OCCURRENCE AND FATE OF ARTIFICIAL SWEETENERS AND PERFLUORINATED COMPOUNDS IN A WASTEWATER RECLAMATION PLANT IN SINGAPORE

GAN JIE

NATIONAL UNIVERSITY OF SINGAPORE

2014

OCCURRENCE AND FATE OF ARTIFICIAL SWEETENERS AND PERFLUORINATED COMPOUNDS IN A WASTEWATER RECLAMATION PLANT IN SINGAPORE

GAN JIE

(B.Eng.(Hons), NUS)

A THESIS SUBMITTED
FOR THE DEGREE OF MASTER OF ENGINEERING

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

NATIONAL UNIVERSITY OF SINGAPORE

2014

DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Gan Jie
10 July 2014

Acknowledgements

I owe my deepest appreciation and gratitude to my supervisor, Associate Professor Gin Yew-Hoong, Karina, whose constructive guidance and invaluable support throughout the entire project enabled me to gain a fruitful experience. Her kindness and encouragement in both research and life have kept me optimistic and enthusiastic on research and study.

In addition, I would like to express my gratitude to Professor Martin Reinhard and research fellow Dr. Nguyen Viet Tung for their insightful suggestions on my research. Professor Reinhard's enthusiasm in science inspired me tremendously to think deeply and his profound knowledge enhanced my understanding on research topics. Dr. Nguyen taught me a lot on the knowledge background and instrument usage from the beginning of the project and he was always willing to help me whenever I was faced with any difficulty on my research work.

Next, I am heartily thankful to the whole group of A/P Gin for useful suggestions, consumables purchase and fantastic group gatherings which made me conduct experiments smoothly in a warm and happy mood. Especially, I would like to express my big thanks to You Luhua, Chen Huiting and Ling Ran. They kept encouraging me when I was upset on experiments, giving useful advice on experiment improvements and data analysis and providing prompt help whenever I needed it.

Furthermore, I am grateful to all laboratory and administrative staff in Environmental Engineering, especially Ms. Chia Yuit Ching, Susan, Mr. Sit Beng Chiat, Mr. Sukiantor Bin Tokiman and Ms. Phyllis Lim Bee Hoon for their good laboratory and project management and help in field samplings.

Moreover, I would like to express my thanks to Professor Li Fong Yau, Sam and Associate Professor Hu Jiangyong for their generous sharing of equipments which facilitated the smoothness of my project.

In addition, I am thankful to NUS Environmental Research Institute and the research staff and laboratory staff there for their company and well management to keep a tidy and safe working environment.

I appreciate continuous support from PUB, especially Dr. Ankur Duarah and Dr. Lifeng Zhang for their project coordination.

Furthermore, I am sincerely grateful to my dearest family members for their encouragement and my friends, especially Miss Wang Bingyu for their continuous care and support during the program.

Lastly, I offer my regards to all of those who supported me in any respect and made this thesis possible.

Table of Contents

Summary

Water scarcity can cause health issues, constrain economic growth and promote social unrest. As a result, wastewater reclamation is an important solution, especially for water scarce countries such as Singapore. In Singapore, reclaimed water, i.e. NEWater, is considered as one of the national taps for water supply, where effluent from domestic wastewater is used as the inflow into NEWater reclamation plants. However, rapid economic development and change in life styles have led to the continuous release of emerging organic contaminants (EOCs) into wastewater, which poses new challenges in wastewater treatment. Artificial sweeteners (ASs) and perfluorinated compounds (PFCs), as two typical classes of EOCs, were studied in the present research because they are refractory compounds that are ubiquitous in the environment and (potentially) toxic to human health and/or ecology.

