (98–100%), hydrochloric acid (HCl, 37%), and HPLC-grade methanol and acetonitrile were purchased from Merck (Singapore). Milli-Q water was freshly taken from an integral water purification system (Millipore GmbH). Individual stock solution (1 g/L) of each standard and IS were prepared in methanol and stored at $-20^\circ C$. Working standard mixtures were freshly prepared by mixing and diluting the stock solutions with methanol and stored at $4^\circ C$.

The target cytostatic compounds were selected mainly due to their high environmental relevance and suspected ecotoxicity (Zhang et al., 2013a). Several analytes such as Mel, Mer, Flu, Bic, Pro, and Mit were scarcely investigated in previous studies (Gomez-Canela et al., 2013; Martin et al., 2011; Negreira et al., 2013; Yin et al., 2010). The chemical structure and major physicochemical properties of the target compounds are presented in **Table 2-2**. For safety considerations, researcher working with cytostatic drugs in laboratory should wear sufficient personal protective equipment, and all cytostatic-containing waste should be disposed as hazardous waste.

5.2.2 Sample filtration and SPE optimization

Surface water was collected from Pandan reservoir located in the west region of Singapore ($1^\circ 18' 50'' N$, $103^\circ 44' 30'' E$) which provides non-potable water for local industrial use. Influent and effluent wastewaters were sampled from Changi water

reclamation plant located at the eastern end of Singapore (1°18'54"N, 104°0'12"E), one of the largest reclamation facilities in the world, in January, 2014. All samples were centrifuged at 8000 rpm for 20 min to remove suspended particles and the supernatants were further purified by filtration and SPE within 48 h of collection. The potential loss of analytes during sample filtration was evaluated by percolating 2 mL pH-adjusted spiked water (100 µg/L of each analyte) through different disposable syringe membrane filters (0.45-µm pore size) including cellulose acetate (CA), nylon, poly (tetrafluoroethylene) (PTFE), poly (vinylidene fluoride) (PVDF), and glass microfibre (Whatman, Maidstone, UK).

SPE procedure was first optimized with five different polymeric sorbents, i.e., Oasis HLB, AttractSPE W/O, SiliaPrep XHLB, HyperSep Retain PEP and Bond ElutPlexa (see sorbents specifications in **Table 5-1**). The commercial polymer-based sorbents promisingly hold a hydrophilic-hydrophobic balance (HLB) for acidic, basic and neutral compounds even without pH adjustment. All cartridges (bed mass 60 mg, 3 mL) were conditioned with 2 mL methanol followed by 2 mL deionized water (pH-adjusted) at a flow rate of 1 mL/min. The tests were carried out with 100-mL aliquots of deionized water spiked with 2.0 µg/L of each analyte without pH adjustment (pH~7.3) and with pH adjusted to 2 and 4 with 1 M hydrochloric acid for comparison purpose. The spiked water was percolated through the conditioned cartridges at 5 mL/min assisted by a Visiprep 12-port vacuum manifold (Sigma-Aldrich). The loaded cartridges were dried for 15 min under gentle vacuum without a washing step. The elution was then supplemented with 1+1+1 mL methanol (with separate collection) within 6 min to investigate the minimum elution volume needed for each SPE sorbent (60mg), and all extracts were analyzed under the optimum LC–MS/MS conditions. Further, the wash solvent and volume were optimized for the selected SPE cartridge, on which 100 mL of deionized water or influent wastewater (as worst matrix scenario) spiked with 2.0 µg/L of each analyte was loaded. The loaded cartridges were then washed with 2 mL (as recommended by vendors) pH-adjusted water containing 0, 1, 2, or 5% (v/v) methanol to remove matrix components without compromising analyte retention. At least duplicate tests were conducted for quality control purposes.

Table 5-1 Specifications of five different polymeric sorbents used for sample extraction in this study.

Brand	SPE sorbents	Particle size (μm)	Pore size ($\text{\AA}$)	Surface area (m^2/g)	Manufacturer
Oasis HLB	Poly(styrene-divinylbenzene-N-vinyl pyrrolidone) terpolymer	30	80	800	Waters
AttractSPE W/O	Neutral polymeric reversed phase with patented polar functionalities	40	50-80	800	Polyintell
HyperSep Retain PEP	Neutral polystyrene-divinylbenzene with urea functionalities	51	79	626	Thermo Scientific
Bond Elut Plexa	Neutral polystyrene-divinylbenzene with an amide-free hydroxylated surface to minimize binding of proteins and lipids	46	177	550	Agilent
SiliaPrepX HLB	A wettable copolymer presenting HLB property and high stability in organic solvents	40	110	850	Silicycle

5.2.3 LC–ESI–MS/MS method

Aqueous phase (A phase) with or without modifiers added (i.e., 0.1% v/v formic acid with/without 5 mmol/L ammonium formate, or 0.1% acetate acid with/without 5 mmol/L ammonium acetate) coupled with methanol or acetonitrile as organic phase (B phase) were tested to yield selective chromatographic separation for the multi-class cytostatic compounds and high intensity of MRM spectrum. LC separation was performed at a flow rate of 0.2 mL/min on a Shimadzu Ultifine10 AD system, consisting of two binary pumps, an auto-sampler, a thermo-stated column oven and a system communication controller. Seven different reverse-phase columns were tested, including Shim-pack VP-ODS (150 mm $\times$ 2.0 mm, i.d. 4.6 μm) from Shimadzu, Eclipse XDB C18 (150 mm $\times$ 4.6 mm, 5 μm) from Agilent, and Luna C18(2) (75 mm $\times$ 2.0 mm, 3 μm), Synergi Fusion C18 (150 mm $\times$ 4.6 mm, 4 μm), Hydro-RP (150 mm $\times$ 4.6 mm, 4 μm) and Polar-RP (100 mm $\times$ 3.0 mm, 2.5 μm) supplied by Phenomenex, and XBridgeBEH C8 (100 mm $\times$ 2.1 mm, 3.5 μm) by Waters. Column temperature was maintained at 25 $^{\circ}\text{C}$, while the sample injection volume was 10 μL .

MS measurements were carried out with an LC–MS 8030 triple quadrupole mass spectrometer (QqQ MS, Shimadzu) equipped with a dual-polarity electrospray ionization (ESI) interface. System operation and data acquisition and processing were performed with LabSolutions V5.42 software (Shimadzu). QqQ MS parameters were automatically tuned by using direct injection of 10 μL of individual standard solution at a flow rate of 200 $\mu\text{L}/\text{min}$ (50:50/0.1 methanol/water/formic acid). The following MS conditions were

used: ion-spray capillary tube voltage 4500 V, nebulizing gas 3 L/min, drying gas 15 L/min, desolvation line 250 °C, heat block 400 °C, collision gas pressure (Argon) 350 kPa. The analytes were detected in multiple reaction monitoring (MRM) mode using the two most intense ion transitions for quantification and qualification, respectively. The ratio of the two selected fragment ions and the chromatographic retention time (RT) were also used to identify the target compounds. A short dwell time of 20 msec was set for all 36 MRM transitions in order to shorten the loop time without resolution or sensitivity loss.

5.2.4 Matrix effect mitigation and method validation

Matrix effects were investigated using different sample extracts spiked with cytostatic standard mixture at 100 µg/L (n=3). SPE were conducted with Oasis HLB sorbent for different types of environmental samples. The detected response for one specific analyte (*i*) spiked in sample extracts (subtracted by that of non-spiked extracts) was compared to that of same concentration of analyte in methanol. As defined by eq 5-1, a higher response ($\theta > 1$) indicates matrix enhancement, while a lower response ($\theta < 1$) means matrix suppression. In a further step, sample extracts were spiked with the cytostatic standard mixture and three available isotopically labelled compounds as IS (all at 100 µg/L) for LC–MS/MS analysis. Peak areas of analytes were then corrected by those of IS to evaluate the efficacy of using IS for correcting matrix effects. In the case that all analytes and IS were spiked at a same concentration (i.e., 100 µg/L), the degree of matrix effect corrected for one specific analyte (*i*) by a specific IS (*j*) can be defined as eq 5-2.

$$\text{matrix effect } (\theta, \%) = A_{i,\text{matrix}}/A_{i,\text{std}} \times 100 \quad \dots\dots\dots (5-1)$$

$$\text{matrix correction } (\varphi, \%) = (A_i/A_j)_{\text{matrix}}/(A_i/A_j)_{\text{std}} \times 100 \quad \dots\dots\dots (5-2)$$

where A_{matrix} is the peak area of an analyte (or IS) in one specific environmental matrix, and A_{std} is that in methanol. A φ -value close to 1 indicates that matrix effect can be calibrated for an analyte by using the corresponding IS, while a φ -value deviating from 1 means the failure of using that IS for calibration.

Internal calibration curves were built up for all compounds at nine concentration levels ranging from 1 to 1000 µg/L using the three isotopically labelled cytostatic compounds as possible IS. In order to demonstrate the robustness of the analytical protocol, the method performance was evaluated in terms of accuracy (process recovery), repeatability, linearity, sensitivity, and matrix effects (ion suppression or enhancement). Process recovery (mean ± SD) was determined in triplicate at two spiking levels (20 and 200 ng/L) in different environmental matrices. The process recovery was calculated by comparing the peak areas obtained from the spiked samples (subtracted by non-spiked samples) as percentages of those obtained in the direct chromatographic injection of equivalent amounts of the standard mixture, relative to that of associated IS. The method detection limits (MLD) and quantification (MLQ) were determined at the lowest spiking level in individual calibration curve giving a signal-to-noise (S/N) ratio of 3 and 10 of the first selected reaction monitoring (MRM 1) response, respectively. In addition, procedural blanks (pure water) and control samples (spiked water at 100 µg/L) were run in between samples for quality control purpose. The internal calibration method was applied for the trace analysis of cytostatic drugs in environmental samples.

5.3 LC–ESI–MS/MS Optimization

5.3.1 Electrospray ionization and MRM transitions

Figure 5-1 illustrates the effect of mobile phase on the soft ionization of cytostatic molecules in ESI chamber. The liquid-phase chemistry (i.e., organic phase, pH and ammonium additives) plays a key role in ion formation by influencing the proton affinity (or gas-phase basicity) of analytes. ESI was operated in both positive and negative modes. As expected, most cytostatics formed protonated precursor ions (PI), while 5-FU, Flu, Bic, and Mit preferred to form deprotonated ions in negative mode under all conditions. Some compounds such as Mer and Aza formed deprotonated ions under neutral conditions but strong $[M+H]^+$ ions in acidified mobile phase. Gem, MC, Pac, IF and CP also formed sodium and/or ammonium adducts but with relatively low intensity. More specifically, the acidification of the binary phase significantly increased the signal intensity of positive ions but reduced the negative ions up to 60%. Sodium adduct formation was inhibited as well. Acetic acid performed equivalently with formic

acid in enhancing the positive ions, although the former is slightly less acidic (pH 3.2 versus 2.8, at 0.1% v/v). By comparison, formic acid was particularly favorable in increasing the PI intensity of Mit (E^-), Gem, Mel and Pro, while acetic acid benefited Aza, Cyt and Flu (E^-). In addition, methanol was generally a better organic mobile phase than acetonitrile for most analytes. Compared to acetonitrile, methanol possesses a much lower boiling point (64.7 versus 82 °C) and a higher vapour pressure, which favored its desolvation away from the spraying mists in ESI. Further addition of respective ammonium salts (formate or acetate) to the acidic mobile phase slightly reduced the PI signals probably due to an increased ionic matrix, but induced 1~4 fold increase in PI intensity for CP, IF and Mit. As such, water (phase A) and methanol (phase B) both acidified with 0.1% (v/v) formic acid were used as the ideal binary phases in this study. Overall, most cytostatic molecules except 5-FU and Pac were effectively ionized in ESI in view of the high PI signal intensity.

Figure 5-1 Effect of mobile-phase chemistry on precursor ion formation during ESI with direct injection of 10 μ L individual standard (1 mg/L). Mobile phase chemistry: (a) neutral versus acidic methanol; (b) methanol with different acids; (c) acidic acetonitrile versus acidic methanol; (d) acidic methanol with 5 mmol/L respective ammonium salts. FA: formic acid; AA: acetic acid.

The positively or negatively charged ions of target compounds and co-eluted matrix components were sucked into the high vacuum triple-quadrupole mass spectrometer. Specific PI was then selected for each target compound by the first quadrupole (Q1 scan) according to the mass-to-charge ratio (m/z) and was fragmented into multiple characteristic transition ions (also called daughter ions) by collision with energetic Argon gas molecules in the collection cell (q2). Two major transition ions of each compound were selected by the third quadrupole (Q3 scan) for quantification and qualification purposes. The signal ratio of the two selected reaction monitoring (MRM 1/MRM 2) facilitates to identify the parent compounds. Some cytostatic compounds such as 5-FU and Pac experienced relatively poor fragmentation. The selected MRM transitions for all target compounds and three IS were summarized in **Table 5-2**.

Table 5-2 MRM transitions for LC–MS/MS determination of 18 cytostatic drugs.

Compound	MW (g/mol)	MRM 1 for quantification (m/z) (CE, eV)	MRM 2 for qualification (m/z) (CE, eV)	MRM 1/MRM 2
<i>ESI in positive mode</i>				
Mer	152	153.0>119.0 (-25)	153.0>92.0 (-35)	2.8
Pro	221	222.2>191.2 (-15)	222.2>177.2 (-15)	1.2
Cyt	243	244.1>112.1 (-15)	244.1>95.1 (-45)	8.5
Cyt- ¹³ C ¹⁵ N ₂	246	247.2>115.1 (-15)	247.2>97.1 (-45)	11.4
IF	260	261.1>92.0 (-30)	261.1>63.1 (-45)	2.1
CP	260	261.1>140.0 (-20)	261.1>63.1 (-40)	1.3
CP-d ₈	268	269.2>147.1 (-25)	269.2>67.1 (-10)	1.7
Gem	263	264.2>112.1 (-15)	264.2>223.1 (-10)	>2000
Aza	277	278.0>142.0 (-10)	278.0>232.1 (-15)	2.8
Mel	304	305.2>288.1 (-15)	305.2>246.2 (-25)	2.1
MC	334	335.2>242.2 (-15)	335.2>131.1 (-50)	16.4
Tam	371	372.3>72.2 (-25)	372.3>44.2 (-50)	10.1
Met	454	455.3>308.2 (-20)	455.3>134.1 (-40)	3.4
Dox	544	544.2>397.2 (-15)	544.2>130.2 (-15)	1.4
Epi	544	544.2>130.1 (-20)	544.2>86.2 (-35)	1.8
Pac	854	854.2>105.2 (-45)	854.2>286.2 (-20)	1.6
<i>ESI in negative mode</i>				
5-FU	130	129.0>42.0 (25)	129.0>86.1 (25)	8.4
Flu	276	275.0>202.1 (25)	275.0>205.1 (25)	2.1
Flu-d ₇	283	282.1>202.1 (25)	282.1>206.1 (25)	2.5
Mit	322	321.0>275.1 (15)	321.0>202.1 (30)	7.6
Bic	430	429.0>255.2 (15)	429.0>185.1 (40)	2.0

5.3.2 Liquid chromatography optimization

Seven different reverse-phase columns were tested to provide chromatographic separation of 18 cytostatic compounds under optimum mobile phase gradients. Overall, Luna 3u C18(2), Shim-pack VP-ODS, and Xbridge C8 displayed effective separation

and chromatograms for most analytes. **Figure 5-2** shows the typical MRM spectra for 18 cytostatic compounds effectively separated by the Luna column. With an optimized methanol–water gradient, the LC separation was accomplished in merely 8-min elution. Nevertheless, tailed or subsidiary peaks appeared occasionally for 5-FU, Mer and Met, which constrained the later quantification. By comparison, other three analytical columns showed inferior performance in cytostatic separation. The stationary phase in Synergi Polar-RP column was ether-linked phenyl with polar end capping, only providing fair retention to polar and aromatic compounds. Fusion- and Hydro-RP columns also involved the polar-enhanced C18 phase, but they were still rather hydrophobic and could only elute out some of the extremely polar compounds under the current binary elution conditions. Running with more aqueous mobile phase might improve the elution efficacy. Similarly, the double alkyl end capped Eclipse phase was very hydrophobic and only polar analytes could be eluted out within 15 min.

Figure 5-2 Typical MRM spectra for 18 cytostatic standards (100 $\mu\text{g/L}$). Injection volume 10 μL . LC–MS/MS was run with a Luna 3u C18 column and methanol–water gradient (+ 0.1% formic acid): 10–50% B in 0.1 min; 8 min, 90% B; 8.1–11 min, 100% B; 12–15 min, 10% B.

As illustrated in **Figure 5-3**, a significant correlation ($R^2 = 0.66$) was found between LC retention times (by Luna column) of cytostatic compounds with their logP values. The more hydrophobic compounds with $\log P > 2$ were eluted out at last. It was implied that

hydrophobic interaction with C8 or C18-bonded stationary phase was the major interaction force in molecular recognition during the LC separation, as most analytes remain as neutral molecules in the acidic mobile phase. As such, the target compounds can be divided into three groups according to their hydrophobicity-associated retention times in the analytical column, except for MC and Mel. However, *Pearson* correlation was weak ($R^2 = 0.24$) between LC retention times and cytostatic molecular weights.

Figure 5-3 Relationship between chromatographic retention times and the octanol-water partition coefficients of 18 target cytostatic drugs. The diameters of circles indicate analytes' molecular weights. LC-MS/MS was run with a Luna 3u C18 column and methanol–water gradient (+ 0.1% formic acid): 10–50% B in 0.1 min; 8 min, 90% B; 8.1–11 min, 100% B; 12–15 min, 10% B.

5.4 Sample Pretreatments

5.4.1 Sample filtration

The filtration tests were conducted by percolating spiked water (pH adjusted to 7 and 2) through different membrane filters, and the filtration recoveries (in terms of MRM 1 signal reduction) for all target compounds are showed in **Figure 5-4**. In most cases, Flu, Mit, Bic, Tam, Epi, Dox and Pac were subjected to relative high filtration loss in neutral conditions, which was likely due to their strong hydrophobic property (logP 1.27–7.88). Sample acidification to pH 2 significantly increased the filtration recovery for most cytostatic compounds. This leads to the recommendation that pH adjustment should be done prior to sample filtration. At pH 2, GMF filter showed satisfactory recoveries (63–111%) for nearly all analytes except Tam. PTFE filter also exhibited comparable filtration recoveries for most analytes (except Tam, Bic, Epi, Dox, and Pac), followed

by CA and nylon filters. In comparison, the PVDF filter showed poor recoveries for seven out of the 18 target compounds and might not be suitable for cytostatic compound-containing sample filtration. The results indicate the potential loss of cytostatic compounds during sample treatment, which calls for extra effort to minimize measurement bias. In this study, all water/wastewater samples were acidified to pH 2 using HCl and filtered through 0.45 μ m GMF filters prior to SPE.

Figure 5-4 Filtration recoveries for cytostatic compounds passing through different 0.45- μ m membrane filters at pH 7 (a) and pH 2 (b).

5.4.2 Stepwise SPE optimization

Figure 5-5 presents the performance of Oasis HLB and other four SPE sorbents in retaining multi-class cytostatic compounds under neutral and acidified conditions. The polymeric sorbents yielded satisfactory recoveries for 13 of the 18 target compounds in most cases. The polar-enhanced polymeric Oasis HLB has been proven to have better retention to cytostatic drugs in wastewaters in comparison to reverse-phased or ion-exchangeable sorbents such as Chromabond tetracycline (Martin et al., 2011), Isolute C18/HCX (Negreira et al., 2013), and Oasis MCX/MAX (Ferrando-Climent et al., 2013; Martin et al., 2011). However, Oasis HLB (copolymer of divinylbenzene and N-vinylpyrrolidone) and other four polymeric sorbents showed only little retention to 5-FU, Mer, Pro, Cyt, Gem, and MC (at pH 2). This was likely because these compounds are rather hydrophilic ($\log P = -1.93 \sim 0.77$) and could be easily flushed off the SPE

cartridges during sample loading. Other SPE sorbents with ion-exchange capability such as hydroxylated highly-cross linked polystyrene-divinyl-benzene copolymer of Isolute ENV⁺ might provide better retention for these compounds (Kovalova et al., 2009).

Figure 5-5 SPE recoveries for cytosstatic drugs with five different polymeric sorbents at variable pH.

There was no significant difference in extracting these polar compounds using either copolymeric sorbents with a polar monomer (i.e., Oasis HLB, SiliaPrepX HLB) or hydrophobic sorbents functionalized with a polar moiety (i.e., AttractSPE W/O, HyperSep, and Bond ElutPlexa). Negreira et al. (2013) also compared the SPE recoveries for 13 cytosstatics and 4 metabolites with five different sorbents at neutral condition. The results showed that Oasis HLB and PLRP-s (cross-linked styrene-divinylbenzene) showed generally preferred performance and better repeatability. The reported recovery efficiencies were comparable to those observed in this study.