The occurrence and fate of ASs and PFCs were investigated by monitoring both the dissolved and suspended solid phase concentrations in wastewater samples collected from 9 different points along treatment trains at a wastewater treatment plant (WWTP) in Singapore. All the targeted ASs and PFCs were detected. The ASs included ACE, SUC, CYC and SAC with total influent concentrations of around 66.82 ppb and removal efficiency of 84%. The majority of ASs was present in the dissolved phase due to their high solubility. CYC and SAC were dominant in the influents but ACE and SUC were dominant in the effluents, because the latter are more resistant to biological processes. Regardless of the potentially low sorption tendency of ASs, sorption and sedimentation of suspended solids were suggested to be the main removal mechanism for ACE and SUC in the WWTP. In addition, 8

PFCs were analyzed, including carboxylic acids, sulfonates and derivatives with different C-F chain lengths. The influents carried approximately 197.6 ppt total PFCs with PFOS and PFBA as the dominant species. The removal rates for PFCs in the WWTP were less than 43%. Both effluent and sludge were considered significant sinks for PFCs, especially MLSS which contained much higher PFCs content.

In addition, bench-scale experiments were conducted to confirm the sorption affinities of selected ASs and PFCs on activated biomass. For ASs, wet biomass with and without inhibition by NaN_3 were tested. An 18% removal of SUC was achieved in 17 days. However, no sorption of ACE was observed for tests with inhibited biomass. In comparison, a 70% reduction was observed for ACE in aqueous solution for tests without NaN_3-inhibition, indicating high biodegradation potential. Furthermore, most PFCs showed high sorption uptake on activated biomass, except for PFBA, PFHxA and PFBS with shorter C-F chain lengths. Compared to the NaN_3-inhibited dried biomass, lyophilization-heat treated (USEPA-method) sludge showed longer equilibrium time (<1day) and higher sorption capacity for PFCs. This demonstrates that different pre-treatment methods for biomass in sorption studies can affect the interpretation of results. Overall, however, our study showed that within the same family, compounds with longer C-F chain length had higher sorption affinity for biomass, highlighting hydrophobic interactions. Furthermore, sulfonates showed higher sorption capacity than carboxylic acids.

List of Tables

List of Figures

Nomenclature

6:2 FTS	6:2 Fluorotelomer sulfonate
8:2 FtOH	8:2 Fluorotelomeralkohol
A1/B1	Primary settled sewage in Southworks/Northworks
A2/B2	Mixed liquor suspended solids in Southworks/Northworks
A3/B3	Effluent/Membrane permeate in Southworks/Northworks
A4/B4	Return activated sludge in Southworks/Northworks
ACE	Acesulfame
ACE-K	Acesulfame potassium
ADI	Acceptable daily intake (mg/kg body weight)
AS	Artificial sweeteners
ASP	Aspartame
BT	Benzothiazole
BTr	Benzotriazole
C_0	Initial concentration of analytes in the aqueous phase
CAS	Conventional activated sludge
CAS No.	Chemical Abstracts Service registry number
CE	Collision energy
C_s	Dried mixed liquor suspended solids concentration (g/L)
C_t	Concentration of analytes in the aqueous phase at time t

CYC	Cyclamate
d	day
DCF	Diclofenac
DEHP	Bis (2-ethylhexyl) phthalate
DI	Deionized water
DP	Dissolved phase
DWI	Drinking Water Inspectorate
E1	Estrone
EC	The European Council
EFSA	European Food Safety Authority
EOCs	Emerging organic contaminants
EP	The European Council
ESI	Electrospray ionization
EU	European Union
eV	Electron Volt
FDA	The U.S. Food and Drug Administration
FOA	The Food and Agriculture Organization of the United Nations
FOSA	Fluorooctane sulfonamide
FOSAA	Perfluorooctanesulfonamidoacetate
FOSE	Fluorooctane sulfonamidoethanols
FtOH	Fluorotelomer alcohol