Sample acidification significantly improved the recovery of most cytosstatic compounds in nearly all cases (except Pro, MC). This was mainly because the ionic cytosstatic compounds ($pK_a < 7$) became neutral molecules with increased hydrophobicity under acidic conditions. It was also interesting to find that MC exhibited extremely low

recovery by all SPE sorbents at pH 2. This was probably because MC underwent rapid degradation in acidic medium and could retain its activity only in neutral and basic conditions (Beijnen and Underberg, 1985). In this study, the Oasis HLB sorbent was selected for latter sample extraction, but other polar-enhanced polymeric sorbents can also be used since they had similar performance. The sample pH was adjusted to 2 (unfavorable for MC) before SPE to improve recovery of cytostatic compounds during sample filtration and extraction, especially those with relatively low pKa values.

The use of methanol as elution solvent was also minimized to effectively elute out cytostatic compounds from Oasis HLB cartridges. The results shows that cytostatic compounds (except 5-FU, Mer, Pro and MC due to low retention) were almost completely eluted out by the first millilitre of methanol. In this case, the final extract can be directly injected for LC–MS/MS analysis without further evaporation of surplus methanol. The results indicates that the SPE recovery for hydrophobic compounds such as Flu, Mit and Bic was significantly decreased (up to 20%) when increasing methanol content in wash solvent from 0 to 5%. However, the effect of increasing methanol content in the wash solvent was marginal for more hydrophilic compounds. In real-world applications, a sample volume of 500 mL was used to extend the detection limits down to the ng/L level, the chosen sorbent with a larger cartridge bed mass (220 mg, 6 mL) was employed in the final analytical protocol, in which 500 mL sample was extracted and a final extract volume of 500 µL was reconstituted for LC–MS/MS analysis. The optimum parameters for SPE were properly adjusted. Generally, 50 µL of IS mixture (1 mg/L) was spiked into 500 mL sample to achieve a IS spike level of 100 ng/L.

5.5 Matrix Suppression and Compensation

5.5.1 Matrix suppression and sample extract dilution

Figure 5-6 presents the matrix effects on cytostatic compounds spiked into different sample extracts with a 1000-fold concentration (without further extract dilution). All compounds encountered signal suppression in both water and wastewater extracts. In particular, Flu, Mit, Bic, Epi, and Dox encountered relatively severe signal suppression

in most cases, which was likely due to their hydrophobic properties (logP 2.82–5.39). The signal suppression might be attributed to the hydrophobic interaction between trace cytostatic molecules and NOM present in water and wastewater. Compared to the SW extract, wastewater extracts induced higher signal reduction to all cytostatic compounds mainly due to more complex matrices. The major characteristics of the water and wastewater samples are showed in **Table 5-3**.

Figure 5-6 Ion suppression of cytostatic compounds in different sample extracts.

Table 5-3 Characteristics of surface water, STP influent and effluent wastewater.

Component	Surface water	Effluent	Influent
pH	6.8	6.5	6.8
EC, mS/cm	315.6	829	1020
COD, mg/L	12.6	37	160
TN, mg/L	0.2	4.9	48
NH ₃ , mg/L	0	3.4	47.7
NO ₃ , mg/L	0.06	0.3	0.2
TP, mg/L	0.02	4	17.8
SO ₄ , mg/L	21.6	54.8	68.1
Cl, mg/L	46.6	100.5	143.8
Na, mg/L	32.6	79.6	78.4
K, mg/L	6	17.2	18.1
Mg, mg/L	3.8	4.3	4.8
Ca, mg/L	23.8	25.6	27.3
Si, mg/L	0.5	4.3	5.9

However, the mechanisms of causing matrix effects in ESI and mass spectrometer remain largely unclear. Various matrix components such as ions and NOM could co-elute into sample extracts and compete with cytostatic compounds during protonation/deprotonation reactions in the ESI chamber. The matrices might also alter the favorable PI being formed during ESI. For example, the two antibiotic epimers Dox

and Epi formed both [544.2] m/z and [545.2] m/z ions in positive mode whilst their relative amounts were affected by the mobile phase chemistry and co-existing matrix components.

Figure 5-7 Mitigation of matrix effects by diluting sample extracts (a, surface water; b, STP-effluent; c, STP-influent) at different dilution factors.

One simple approach to reduce matrix effects is to use extract dilution. **Figure 5-7** also illustrates the attenuation of matrix effects by diluting sample extracts with methanol in a wide range of dilution factors (1~20). With greater dilution to sample extracts, less

signal suppression was fulfilled for most cytostatic compounds. Negreira et al. (2013) did not successfully removed signal suppression via 1:2 sample dilution during the online SPE–LC–MS/MS analysis of 17 cytostatic compounds and metabolites in wastewater samples. Generally, a high dilution factor of more than 10 should be applied to satisfactorily remove the matrix effects of the SW extract, and more than 20 of wastewater extracts. However, such high dilution would greatly sacrifice the detection limits of the developed SPE–LC–MS/MS method. Therefore, it is not recommended to abate the matrix effects simply by diluting the sample extracts.

5.5.2 Matrix compensation by isotope dilution calibration

Alternatively, matrix effects could be compensated by using the correct IS. It is ideal to engage an isotope-labelled analogue as respective IS for each target analyte, because the paired compounds performed similarly during sample pretreatments, liquid chromatography, and electrospray mass spectrometry response (Ferrando-Climent et al., 2013; Hewavitharana, 2011; Llewellyn et al., 2011; Negreira et al., 2013; Vanderford and Snyder, 2006). However, such analogues are usually rather expensive and might not be commercially available. Hence, it is practically important to select one or more appropriate IS to compensate matrix effects for multi-class cytostatic drugs with a variety of physicochemical properties. **Figure 5-8** illustrates the correction of matrix effects by using CP-d₈ (in positive mode) and Flu-d₇ (in negative mode) as IS.

Figure 5-8 Correction of matrix effects with two internal standards CP-d₈ and Flu-d₇ in three different environmental matrices. Flu-d₇ was particularly used for ion suppression correction for Flu, Mit, Bic, Epi and Dox.

More specifically, Flu-d₇ was used to correct matrix effects for Flu (E⁻), Mit (E⁻), Bic (E⁻), Aza, Epi and Dox, which suffered from relatively severe ionization suppression as showed in **Figure 5-8**. CP-d₈ satisfactorily compensated for the matrix effects for the remaining 12 cytostatic compounds in most cases ($\phi = 50\text{--}150\%$). It was also noticed that although Cyt-¹³C¹⁵N₂ showed comparable performance as CP-d₈ in correcting matrix effects for most compounds, it suffered from poor SPE recovery (similar to Cyt) and it was therefore, not considered suitable for calibration purpose. Although the three isotope-labelled compounds were carefully selected as possible IS with distinct hydrophobicity and retention times, they did not specifically favor the abatement of matrix effects for those analytes with similar hydrophobicity. As such, CP-d₈ and Flu-d₇ together performed well in matrix compensation for the selected group of cytostatic drugs using ESI in both positive and negative modes. In addition, CP-d₈ was more chemically stable and encountered less matrix suppression in different sample extracts compared to Flu-d₇ (Negreira et al., 2014b). Therefore, it may still serve as a robust single IS for trace determination of multi-class cytostatic compounds when Flu-d₇ is not available.

Other isotope labelled or non-labelled pharmaceutical compounds could also be used as efficient IS, such as methotrexate-d₃¹³C and phenacetin (Martin et al., 2011; Nussbaumer et al., 2010). Martin et al. (2011) reported a SPE–LC–MS/MS method for the trace detection of 14 commonly used cytostatic drugs in water and wastewater samples using phenacetin-ethoxy-1-¹³C as single IS; however, process recoveries were rather poor for some polar analytes such as cytarabine and methotrexate. Nussbaumer et al. (2010) also developed a similar LC–MS/MS method for the simultaneous detection of 10 cytostatics in wiped samples in pharmacy using methotrexate-d₃¹³C as IS. More recently, Negreira et al. (2013) successfully built up an on-line SPE–LC–MS/MS method for the trace determination of 13 cytostatics and 4 metabolites in environmental samples based on isotope dilution calibration with 15 isotope-labelled analogues as IS (specific for each cytostatic analytes). Marín et al. (2009) pointed out that only those analytes with response corrected by their own isotope-labelled analogues could be accurately quantified in all matrix samples (with high sensitivity and reliability).

5.6 Method Validation and Application

5.6.1 Method robustness

Table 5-4 displays the quality parameters obtained by the SPE–LC–MS/MS quantification method using CP-d₈ and Flu-d₇ as dual IS. Linear concentration levels ($n \geq 5$) ranged from 10 to 1000 µg/L for most compounds with good linearity ($R^2 > 0.99$). Nonlinear mass–response relationship was found for Pro and Bic at high concentrations and for Flu and Mit at low concentrations. The instrumental detection limits (IDL) for most compounds (spiked in deionized water) ranged from 0.02 to 1 µg/L except for 5-FU, Mer, Epi, Dox and Mit, which had relatively higher LOD up to 6.23 µg/L. The instrumental quantification limit (IQL) was 0.06 for Tam and 19.0 for 5-FU. The intra-day precision ranged from 1.0 to 12.1% at a low spike level (20 µg/L) and 2.4 to 13.2 % at a high spike level (200 µg/L), respectively. Furthermore, the inter-day repeatability ranged from 2.3 to 24.0 % and 2.0 to 14.8 % for the two spike levels, which indicated relatively stable instrumental performance. However, many cytostatic compounds were not very stable during sample storage (Negreira et al., 2014b). Hence, it is recommended that the calibration curve is reconstructed every week to avoid variability of MS responses over time.

Table 5-4 Method quality parameters for the simultaneous analysis of 18 cytostatic drugs (spiked in deionized water) by LC–MS/MS.

Compound	RT (min)	Linear range (µg/L)	Linearity R^2	IDL (µg/L)	IQL (µg/L)	Intra-day precision (RSD %, n=5)		Inter-day repeatability (RSD %, n=5)	
						20 µg/L	200 µg/L	20 µg/L	200 µg/L
Cyt	1.09	10–1000	0.9969	0.35	1.06	9.8	4.0	15.8	10.6
Pro	1.23	10–200	0.9945	0.77	2.34	4.6	2.5	4.8	6.1
Gem	1.22	20–500	0.9994	0.12	0.35	12.0	9.5	15.6	2.5
Met	1.24	10–500	0.9943	0.21	0.63	10.9	7.2	18.7	2.8
5-FU	1.30	20–500	0.9966	6.23	19.0	12.1	13.2	24.0	14.8
Mer	1.45	20–1000	0.9996	2.85	8.65	4.6	7.6	15.2	9.3
Aza	4.13	10–1000	0.9979	0.11	0.33	5.1	2.4	13.5	6.5
MC	4.12	1–1000	0.9990	0.08	0.24	2.0	3.0	11.6	11.4
Dox	4.53	10–1000	0.9994	3.05	9.24	6.4	5.4	8.7	4.6
Epi	4.64	10–1000	0.9997	1.16	3.52	1.2	2.9	9.6	8.1
IF	5.22	10–1000	0.9971	0.04	0.13	7.4	2.9	10.7	2.0
Mel	5.44	10–1000	0.9959	0.24	0.73	4.7	3.9	3.5	4.6
CP	5.49	10–1000	0.9958	0.26	0.78	4.3	3.7	8.2	4.4
Tam	6.39	1–1000	0.9985	0.02	0.06	7.7	2.9	4.2	2.7
Bic	7.08	10–200	0.9989	0.03	0.09	8.3	3.0	9.9	5.2
Flu	7.63	50–1000	0.9920	0.02	0.07	1.0	3.8	11.5	8.4
Mit	7.61	50–500	0.9970	2.31	6.99	1.8	3.5	2.3	3.9
Pac	8.05	10–1000	0.9995	0.80	2.43	4.7	3.9	14.7	6.4

Table 5-5 summarizes the process recovery and method detection limits (MDL) for the SPE–LC–MS/MS method developed. The process recovery for each analyte considers SPE loss, ionization suppression and instrumental variability, while the MDL mainly depends on the volume of sample extracted and the complexity of sample matrices. The isotope dilution method was successfully used to correct matrix effects for 12 analytes with process recovery ranging from 33.1–137.6 ng/L in SW, 15.0–149.7 ng/L in STP-effluent, and 13.0–143.3 ng/L in STP-influent. Based on a high sample concentration factor of 1000, low MDL was achieved for all target compounds with a range of 0.33–28.9, 0.44–31.2, and 1.23–40.8 ng/L in SW, effluent and influent, respectively. The method quantification limits (MQL, data not shown) could be simply obtained from the MDL according to their multiple relationships with S/N ratio. It is noticeable that although internal calibration curves could be constructed for Cyt, Pro, Gem, 5-FU, Mer and MC (Table 5-4), these polar compounds subjected to poor process recoveries (0~20% in most cases) mainly due to severe SPE loss and therefore, their concentrations in real samples could not be quantified in this study. As such, further study is needed to choose more specific SPE sorbents for these compounds in order to successfully determine their concentrations in environmental matrices by LC–MS/MS. An alternative approach for the environmental analysis is to apply direct injection of larger-volume environmental samples onto LC instrument without SPE procedure, which might fulfil comparable analytical results as the SPE-based method (Backe and Field, 2012).

Table 5-5 Process recoveries and method detection limits for the trace determination of 12 cytostatic drugs by SPE–LC–MS/MS in different environmental matrices at two spiking levels.

Compound	Process recovery (mean $\pm$ SD, %) (n=3) ^a						MDL (ng/L)		
	SW (20 ng/L)	SW (200 ng/L)	Effluent (20 ng/L)	Effluent (200 ng/L)	Influent (20 ng/L)	Influent (200 ng/L)	SW	Effl.	Inf.
CP-d ₈ (IS)									
CP	87.6 (2.5)	103.2 (1.7)	138.2 (13.7)	102.2 (4.4)	102.8 (9.0)	118.2 (14.9)	8.18	13.2	16.8
IF	80.5 (1.0)	89.4 (1.8)	111.9 (11.4)	83.4 (0.5)	69.2 (11.7)	86.4 (16.7)	1.63	3.50	5.17
Tam	68.8 (8.0)	89.1 (6.9)	41.2 (6.3)	40.3 (3.1)	19.4 (0.2)	29.1 (3.9)	0.33	0.58	0.90
Met	72.3 (3.1)	60.0 (0.5)	88.1 (14.4)	50.3 (4.2)	53.4 (8.3)	56.2 (15.6)	0.90	1.54	3.31
Pac	87.6 (6.6)	45.1 (10.3)	149.7 (3.6)	137.1 (23.5)	126.0 (8.4)	90.4 (5.1)	12.9	17.1	24.3
Mel	105.0 (7.2)	98.2 (6.3)	109.2 (11.7)	102.8 (13.1)	102.2 (10.1)	104.9 (3.7)	5.63	8.10	12.2
Flu-d ₇ (IS)									
Flu	122.6 (4.7)	107.5 (10.8)	132.2 (52.4)	91.1 (21.2)	83.6 (17.3)	71.5 (15.3)	4.63	8.44	19.3
Aza	137.6 (24.1)	97.8 (18.0)	106.7 (18.7)	87.4 (11.2)	47.1 (11.2)	52.1 (7.3)	1.39	12.6	26.5
Mit	77.3 (14.8)	87.8 (10.8)	80.2 (12.3)	87.4 (13.6)	41.5 (5.4)	45.9 (8.6)	14.7	24.0	36.7
Bic	36.1 (6.0)	51.1 (2.6)	20.6 (1.7)	15.0 (2.7)	13.0 (2.5)	13.7 (3.3)	0.90	11.7	13.8
Epi	59.4 (8.9)	33.1 (3.1)	44.2 (10.5)	37.2 (3.4)	85.3 (9.6)	76.1 (6.4)	13.5	22.6	31.7
Dox	101.7 (37.1)	56.7 (5.8)	108.7 (14.5)	54.4 (0.5)	84.4 (5.9)	78.2 (2.8)	11.5	24.8	27.8

Note: ^a Each 500-mL water/wastewater sample was concentrated to 500 μ L extract using Oasis HLB cartridges (220 mg, 6 cc).

5.6.2 Detection of cytostatic drugs in real samples

The optimized SPE–LC–MS/MS method with dual isotope standard calibration was applied to determine the trace concentration levels of cytostatic residue in tropical lake water, influent and effluent wastewater of a central STP and the results are shown in **Table 5-6**. Most compounds were detected at concentration levels below their respective MDL. Interestingly, the two hormone antagonists Tam and Bic were detected in all water and wastewater samples at concentration levels of 11~91 and 32~146 ng/L, respectively.

Tam is widely used for the treatment of hormone receptor-positive breast cancer in pre- and post-menopausal women, while Bic is an oral non-steroidal anti-androgen against prostate cancer. Breast cancer and prostate cancer are recently reported by Singapore Cancer Registry as the top frequent cancers in local females and males, respectively. Both cytostatic drugs appeared to experience limited removal efficiency in wastewater treatment, thereafter likely escaping into effluent-impacting surface water where natural degradation such as solar degradation seems to be ineffective. In addition, the antimetabolite Met was merely detected in influent wastewater at a trace concentration level of 3.6 ng/L. It is noticeable that although Cyt cannot be accurately determined due to extremely low process recovery, it was still detectable at ng/L-levels (> MDL) in both influent and effluent wastewater, indicating incomplete removal during wastewater treatment.

Table 5-6 Detection of cytostatic drugs in tropical lake water, STP influent and effluent wastewater.

Compound (ng/L)	Lake water		Effluent		Influent	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
Tam	10.7	0.1	90.7	10.2	87.0	5.4
Bic	32.2	0.4	38.4	10.3	146	1.0
Met	<MDL	–	<MDL	–	3.6	2.8

Note: RSD indicates relative standard deviation from triplicate sample analysis.

5.7 Chapter Conclusions

The continuous discharge of cytostatic drugs to the environment potentially harms the ecosystem and even human health when entering into the water purification system. The

simultaneous detection of multiple cytostatic compounds is a practical prerequisite for environmental screening and routine laboratory analysis. In this work, a robust analytical method was developed and validated for the trace determination of multi-class cytostatic drugs in environmental matrices based on polymeric solid-phase extraction and liquid chromatography tandem mass spectrometry equipped with polarity-switching electrospray ionization. The method was intensively optimized for sample pretreatments, instrumental conditions, and calibration approaches (extract dilution and internal calibration) in order to enhance the analytes' recoveries and to mitigate matrix effects. Twelve of the 18 target compounds were reliably quantified by multiple-reaction monitoring with dual isotope-labelled internal standards. Several polymeric sorbents with a hydrophilic-hydrophobic balance provided comparably high recoveries for relatively hydrophobic compounds, but low recoveries for polar compounds. Chromatographic separation was accomplished with C8 or C18-bonded reverse-phase columns with acidic (+ 0.1% v/v formic acid) methanol–water gradient elution. The matrix spike recoveries for these cytostatic compounds were between 13–126% for influent wastewater, 15–150% for effluent wastewater, and 33–138% for lake water with respective method detection limits down to 0.90–36.7, 0.58–24.8, and 0.33–13.5 ng/L.

The optimized method was applied for cytostatic screening in tropical aquatic environment where two hormone antagonists – tamoxifen and bicalutamide – were detected at 11–91 and 32–146 ng/L, respectively, indicating their incomplete removal during wastewater treatment and even trace presence in surface water. Further study is ongoing to carry out a nationwide environmental screening for cytostatic drugs (as well as their metabolites) by using the SPE–LC–MS/MS method developed. This study also aims to raise public concerns and precautionary actions to eliminate these detectable cytostatic drugs from the aquatic environment although their long-term fate in the environment is still beyond the knowledge of current science.

CHAPTER 6 REMOVAL OF CYTOSTATIC DRUGS BY FORWARD OSMOSIS PROCESS

6.1 Introduction

The environmental analysis in Chapter 5 showed that multiple cytostatic drugs appeared in STP wastewater and effluent-impacting surface water. The intact compounds and metabolites as well as intermediate transformation products can be still biologically active and harmful to the environment (Besse et al., 2012; Johnson et al., 2008; Parrella et al., 2014b; Rowney et al., 2009; Toolaram et al., 2014). Although their fate in the environment remains largely unclear, precautionary actions should be taken to eliminate these micropollutants by engaging either appropriate tertiary treatment to STP effluent wastewater or enhanced water purification techniques.

One of the major challenges in removing multi-class cytostatic drugs is their wide variety of physicochemical properties. The removal efficiencies for different compounds can be rather variable within a stand-alone treatment process. For example, hydrophobic tamoxifen and irinotecan ($\log P > 2$) could be effectively absorbed by powder or granular activated carbon; however, physical adsorption seems ineffective towards relatively polar cytostatic compounds (Verliefde et al., 2007). Some cytostatic drugs such as tamoxifen can be chemically destroyed during ozone treatment and AOP assisted with ultraviolet irradiation and/or photo-reactive catalysts, while others such as cyclophosphamide performed rather recalcitrant during these processes and might generate intermediate mutagens (Chen et al., 2008; Garcia-Ac et al., 2010; Hansel et al., 1997; Lester et al., 2011).