g	Earth's gravitational acceleration
g/mg-d	Gram/milligram per day
GAC	Granular activated carbon
HDPE	High-density polyethylene
IBD	Inflammatory bowel disease
IDL	Instrument detection limit
Inf	Influent wastewater
K_2HPO_4	Dipotassium phosphate
K_d	Distribution coefficient (L/g)
Kg	Kilogram
KH_2PO_4	Monopotassium phosphate
km^2	Square kilometer
km^3	Cubic kilometer
K_{ow}	Octanol-water partition coefficient
L	Liter
LC	Liquid chromatography
LC-ESI-MS/MS	Liquid chromatography-electrospray ionization-multi-stage mass spectrometry
LD50	Median lethal dose
LOD	Limit of detection
LOQ	Limit of quantification
LTM	Liquid treatment module

m/z	Mass to charge number ratio
M-WWTPs	Municipal wastewater treatment plants
MBR	Membrane bioreactor
MDL	Method detection limit
mg/g	Milligram/gram
mg/L	Milligram/liter
min	Minute or Minutes
mL	Milliliter
MLD	Million liter per day
MLE	Modified Ludzack-Ettinger
MLSS	Mixed liquor suspended solids
Mm	Millimeter
mM	Millimolar
MRM	Multiple reaction monitoring
N-EtFOSA	N-ethyl perfluorooctane sulfonamide
N-EtFOSAA	N-ethyl perfluorooctane sulfonamido acetic acid
N-EtFOSE	N-ethyl perfluorooctane sulfonamidoethanol
NaN_3	Sodium azide
ng/g dw	Nanogram analyte per gram of dry weight of solid
ng/L	Nanogram per liter
CH_3COONH_4	Ammonium acetate

NIEHS	National Institute of Environmental Health Sciences
NP	Nonylphenol
n.r.	Not reported
OECD	The Organization for Economic Co-operation and Development
PFBA	Perfluorobutyric acid
PFBS	Perfluorobutane sulfonate
PFCAs	Perfluorinated carboxylic acids
PFCs	Perfluorinated compounds
PFDA	Perfluorodecanoic acid
PFDOA	Perfluorododecanoic acid
PFDPA	Perfluorodecylphosphonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFOSA	Perfluorooctane sulfonamide
PFSAs	Perfluorinated sulfonic acids
pK_a	Acid dissociation constant
POPs	Persistent organic pollutants
PP	Polypropylene

ppb	Parts per billion
PPCPs	Pharmaceuticals and personal care products
ppm	Parts per million
ppt	Parts per trillion
PTFE	Poly-tetrafluoroethylene
PUB	Public Utilities Board
RAS	Return activated sludge
rpm	Revolutions per minute
S/L	Solid to liquid ratio
SAC	Saccharin
SD	Standard deviation
SEM	Scanning electron microscopy
SP	Suspended solid phase
SPE	Solid phase extraction
SRT	Sludge retention time
STDEV	Standard deviation
SUC	Sucralose
t	Time
T	Temperature
TBBPA	Tetrabromobisphenol A
TBTO	Bis(tributyltin)oxide

TCS	Triclosan
TSS	Total suspended solids
HPA	UK Health Protection Agency
UHPLC/MS/MS	Ultra high-performance liquid chromatography tandem mass spectrometry
USEPA	US Environmental Protection Agency
USEPA OPPT	USEPA's Office of Pollution Prevention and Toxics
UV	Ultraviolet
V	Voltage
WRPs	Water reclamation plants
WWTPs	Wastewater treatment plants
µg/l	Microgram/liter
µm	Micrometer
µmol/g	Micromole/gram
℃	Degrees Celsius

Chapter 1. Introduction

1.1. Backgrounds of WRPs and EOCs

Currently, many countries and regions in the world are threatened seriously by severe water shortage. To illustrate, in 2005, 35% of the population of the OECD and 44% worldwide were living in areas characterized by severe water stress, and by 2030, the number of people is expected to increase to an estimated 3.9 billion people or 47% of the world population, mostly in non-OECD countries (The Organisation for Economic Co-operation and Development, 2007; Stahl et al., 2009). Since water scarcity could constrain economic growth and promote social unrest and tension between countries, human beings have been exploring and advancing technologies to utilize all available water resources, and wastewater reclamation has evolved as an important solution.