Nowadays, membrane separation technologies have been widely integrated into urban water supply systems especially in cities featuring water scarcity. Particularly, the osmotically driven FO membrane process has gained renewed interests for water reclamation and wastewater treatment purposes over the last decade, mainly because FO is promising to generate MP-free permeate with low energy demand and membrane fouling tendency (Cath et al., 2006; Zhao et al., 2012). A bench-scale FO process was

capable of effectively rejecting natural steroid hormones (Cartinella et al., 2006), non-steroidal anti-inflammatory drugs (NSAIDs) (Hancock et al., 2011; Jin et al., 2012a; Linares et al., 2011; Xie et al., 2012a; Xie et al., 2012b), and heavy metals (Jin et al., 2012b; Jin et al., 2011) from waters and wastewaters. FO was also used to significantly improve the effluent quality in a submerged FO membrane bioreactor (Achilli et al., 2009). For more, a hybrid FO–RO process was successfully applied for water reclamation from impaired water (Cath et al., 2005; Cath et al., 2010; Hancock et al., 2013), in which FO assisted to remove unwanted components present in the impaired water as a pretreatment unit. Although such information on MP removal by FO process has been increasingly reported in recent years, cytostatic drugs were not included as target contaminants. FO process remains largely unclear in terms of MP rejection characterization and mechanism elucidation compared to pressure-driven processes such as reverse osmosis (RO) and nanofiltration (NF) that have been widely applied on lab, pilot and full scales (Bellona et al., 2004; Coday et al., 2014; Kimura et al., 2003b; Radjenović et al., 2008).

This study aimed to investigate the effectiveness of using FO process (compared to RO) for removing a selected group of cytostatic drugs as a function of their physicochemical properties. The FO performance in terms of water flux, reverse salt flux, and cytostatic rejection was compared between thin-film composite (TFC) polyamide membrane and asymmetric cellulose triacetate (CTA) membrane under variable feed and draw conditions (pH, draw solution concentration, matrix components). This present work emphasized to explore the chemical structure – dependent mechanisms of cytostatic rejection during FO process. The determination of multi-class cytostatic compounds spiked at environmental relevant concentration levels was based on solid-phase extraction (SPE) and high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS). To our knowledge, this is the first study addressing the removal of trace-level cytostatic drugs by engaging a stand-alone FO process. The results are practically important when integrating FO technology to strengthen sustainable management of local water resources and wastewater.

6.2 Materials and Methods

6.2.1 Chemicals and solutions

A selected group of 13 cytostatic drugs of high environmental relevance were tested in this study, including azathioprine (Aza), bicalutamide (Bic), cyclophosphamide monohydrate (CP), doxorubicin hydrochloride (Dox), epirubicin hydrochloride (Epi), flutamide (Flu), ifosfamide monohydrate (IF), melphalan hydrochloride (Mel), methotrexate hydrochloride (Met), mitomycin C (MC), mitotane (Mit), paclitaxel (Pac), and tamoxifen citrate (Tam) supplied by Sigma–Aldrich (Singapore) with high purity ($\geq 98\%$). Individual stock solution (1.0 g/L) was prepared in HPLC-grade methanol (Merck, Singapore) and stored at -20°C . Cyclophosphamide- d_8 (CP- d_8) supplied by TLC PharmaChem (Ontario, Canada) was used as internal standard for calibration. The main physicochemical properties of the target compounds are included in **Table 2-2**.

The FO experiments were conducted using a background electrolyte containing 20 mmol/L sodium chloride and 1 mmol/L sodium bicarbonate as the feed solution (FS). A mixture of cytostatic drugs was spiked into FS to reach an environmentally relevant concentration level ($\sim 5\text{ }\mu\text{g/L}$) of individual target compound. The use of background electrolyte avoided a significant change of ionic strength in FS due to reverse salt flux and cytostatic permeation. The pH of spike FS was further adjusted to 4 and 9 with 1 M hydrochloric acid and sodium hydroxide solution to investigate the pH influence on membrane performance. Secondary effluent sampled from a local activated sludge STP was also tested as FS to elucidate the matrix effects on cytostatic rejection by FO membranes. Draw solution (DS) was prepared by dissolving sodium chloride into deionized water at concentration levels of 1 and 2 M. The deionized water was freshly taken from an integral water purification system (Millipore GmbH, Germany). All the chemicals used were reagent grade and obtained from Merck (Singapore) unless otherwise specified.

6.2.2 Bench-scale FO experiments

Two types of commercial flat-sheet FO membranes (Hydration Technology Innovations, HTI, Albany, OR) were used in the current study. One was made of cellulose triacetate (CTA) that consists of a dense active layer and a porous support layer with embedded

polyester woven mesh (Zhang et al., 2014). The other was a thin-film composite (TFC) membrane with a thin and dense polyamide active layer formed on a polysulfone substrate layer that was casted on a polyester woven mesh. All membrane samples were soaked in deionized water and stored in 4 °C prior to FO experiments. The FO membrane structures were observed under field emission scanning electron microscopy (FE–SEM, JSM-7600F, JEOL, Japan).

The membrane surface hydrophilicity was characterized by contact angle measurement, while the intrinsic separation properties such as molecular weight cut-offs (MWCO), water permeability (A value) and salt permeability (B_s value) were determined in a pressurized crossflow RO filtration test unit (Zhang et al., 2014). Briefly, sessile-drop contact angle on dry membrane surfaces were measured with a video contact angle system (VCA Optima, AST Products Inc, MA) using 5- μ L droplets of the background electrolyte solution (contact time 30 sec). The MWCO of both FO membranes was characterized by determining the rejection efficiencies for a mixture of polyethylene glycols (PEG-200 and PEG-400, Sigma-Aldrich, Singapore) in bench RO testing. The applied hydraulic pressure was changed in a range from 50 to 250 psi. The concentrations of PEG compounds in feed water and permeate were measured by scanning their positively-charged precursor ions with an LC–MS 8030 triple-quadruple mass spectrometer (Simadzu, Japan) using 50:50 methanol/water as eluent (injection volume 10 μ L). Typical mass spectrums of the PEG mixture in feed water and permeate were showed in the **Figure 6-1**. The calculated rejection efficiencies of variable PEG species served as reference lines to elucidate the size exclusion mechanism for cytostatic rejection by FO membranes.

The crossflow FO system used was similar to the one described in our previous study (Jin et al., 2011; Tang et al., 2010; Zhang et al., 2014). Briefly, a membrane coupon with an effective area of 42 cm² was placed in the acrylic membrane cell with symmetric DS flow channel and FS flow channel. Both DS and FS flow channels were filled with net-type spacers to support the membrane and enhance the flow turbulence and solution mixing. The FS and DS, both with an initial volume of 3 L, were recirculated independently at a cross-flow velocity of 20 cm/s with two digital gear

pumps (Cole-Parmer, US). An analytical balance (UX6200H, Shimadzu) interfaced with a computer-based data logging system was used to record the FS weight decrease over time for water flux calculation. Solution pH and electrical conductivity (EC) were manually monitored with a portable pH/EC meter (SG 23, Mettler Toledo, Germany).

Figure 6-1 A typical mass spectrum of PEG 200/400 mixture detected in spike feed water (at 0.1 $\mu\text{L/L}$) (a) and RO permeate (b) by LC–MS/MS operated in Q1 scan mode. Injection volume 10 μL .

The penetration of cytostatic compounds through the membrane was determined by measuring their increasing concentrations in DS at designated time intervals. A volume of 100 ml of DS was collected for cytostatic drugs analysis when 100 g water was transported from FS to DS according to the balance recording. Each batch experiment was run for 6–10 h to obtain six DS samples. Before each batch experiment, the spike FS was circulated overnight (> 12 h) at the active-layer side to saturate cytostatic adsorption to membrane and the entire FO system, while the support layer surface was soaked idly in a small volume of drug-free FS isolated in a short loop. Chemical adsorption to experimental apparatus was minimized by using stainless steel pipe, silicone tubing, and glass containers. The reduction of cytostatic concentration in the FS

during this period was determined for mass balance analysis. The FO experiments were conducted in an air-conditioned laboratory ($\sim 23\pm 1^\circ\text{C}$). All the FO experiments were carried out with the membrane active layer facing FS for better flux stability and lower fouling tendency in this membrane orientation (Tang et al., 2010; Zhao et al., 2011).

6.2.3 Analytical and theoretical methods

Cytostatic drugs in FS and DS samples were determined by SPE–LC–MS/MS analysis modified from the method described in Chapter 5. SEP was performed with Phenomenex Strata-X polymeric sorbent (3cc, 60 mg) (Torrance, CA) and high extraction recoveries (86–128%) was achieved for all analytes at pH 4 as showed in **Figure 6-2**. Small aliquots of internal standard solution was spiked into each sample extract (1 mL) to obtain a spiking level of 100 $\mu\text{g/L}$. Chromatographic separation of cytostatic drugs was fulfilled by a Phenomenex Luna C18(2) column (75mm $\times$ 2.0mm id, 3 μm) preceded by a Gemini-NX C18 guard column (4 mm $\times$ 3 mm id) (Torrance, CA) with methanol–water gradient elution (+0.1% formic acid) at a flow rate of 0.2 mL/min. The injection volume was 10 μL . MS measurements were carried out in both positive and negative ESI modes. Multiple-reaction monitoring (MRM) was applied to obtain two fragment ion transitions for both quantification and qualification purposes (see **Table 5-2**). A short dwell time of 20 msec was set for all 26 MRM transitions to shorten the analysis loop time without resolution or sensitivity loss. Based on 100 mL of sample extracted and a final extract volume of 1 mL, the method detection limits for all target compounds were in the range of 0.2 $\sim$ 30 ng/L.

Figure 6-2 SPE recoveries for cytostatic compounds with Phenomenex Strata-X C18 sorbent (3cc, 60 mg) at variable pH.

The water flux (J_w) across FO membrane was experimentally determined by the weight decrease of the FS over time. An FO process operated in the AL-FS orientation usually suffers from severe dilutive internal concentration polarization (ICP) within its porous support layer. Theoretically, the solute and water transport in an FO process follows the classical solution–diffusion process in the nonporous selective layer and the diffusion-convection transport of salt in the porous support layer (Tang et al., 2010). Eq 4-2 can be used to determine the water flux in the FO process operated in the AL–FS orientation (Tang et al., 2010), where K_m represents the effective mass transfer coefficient of salt within the membrane support layer and is defined as the ratio of the salt diffusion coefficient (D) over the membrane structural parameter (S). The structural parameter describes the equivalent ‘boundary layer thickness’ of the porous support layer of the FO membrane and depends on the actual thickness, porosity, and tortuosity of the support layer (Tang et al., 2010). On the other hand, the rejection of cytostatic compounds in the FO process can be experimentally determined by eq 6-1 (similar to eq 4-4) following Jin et al. (2011).

$$R = 1 - \frac{J_s}{J_w \cdot C_{f,s}} \quad \dots\dots\dots (6-1)$$

where $C_{f,s}$ is the solute concentration in bulk feed; J_s is the solute flux determined from the regression slope of the total solute permeate across unit membrane (g/m^2) plotted versus time. Eq 6-1 is consistent with the classical definition of membrane rejection (i.e., for RO), where the ratio J_s/J_w represents the effective concentration of solute passing through the membrane. The adverse salt flux was determined by the measurement of increasing electrical conductivity in FS (corrected by volume reduction).

6.3 Membrane Characterization and Water Flux

The characteristics of both FO membranes are summarized in **Table 6-1**. Despite its greater thickness (see FE-SEM images in **Figure 6-3**), the TFC membrane exhibited a generally higher mass transfer coefficient. Both membrane surfaces were negatively charged in the pH range of 4–9 and became more negative with increasing feed pH (Jin et al., 2012a; Xie et al., 2014). Compared with CTA, the TFC polyamide membrane was consistently more negatively charged and might consequently induce stronger

electrostatic interaction with charged or highly polar species in FS. The contact angle measurements clearly showed that the TFC membrane possessed a more hydrophilic surface. Increasing feed pH from 4 to 9 enhanced the permeability of TFC membrane by 21%. At alkaline pH, the TFC membrane became more hydrophilic and negatively charged (see **Table 6-1**), which partially explained its relatively higher water permeability coefficient. For the CTA membrane, however, the feed pH showed only tender influence on its electronegativity and hydrophobicity. Consequently, the CTA membrane remained a stable water permeability coefficient under different pH conditions. Besides, the TFC membrane exhibited a higher salt permeability coefficient. Its salt permeability decreased by half with an increased pH in the FS, probably due to a more electronegative membrane surface. By comparison, the salt permeability of the CTA membrane was also not affected by the changing pH condition in FS.

Table 6-1 Characteristics of the commercial TFC and CTA FO membranes used in this study.

FO membrane (feed pH)	TFC			CTA		
	pH 4	pH 7	pH 9	pH 4	pH 7	pH 9
Total thickness (μm)	120–132			59–83		
K_m^a (m/s)	3.11×10^{-6}	3.16×10^{-6}	4.07×10^{-6}	2.49×10^{-6}	2.88×10^{-6}	2.63×10^{-6}
Zeta potential (mV) ^b	$-6 \sim -2$	$-17 \sim -8$	$-15 \sim -22$	$-2 \sim 2$	$-6 \sim -4$	$-6 \sim -12$
Contact angle (deg)	37 ± 6	40 ± 7	34 ± 4	56 ± 7	57 ± 3	55 ± 6
Water permeability, A ($\times 10^{-12}$ m/s·Pa) ^c	8.22 ± 0.91	9.25 ± 0.83	9.93 ± 1.21	3.89 ± 0.56	3.91 ± 0.48	3.89 ± 0.47
NaCl permeability, B_s ($\times 10^{-7}$ m/s) ^c	19.9 ± 7.65	17.6 ± 7.83	9.23 ± 3.83	4.31 ± 1.75	4.41 ± 0.92	4.12 ± 1.00

Notes: ^a K_m represents the effective mass transfer coefficient of salt in the membrane support layer, determined by fitting the FO experimental data in eq 4-2; ^b refers to the literature with certain data variations due to the differences of test conditions and membrane synthesis process (Jin et al., 2012a; Xie et al., 2014); ^c tested in crossflow RO filtration mode with 20 mmol/L NaCl as feed solution under an applied hydraulic pressure ranging from 50 – 250 psi.

Figure 6-4 shows the average water flux across the TFC and CTA membranes at variable pH in the FS. Using a 1 M NaCl draw solution and the background electrolyte solution as FS, the TFC membrane produced a slightly higher water flux compared to the CTA membrane (18.3 versus 16.9 L/m²h). This can be explained by the higher A and K_m values of the TFC membrane as showed in **Table 6-1**. In addition, there was also a surge of water flux across the TFC membrane with an alkaline pH in the FS, which was attributed to the increased water permeability of the active layer and an improved K_m value in the porous layer (**Table 6-1**). On the contrary, the pH condition in the FS showed limited effect on the CTA membrane's water flux. Besides, a similar

reverse salt flux of $\sim 170 \text{ mmol/m}^2\text{h}$ was observed for the TFC and CTA membranes with 1 M NaCl draw solution. The pH condition in the FS showed minor influence on the reverse salt flux of both membranes.

Figure 6-3 FE-SEM images of cross-sectional structure and active surfaces of the TFC (a, b) and CTA (c, d) FO membranes.

Figure 6-4 Average water fluxes across the TFC and CTA FO membranes at different pH in the feed solution. FO conditions: feed solution, 20 mM NaCl and 1 mM NaHCO₃; draw solution, 1 M NaCl; membrane orientation, AL-FS; temperature, $23 \pm 1^\circ\text{C}$.

6.4 Ionization States of Analytes in Feed

The cytostatic drugs were expected to be completely dissolved in the FS in consideration of low concentration levels at 5 µg/L. The ionization states of analytes, which can strongly affect the FO rejecting behaviors, rely mainly on their intrinsic acid dissociation constants (pKa) in comparison to the pH of surrounding feed solution. As explained below in detail, the 13 target cytostatics can be further divided into 4 groups according to their ionization states at neutral pH:

- Anions (pKa < 4): CP, IF, and MC;
- Cations (pKa 7~9): Dox, Epi, and Tam (partially become neutral at pH 9);
- Nonionic molecules: Bic, Flu, Mit, and Pac, as well as Aza (though pKa 8.2).
Note that Aza is neutral at pH 4–7, but it exists dominantly as anion at pH 9.
- Zwitterions: Mel and Met.

The existing forms of cytostatic compounds in the FS mainly depend on their *Brønsted–Lowry* acid–base reactions occurred in the aqueous phase (described as pKa). The two important alkylating agents CP and IF are structural isomers, both are weak acids with relatively low pKa values and therefore mainly exist in negatively charged forms in the experimental pH range. As such, a weak interaction was expected to occur between these two hydrophilic anionic compounds with the membrane surfaces (Wang et al., 2009). Similarly, the hydrophilic MC molecule is readily deprotonated at positions of the basic 7-amino group, N-4 nitrogen, and aziridine nitrogen with low pKa values (Brittain, 2007). Consequently, MC also exists mainly as anionic molecules in the feed solution in the entire experimental pH range.

Figure 6-5 illustrates the existing forms of Mel and Met in the FS. The aromatic nitrogen mustard Mel exists as a zwitterion in the feed solution due to its amino acid structure, which has a pKa of 1.83 and 9.13 for the carboxylic acid (α -COOH) and amine (α -NH₃⁺) functional groups, respectively (Fasman and Sober, 1977). Hence, Mel was almost totally ionized in neutral condition, and the positively charged primary amine group might favor its electrostatic attraction to negatively charged membrane surfaces. Similarly, Met is also a zwitterion due to co-existence of two carboxylic acid groups (α -COOH) and two primary amines (α -NH₃⁺) as well as a tertiary amine in its structure. The positively charged amine moieties might selectively induce strong

membrane adsorption to the negatively charged FO membranes in the experimental pH range. It is worth noting that the nitrogen atom in amide structure ($R'-CO-NH-R''$) is not basic due to delocalization of the lone pair into an adjacent $C=N$ double bond.

Figure 6-5 Simultaneous electrostatic repulsion and attraction of two zwitterionic cytostatic compounds (Mel and Met) with the negatively charged FO membranes.

The hydrophilic Aza molecule is a weak base due to the limited liberation of the tautomeric proton at the purine ring, and can undergo degradation in alkali medium by the cleavage of the sulfide bond (Connors, 1986). Aza therefore existed mainly as unionized neutral molecules at acidic-to-neutral conditions and slightly deprotonated as an anionic form in an alkaline medium. As such, weak membrane adsorption was expected for this compound. Dox and Epi are two stereoisomeric anthracyclines, both having a primary amine ($\alpha-NH_3^+$) in the daunosamine moiety with a basic pK_a . They are commercially available in hydrochloride formulations, i.e., doxorubicin·HCl and epirubicin·HCl. Dox and Epi were likely dissolved as cationic molecules in the FS within the experimental pH range.

Similarly, Tam existed mainly in its conjugated acid form ($M \cdot H^+$) as cationic molecules in the FS. This was likely to occur because the Tam molecule has a tertiary amine ($R'-NMe_2$) moiety and an ether ($R'-O-R''$) group, where the N and O atoms are highly electronegative due to the presence of unshared electron pairs and readily form hydrogen bonds with free protons released from its citrate chelate complex (commercial

formulation as tamoxifen citrate). However, unlike Tam, the hydrophobic Pac, Flu, Bic, and Mit existed dominantly in their unionized forms at pH 4~9 as they have much higher pKa values (≥ 12), while the electron-withdrawing properties of halogens (fluorine and chlorine) and highly polarized ester groups present in structure were likely to induce electrostatic repulsion against the negatively charged membrane surfaces.

6.5 Membrane Adsorption

Prior to FO rejection tests, the FO membranes were conditioned overnight in the background electrolyte solution at both sides, but with spike cytostatic drugs only at the active-layer side. **Figure 6-6** illustrates the loss of cytostatic compounds during this pre-conditioning process at neutral pH, which was likely attributed to adsorption to FO membranes. In general, anionic compounds (CP, IF, MC), and uncharged compounds (Aza, Bic, Flu, Mit, Pac) showed limited adsorption of less than 30% to both TFC and CTA membranes. In contrast, zwitterionic compounds (Mel and Met) and cationic compounds (Epi, Dox, and Tam) were subjected to marked membrane adsorption. A poor correlation was found between membrane adsorption and analytes' hydrophobic properties ($\log P$) ($R^2 = 0.11$ for TFC and 0.08 for CTA).

Figure 6-6 Adsorption of cytostatic compounds to the TFC and CTA FO membranes under neutral condition.

In general, chemical adsorption to membranes can be mainly attributed to hydrophobic interaction and electrostatic attraction (Bellona and Drewes, 2005; Bellona et al., 2004; Kimura et al., 2003a). The strong dependence of adsorption on the compounds' ionization states and its poor correlation with logP values suggest that electrostatic interaction is likely playing a more dominant role in the current study. Specifically, the negatively charged membranes convey an attractive force to cationic compounds including Dox, Epi, and Tam, and therefore significantly enhance their electrostatic adsorption to membrane surfaces. This was also evidenced by the strong tendency to stain the membranes by the red-colored anions Dox and Epi as showed in **Figure 6-7**.

Figure 6-7 SEM images and photos (right top) of the active surface of an original (a) and a used (b) TFC membranes. The difference shows the strong tendency to stain the membrane by the red-colored anions doxorubicin and epirubicin after FO operation.

Similarly, the high adsorption of zwitterionic Mel and Met molecules can be readily explained by the molecule-membrane electrostatic attraction, where the positively charged moieties are preferentially oriented towards the membrane. Overall, the more negatively charged TFC membrane generally exhibited greater adsorption for both cationic and zwitterionic compounds, which is consistent with the electrostatic interaction mechanism. The anionic compounds and uncharged compounds do not suffer from molecule-membrane electrostatic attraction, which explains for their much lower adsorption. Interestingly, these compounds generally had relatively greater adsorption onto the more hydrophobic CTA membrane, revealing the secondary role of hydrophobic interaction in the current study.