Wastewater treatment plants (WWTPs) or wastewater reclamation plants (WRPs) are civil infrastructures designed to purify wastewater for various purposes such as agricultural irrigation or safe disposal to sea/rivers without or with acceptable impacts on human health and ecosystem. Undesired contaminants are removed to at least tolerable levels after treatment through physical, chemical and biological processes. For normal wastewaters, conventional WWTPs involve preliminary and primary treatments (which target the removal of coarse solids and settleable organic and inorganic solids by sedimentation, and eliminate floating materials by skimming) and secondary biological treatment (which aims to treat biodegradable dissolved and colloidal organic residues and nutrients) (Pescod, 1992). Advanced treatment may be employed to remove specific wastewater constituents such

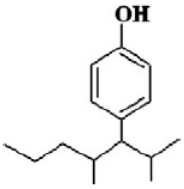
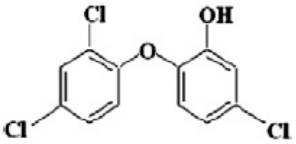
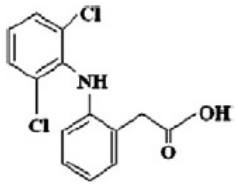
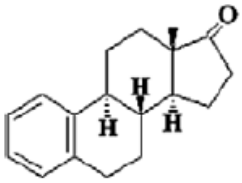
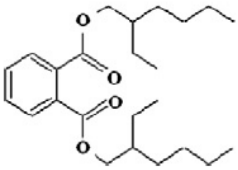
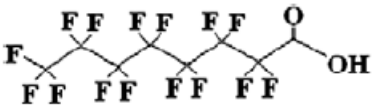
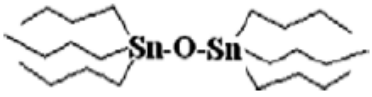
as heavy metals and refractory organics (Pescod, 1992). These processes in a conventional WWTP principally engage sedimentation, biodegradation, sorption, chemical reaction, stripping/volatilization, photolysis and dilution, which could be integrated to achieve a satisfactory quality of reclaimed water.

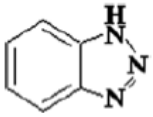
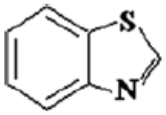
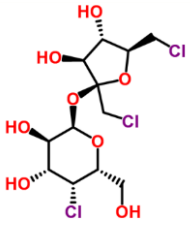
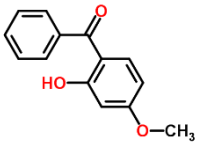
However, rapid economic development and change in life styles have led to continuous production and release of emerging organic contaminants (EOCs), which are defined by U.S. Geological Survey (2014a) as "any synthetic or naturally occurring organic chemicals that are not commonly monitored in the environment but have the potential to enter the environment and cause known or suspected adverse ecological and(or) human health effects". Some of these chemicals are environmentally persistent and are not removed in conventional WWTPs. Furthermore, the risks associated with most EOCs are not fully characterized, with uncertainties and data gaps which prevent the development of regulations and water quality guidelines (Tremblay et al., 2011). However, various sources of EOCs reflect their ubiquity in the environment and one of the most significant sources focuses on municipal wastewater discharges (Tremblay et al., 2011; The United States Geological Survey, 2014b).

A wide range of chemicals are covered in EOCs (typical groups are listed in Table 1.1) and artificial sweeteners (ASs) and perfluorinated compounds (PFCs) are two typical classes (Tremblay et al., 2011; Farre et al., 2012; Stasinakis, 2012). ASs and PFCs have drawn serious attention, because they are refractory compounds that are ubiquitous in the environment (Schröder, 2003; Sáez et al., 2008; Fromel & Knepper, 2010; Lange et al., 2012) and they are (potentially) toxic to human health and/or ecology (Kroger et al., 2006; Hu

& Hu, 2009; Qazi et al., 2009; Zygler et al., 2009; Stahl et al., 2011). PFCs possess high bioaccumulation potential (Stahl et al., 2011) and ASs production is soaring due to diet change (Haley, 2013). However, there is limited knowledge on their ecological and health impacts. It is also not known to what extent they are removed in different parts of the wastewater treatment system.

Table 1.1. Various groups of emerging organic contaminant with one corresponding representative compound (Farre et al., 2012; Stasinakis, 2012; ChemSpider, 2014f, 2014d).

Group	Representative compound (Abbreviation)	Chemical structure
Surfactant	Nonylphenol (NP)	
Personal care product	Triclosan (TCS)	
Pharmaceuticals	Diclofenac (DCF)	
Estrogens	Estrone (E1)	
Phthalate acid esters	Bis (2-ethylhexyl) phthalate (DEHP)	
Perfluorinated compounds	Perfluorooctanoic acid (PFOA)	
Organotins	Bis(tributyltin)oxide (TBTO)	

Brominated flame retardants	Tetrabromobisphenol A (TBBPA)	
Benzotriazoles	Benzotriazole (BTr)	
Benzothiazoles	Benzothiazole (BT)	
Artificial sweeteners	Sucralose (SUC)	
UV filters	Benzophenone-3	

1.2. Objectives and scope

In Singapore, the efficiency and effectiveness of wastewater reclamation is extremely important because it contributes to water supply and consequently helps to relieve the water scarcity problem. Reclaimed wastewater is purified to NEWater for domestic and industrial purposes and sludge from WRPs is incinerated. Leakage of sewage and illegal discharge of industrial effluent may pollute catchment and reservoir water that could deteriorate drinking water quality. As a result, due to the ubiquity and (potential) health and ecological adverse effects of ASs and PFCs, it is important to monitor their fates in WWTPs, and assess their potential for contamination control.

This study is divided into two parts. The first part of the study is to monitor and evaluate the occurrence and fate of ASs and PFCs in a wastewater

reclamation plant in Singapore. The specific objectives are listed below:

a. To develop sample preparation methods and specific analytical methods using UHPLC/MS/MS instrument in order to detect and quantify ASs and PFCs both in the dissolved phase and the suspended solid phase of collected wastewater samples.
b. To conduct field sampling at nine sampling points within the WWTP.

The second part of this study involves bench-scale sorption experiments of selected refractory ASs and PFCs to help understand their behavior observed in the field data. Since the WRP mainly consists of underground facilities that minimize photolysis and volatilization, sorption was hypothesized to be one of the major mechanisms that influenced the fate of EOCs in the plant, compared to the other processes aforementioned. The specific objectives are as follows:

a. To conduct a lab-based sorption kinetic test of ASs (SUC and ACE) to assess their sorption affinity onto freshly collected wet mixed liquor suspended solids in terms of equilibration time and percentage removal of aqueous concentration.
b. To conduct a lab-based sorption kinetic test of PFCs to assess their sorption capacity on mixed liquor suspended solids in terms of equilibration time, percentage removal of aqueous concentration and relative affinities between various PFCs. Fresh biomass collected from the WRP will be treated using two different methods for comparison. One is oven-dryness under normal temperature for 1 day based on literature (Gulnaz et al., 2004), and the other is the USEPA OPPTS 835.1110 method (The Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency, 1998).

These field measurements and laboratory tests will help us to better understand the effectiveness of different treatment units in removing PFCs and ASs and the main mechanisms of their removal.