6.6 FO Rejection of Cytostatic Drugs and Influencing Factors

6.6.1 TFC versus CTA membrane

Figure 6-8 illustrated the solute fluxes (normalized by its driving force, i.e., the feed concentration) and FO rejection efficiencies for target cytostatic compounds at neutral pH. The normalized solute flux (i.e., J_s/C_f in eq 6-1) serves as a good indicator for evaluating the solute permeation potency in FO process. As showed in **Figure 6-8a**, the J_s/C_f value varied in a wide range from zero to around 10 L/m²h for most cytostatic compounds, though higher values were observed for Flu and Mit with the CTA membrane. Generally, both CTA and TFC membranes showed consistently low solute fluxes for anionic compounds (i.e., CP, IF, MC), but relatively high fluxes for most cationic, zwitterionic, and neutral compounds except Met, Tam, and Pac. This reveals the important role of electrostatic interaction in governing the rejection of cytostatic drugs. Nevertheless, comparison between **Figures 6-6** and **6-8a** shows some significant differences. In particular, some nonionic small-molecular-weight compounds Flu (276 Da) and Mit (320 Da) also experienced high normalized solute flux (**Figure 6-8a**) though these compounds had only moderate adsorption (**Figure 6-6**). Such differences can be explained by the important difference between filtration and adsorption – the former is also significantly affected by the size exclusion mechanism (Bellona et al., 2004; Coday et al., 2014).

In order to gain a better understanding of the size exclusion effect, membrane rejection of neutral PEG molecules were performed. The PEG rejection tests revealed that the CTA membrane has a larger MWCO value (MWCO = 220 Da for TFC and 320 Da for CTA, **Figure 6-9**), indicating a bigger pore size in comparison with the TFC membrane. Consistent with the current study, Jin et al. (2012a) also reported that the CTA membrane had a larger pore size (indicated by lower glucose rejection) compared to TFC polyamide membranes. This largely explains the CTA membrane's generally higher solute fluxes and poorer rejection efficiencies for most cytostatic compounds as showed in **Figure 6-8**. With a weaker size-exclusion effect, the relatively 'loose' CTA membrane showed unsatisfactory rejection to six of the 13 target compounds (i.e., Mel, Dox, Epi, Bic, Flu, and Mit) with rejection efficiencies less than 80%. Particularly, the CTA membrane exhibited rather high solute fluxes for the neutral Flu and Mit

molecules, for which the calculated rejection efficiencies turned out to be negative values based on eq 6-1. As discussed earlier, these two molecules are characterized with relatively low MWs (276 Da for Flu and 320 Da for Mit). Furthermore, these molecules are also relatively hydrophobic (logP 3.14 for Flu and 5.84 for Mit). The combination of relatively large MWCO value of the CTA membrane (320 Da), small molecular size, and high logP explains for the peculiar behavior of Flu and Mit when treated by the CTA membrane.

Figure 6-8 Feed-concentration-normalized solute fluxes (a) and FO rejection efficiencies (b) for cytostatic compounds with the TFC polyamide and CTA membranes. The asterisks in (b) indicate negative rejection efficiencies calculated for Flu and Mit based on the eq 6-1.

Figure 6-9. Rejection of PEG compounds in crossflow RO filtration tests at neutral feed pH with the TFC (a) and CTA (b) membranes. A hydraulic pressure of 50 psi was applied on the TFC membrane (and 150 psi for CTA) to produce a comparable water flux of 14 L/m²h (and 16 L/m²h for CTA) as in FO process.

6.6.2 MWCO and size exclusion

Figure 6-10 further presents the cytostatic rejection in FO mode with the CTA and TFC membranes as a function of molecular weights. For comparison purpose, the rejection of neutral hydrophilic PEG molecules tested in RO mode under similar operational conditions is superimposed in **Figure 6-10**. Although there seemed to be a general good agreement with the cytostatic rejection and PEG rejection for most of the compounds, some notable exceptions (Flu, Mit, Mel, Epi, and Dox) with significantly lower rejection compared to the PEG baseline were also observed. These exceptionally low-rejection compounds can be classified into two groups:

- Cationic (Epi and Dox) and/or zwitterionic (Mel) compounds, who suffers from unfavorable molecule-membrane electrostatic attraction;
- Neutral hydrophobic molecules (Flu, Mit, and Bic, logP 3.14 – 5.84), whose reduced rejection (in comparison to PEG) can be attributed to the strong hydrophobic interaction and the absence of electrostatic repulsion.

In contrast, the anionic compounds (CP, IF, and MC) shows consistently high rejection irrespective of their MW values. Similarly, the neutral hydrophilic compound Aza (logP -0.04) maintained high rejection of > 90% despite of its relatively low MW (277 Da). In comparison to the TFC membrane, the CTA membrane not only experienced lower

rejection for a given compound but also greater scattering of the rejection data points around the PGE rejection curve. With a weaker size-exclusion effect, the electrostatic and/or hydrophobic interaction with the negatively charged and medium hydrophobic CTA membrane was likely to play a more dominant role in the rejection of cytostatic compounds (Jin et al., 2012a; Lin and Lee, 2014). The current study suggests much greater risks associated with the use of the CTA membrane for cytostatic compounds rejection.

Figure 6-10 FO and RO rejection efficiencies for cytostatic compounds at neutral feed pH as a function of their molecular weights with the TFC (a) and CTA (b) membranes.

Figure 6-10 also presents the rejection efficiencies of target compounds tested in RO mode, showing comparable but slightly lower rejection efficiencies for most compounds. This disparity was probably attributed to the coupled reverse solute diffusion in the FO process, which might retard the diffusion of organic solutes across the selective layer (Xie et al., 2012a). Alternatively, this could arise from the different mechanical stress environment (low stress condition in FO while high stress condition in RO). On the other hand, the cations Epi and Dox as well as the zwitterion Mel showed an opposite trend, with their RO rejections significantly higher than the respective FO rejections. While the exact mechanism is not well understood, this phenomenon may be attributed to cation exchange under FO mode. That is, the negatively charged membrane acts with ion exchange material and allows the cationic cytostatic compounds to exchange with the cations from the draw solution (Irvine et al., 2013; Lu et al., 2014).

6.6.3 Effect of draw solution

Figure 6-11 illustrated the effect of draw solution concentration on cytostatic rejection efficiencies with the TFC membrane. With an increased draw concentration, the rejection efficiency was significantly improved ($\geq 90\%$ except Mel) for the target cytostatic compounds. This is explained by the significantly increased water flux ($26.7 \text{ L/m}^2\text{h}$) when using 2M NaCl DS, which improves the FO rejection efficiency due to an inversely proportional relationship in between according to eq 6-1. In an FO process, the concurrent solute flux and water flux are considered as two independent processes according to the classical solution-diffusion theory (Jin et al., 2011). The improved water flux favors to dilute the effective concentrations of organic solutes within the support layer, leading to improved rejection. Similar dilution effects have been commonly reported in the RO literature (Fane et al., 2011). In order to ensure a high rejection to unwanted trace organics, a relatively high water flux is therefore suggested to operate the FO process by using appropriate draw solutions.

Figure 6-11 Effect of draw concentration on FO rejection to cytostatic compounds with the TFC polyamide membrane.

6.6.4 Effect of feed pH

Figure 6-12 illustrates the effect of feed pH on trace organic fluxes and FO rejection to cytostatic compounds with the TFC membrane (see Figure 6-13 for CTA). Overall, the anionic cytostatic compounds (CP, IF, and MC) were well rejected at all pHs, suggesting that the electrostatic repulsive force was instrumental in maintaining membrane rejection. Similarly, the rejection of large molecules (e.g., Pac 854 Da) was

little affected by pH due to the size exclusion mechanism. However, significant decrease in rejection occurred for Mel, Flu, and Mit when pH was increased from 4 to 9. In general, the change in rejection can be attributed to the shift in size exclusion and/or electrostatic interactions.

For the neutral molecules Flu and Mit, their solute fluxes increased significantly at pH 9 (Figure 6-12a), suggesting a weaker size exclusion mechanism possibly due to a more loose membrane rejection layer. The hypothesis of loose membrane rejection layer is consistent with the observed higher water flux at pH 9 (Figure 6-4). Compared to the neutral compounds Flu and Mit, the zwitterionic Mel experienced much more severe rejection loss. In addition to a weaker size exclusion effect, this compound also suffers from the enhanced electrostatic attraction due to the more negatively charged TFC membrane surface at pH 9. In contrast, Dox and Epi had greater rejections at pH 9 compared to their respective values at pH 7. The amine functional groups of Dox and Epi have pKa values of 8.2 and 7.7, respectively. Thus, these molecules exist mainly in the form of cations at pH 7 and as neutral hydrophilic compounds at pH 9. The elimination of the unfavorable electrostatic attraction thus explains the improved rejection at pH 9. Consistent with this explanation, membrane adsorption of Dox and Epi was dramatically reduced from > 90% to about 20% when pH increased from 7 to 9.

Figure 6-12 Effect of feed pH on trace organic fluxes (a) and FO rejection (b) to cytosstatic compounds with the TFC membrane. The asterisks indicate that Dox, Epi, Tam, and Aza (with pKa 7~9) change their ionization states at pH 9: Dox (neutral), Epi (neutral), Tam (partially neutral), and Aza (anionic).

Figure 6-13. Effect of feed pH on trace organic fluxes (a) and FO rejection (b) to cytostatic compounds with the CTA membrane. The asterisks indicate that the calculated rejection efficiencies (based on eq 6-1) were negative values for Flu and Mit, and Dox, Epi, Tam, and Aza (with pKa 7~9) change their ionization states at pH 9: Dox (neutral), Epi (neutral), Tam (partially neutral), and Aza (anionic).

6.6.5 Effect of feed matrix

Figure 6-14 compares the FO rejection to cytostatic compounds spiked in two different FS (i.e., electrolyte solution and effluent wastewater) with the TFC membrane. As showed in the Figure 6-4, the FO process with effluent as FS showed a comparable water flux of ~19.1 L/m²h during the 8-hour FO operation, and no significant membrane fouling was observed. Overall, the FO process exhibited improved rejection to cationic and zwitterionic compounds, while little changes were observed for the anionic and nonionic compounds. This enhancement effect can be attributed to the sorption of cationic and zwitterionic compounds by the effluent organic matter, given that typical effluent organic matter is negatively charged (Shon et al., 2006; Tang and Peng, 2011). Overall, the FO process using the TFC membrane could provide generally high rejection (more than 80%) to all target cytostatic compounds in real wastewater matrix. The rejection efficiency can be further improved when hyphenating the FO process with other water and wastewater treatment units such as membrane reactor, RO filtration, or ozone treatment. Further study might be needed to understand how those matrix components affect membrane fouling and the rejection of trace organic solutes in an FO process.

Figure 6-14 Matrix effect on trace organic fluxes (a) and FO rejection (b) to cytostatic compounds with the TFC membrane.

6.7 Implications

This study demonstrated that an FO process can be used to effectively remove various toxic cytostatic compounds from water and wastewater. The rejection efficiency mainly depends on three aspects: 1) the separation properties of FO membranes such as membrane materials and MWCO; 2) the physicochemical properties of trace organics such as molecular weight, charge, and hydrophobicity; 3) the FO operational conditions such as the chemistry of feed solution and operational water flux. Synergistic effects of steric hindrance, electrostatic repulsion/attraction, and hydrophobic interaction take place during the FO rejection towards different trace organic compounds. The present work shows that the TFC membrane generally serves better for MP-removal purpose compared to the CTA membrane. However, particular attention should be paid to cationic and zwitterionic trace organics with high membrane adsorption properties as well as the small hydrophobic nonionic compounds, as they reveal higher potential of penetrating through the FO membranes.

Cytostatic drugs are increasingly consumed to cure cancer patients. Hospital effluents are considered as important point source discharges of cytostatic drugs and other PhACs as well (Langford and Thomas, 2009; Verlicchi et al., 2012a; Zhang et al., 2013a). Precautionary actions are needed to prevent those harmful trace organic contaminants from entering the sewage system. FO process provides a promising technology for this purpose. In real-world applications, a hybrid process hyphenating FO with other

water/wastewater treatment units such as MBR (Holloway et al., 2014; Lay et al., 2011) and RO (Cath et al., 2010) can be used to deal with the cytostatic drugs-containing hospital effluent as well as other MP-containing wastewaters, such as source-separated urine from cancer patients and manufacturing effluent.

In other applications, FO was engaged to concentrate chosen feed solution (e.g., urine and other nutrient-enriched wastewaters) using seawater desalination brine as low-cost draw solution (Holloway et al., 2007; Zhang et al., 2014). FO might provide a robust barrier to prevent various MPs from seeping into the brine-receiving ocean to reduce harm to marine ecosystem. Additional ozone treatment or AOPs can be used to further chemically destroy the trace organics in FO concentrate. FO can be also used to recover fresh water from local brackish water for irrigation proposes using blended fertilizer as draw solution (Phuntsho et al., 2011). In such case, the FO membrane should be carefully selected to effectively prevent MPs from entering into the agricultural food chain. Nowadays, the demand of clean water resource has been fueled by a surging world population. Water reclamation from slightly polluted or brackish water provides a promising way to solve water deficits and alleviate water pollution. A hybrid FO–RO process can be applied for this purpose (Hancock et al., 2013), particularly in view of the low energy demand and membrane fouling tendency of an FO process (Cath et al., 2006; Zhao et al., 2012). In order to ensure safe use of the water product generated from the hybrid process, further study is needed to investigate whether such configuration could effectively barrier various MP including cytostatic trace organics.

6.8 Chapter Conclusions

Cytostatic drugs are increasingly used in cancer chemotherapy in the shade of ramping-up global cancer burden, many of which are carcinogenic and mutagenic compounds. The continuous discharge of cytostatic drugs and metabolites to the environment due to incomplete degradation during wastewater treatment potentially harms the ecosystem and human health. For the first time, the study investigated the removal of a selected group of 13 cytostatic drugs using FO membrane process. The rejection mechanisms were studied with thin-film composite (TFC) polyamide and cellulose triacetate (CTA)

membranes under variable conditions. The TFC membrane produced a relatively higher water flux of 18.3 L/m²h compared to the CTA membrane due to higher water permeability but smaller structural parameter; although both were jeopardized by a similar reverse salt flux of 170 mmol/m²h.

A high proportion of cationic (doxorubicin, epirubicin, and tamoxifen) and zwitterionic (melphalan and methotrexate) compounds were adsorbed to membrane surfaces by electrostatic attraction, resulting in generally higher solute fluxes and lower rejection efficiencies (except tamoxifen). Both membranes exhibited consistently high rejection (> 90%) to anionic cytostatic compounds. The TFC membrane showed generally better rejection to all cytostatics with a greater size-exclusion effect (as indicated by a smaller molecular weight cut-off value). Higher draw solution concentration led to enhanced FO rejection as a result of dilution effect. The presence of effluent matrix in the feed solution generally improved the FO rejection to cationic and zwitterionic compounds. On the other hand, increasing feed pH from 7 to 9 resulted in varied response in rejection as a consequence of complex interplay of size exclusion and electrostatic interaction. The results are practically important when integrating FO technology to strengthen sustainable management of local water resources and wastewater.

CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions and Contributions

This study explores new approaches to improve the current wastewater management practice particularly in terms of nutrient recovery and micropollutant mitigation from both scientific and engineering perspectives. The important findings and scientific contributions are summarized as below.

- 1) This study provides a systematic literature review on current research on the environmental presence and transformation, potential ecotoxicity and various source contributions of cytostatic compounds in the environment. Several research gaps have been identified in this domain. For examples, previous research has focused mainly on hospital effluents but few on household discharges. The latter contributes to the cytostatic residue in the environment as an increasingly important source. More urgently, solid ecotoxicity data is needed to further support or exclude their ‘subtle’ yet chronic effects on the ecosystem. The review also reveals urinary excretion as a major contributor to cytostatic drugs appeared in the water cycle. As such, source separation strategy is promising to improve the current centralized wastewater management in a more sustainable way, especially in terms of nutrient/energy recovery and separate treatment of pharmaceutical residue.
- 2) The present study further evaluated the feasibility and potential limitations of urine source separation in a densely-populated tropical urban setting. Systematical investigations were conducted on urine hydrolysis enhancement, pipe-clogging prevention, odor emission mitigation, and nutrient recovery. Particularly, seawater and desalination brine were successfully explored as cost-effective magnesium sources to recover phosphorus from hydrolyzed urine. The main product was struvite crystals and can be used as good-quality slow-releasing fertilizer. An air stripping process can be used to effectively recover ammonia from source-separated urine and the experimental parameters provided certain guidelines for the development of N-recovery process in industrial-scale.

- 3) This study demonstrated the feasibility of applying FO dewatering process for nutrient recovery from source-separated urine based on the bench-scale experiments and modeling. Seawater or desalination brine can be used as low-cost draw solution in this process to achieve relatively high water fluxes up to 20 L/m²·h and satisfactory rejection of ammonium, phosphate, potassium in hydrolyzed urine. Compared to other urine treatment options, the FO process was cost-effective and environmentally-friendly for nutrient recovery from urban wastewater at source. Based on the FO process, a novel decentralized source-separating sanitation system was designed to convert domestic waste into resources (nutrients and biogas) and minimize environmental discharge. The integral system promises to deal with domestic nutrients and various excreta-derived PhACs at the source rather than the conventional end-of-pipe practice.
- 4) In this work, a robust analytical method was developed and validated for the trace determination of multi-class cytostatic drugs in different environmental matrices based on polymeric solid-phase extraction and liquid chromatography tandem mass spectrometry. For this first time, the optimized method was applied for cytostatic screening in tropical aquatic environment. Two hormone antagonists, i.e., tamoxifen and bicalutamide, were detected at ng/L-levels in STP influent and effluent wastewater samples, indicating incomplete removal during wastewater treatment. This study also aims to raise public concerns and precautionary actions to eliminate these detectable cytostatic drugs from the aquatic environment although their long-term fate in the environment is still beyond the knowledge of current science.
- 5) Relying on the analytical method, this study further investigated the effectiveness of using FO process (compared to RO) for removing a selected group of cytostatic drugs as a function of their physicochemical properties. Special effort was made to elucidate the chemical structure – dependent mechanisms of cytostatic rejection during FO process. To our best knowledge, this was the first study to investigate the removal of a selected group of cytostatic drugs using an FO membrane process. The results are practically important when integrating FO technology to strengthen sustainable management of local water resources and wastewater.

7.2 Recommendations and Future Work

There are important issues worthwhile for further investigation in order to improve the water safety and sustainable wastewater management in an urban setting. The following raises some important questions to be answered in this research area:

- Despite the respective seeming advantages of source separation and treatment technology, a combined strategy may cost-effectively prevent the cytostatic residue from seeping into the environment. However, the combination calls for further evaluation on the associated technological, social-economic and administrative issues at hand. A comprehensive life-cycle impact assessment might be needed to evaluate and optimize the overall system performance at pilot and full scale operation.
- This study has demonstrated the effectiveness of using FO membrane process for the removal of cytostatic drugs. Further study is also worthwhile to investigate and compare the performance in PhACs removal using other important membrane technologies such as pressure-driven nanofiltration and reverse osmosis filtration. Special attention should be paid to the further treatment of PhACs-containing retentate generated from the filtration processes. Ozone treatment and AOPs can be further investigated for this purpose. This is particularly important when using an FO process for both nutrient recovery and PhACs removal purposes (e.g., from source-separated urine). The final concentrated urine products should not contain any PhACs in order to ensure its safe use in agriculture.
- Further fundamental understanding is required on the trace contaminants removal by various technical alternatives, such as advanced MBR, RO/FO, and AOPs. Fair judgment cannot be made on these technologies regarding their intrinsic advantages, drawbacks and development trends.
- Although acute toxicity to aquatic organisms is unlikely to occur at measured environmental concentrations, there is room to improve the current standard ecotoxicity tests in order to better assess the actual impacts of trace organic PhACs

to the ecosystem. Further study is strongly recommended to provide more accurate ecotoxicological information with particular regard to chronic impacts of cytostatic drugs at environmentally relevant concentrations and synergistic toxic effects from coexisting metabolites and other PhACs.

- Current research largely focuses on the intact cytostatic drugs rather than their various pharmaceutically active metabolites. Previous studies have pointed out that those metabolites excreted from human bodies can be still pharmaceutically active with potential environmental impacts not to be negligible. Further study is needed to identify the priority metabolites of various cytostatic drugs and other pharmaceuticals especially in view of their high excretion rates and suspected environmental impacts. These priority cytostatic metabolites should be included in the LC–MS/MS detection method coupled with proper isotope dilution calibration. Based on the analytical method, further study is needed to carry out a nationwide environmental screening for cytostatic drugs and their metabolites.
- Future study might be also interesting to predict the environmental concentrations of various cytostatic drugs and metabolites with the provision of accurate information on pharmaceutical consumption from local hospitals and government agencies. Based on the measured and predicted environmental concentrations, comprehensive environmental risk assessment (ERA) can be conducted for this special group of pharmaceuticals and other PhACs at the national scale. The ERA study will provide strong scientific data support for policy making purpose.

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