Chapter 2. Literature review

2.1. Artificial Sweeteners

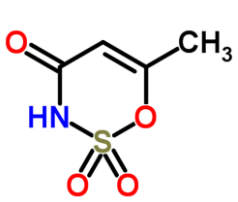
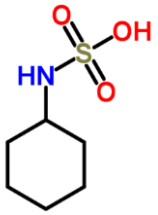
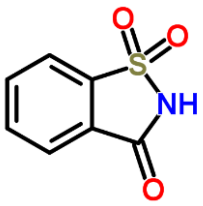
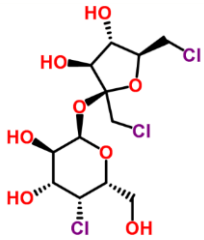
Artificial sweeteners, whose sweetening power is much more intensive than regular sugars, are either synthetic or derived sugar substitutes modified from natural products (Sardesai & Waldshan, 1991; Buerge et al., 2009). They provide no or low calories so that even persons with diabetes could consume them without increase in blood sugar levels (Sardesai & Waldshan, 1991; Zygler et al., 2009). With extra benefits of weight control and tooth-friendliness, they are used widely in low-calories foods and beverages such as table-top sweeteners, chewing gums and so forth (Zygler et al., 2009). They are also added in pharmaceuticals and personal care products such as tooth pastes and mouth washes (Scheurer et al., 2010). The production of artificial sweeteners has been increasing over time. For instance, the supply of high-intensity sweeteners (saccharin, aspartame, acesulfame K, sucralose, stevia products and cyclamate) was estimated to grow from 3.079 million tons in 2002 to a projected 4.201 million tons in 2012, or from 21.2 pounds in 2002 to 26.7 pounds in 2012 on per capita basis (Haley, 2013).

Despite their ubiquity, different artificial sweeteners are authorized for usage in different countries. For example, European Union (EU) authorizes six artificial sweeteners for use (acesulfame K, aspartame, cyclamic acid and its salts, saccharin and its salts, sucralose and neohesperidine dihydrochalcone), while the U.S. excludes cyclamates and neohesperidine dihydrochalcone but includes neotame (European Commission, 2004; Zygler et al., 2009; American Diabetes Association, 2014). Herein, acesulfame, cyclamate, saccharin and

sucralose were selected since they have been detected most frequently in the aquatic environment and wastewater samples (Lange et al., 2012).

Physical and chemical properties of the selected artificial sweeteners are summarized in Table 2.1. They are readily water soluble and unlikely to vaporize and accumulate on fat tissues or hydrophobic phases based on their low Henry's law constant and $\log K_{ow}$ values respectively. They are also mainly excreted without transformation and metabolism after ingestion (Buerge et al., 2009). This implies that they will be found in domestic sewage. Furthermore, among them, sucralose and acesulfame are deemed as good wastewater indicators because of their high concentrations in wastewater (higher than most PPCPs), persistence (more refractory than caffeine), high water solubility, predicted low absorbability to solids and high sensitivity of modern trace analytical methods (Lange et al., 2012). On the contrary, cyclamate and saccharin are subjected to biodegradation in WWTPs but little is known about their transformation by-products (Lange et al., 2012).

Table 2.1. Selected physicochemical properties of the four artificial sweeteners discussed in this study (Lange et al., 2012; ChemSpider, 2014f, 2014b, 2014e, 2014c, 2014a).

	Acesulfame	Cyclamate	Saccharin	Sucralose
CAS no.	33665-90-6	100-88-9	81-07-2	56038-13-2
Full name	6-Methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide	Cyclohexylsulfamic acid	1,2-Benzothiazol-3-ol 1,1-dioxide	1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside
Short name	ACE	CYC	SAC	SUC
Structure[a]				
Molecular formula	$C_4H_5NO_4S$	$C_6H_{13}NO_3S$	$C_7H_5NO_3S$	$C_{12}H_{19}Cl_3O_8$
Molecular weight (g/mol)	163.15	179.24	183.19	397.63
Sugar equivalence	200	30	300	600
Water solubility (g/L)	270 (20 ℃)	1.000; 133	4	4; 283 (20 ℃)
pK_a	2.0	1.9	2.2	11.8
log K_{OW}	-1.33	-1.61	0.91	-1.00 -0.51±0.05
Melting point (℃)[b]	123.25	169.5	228	130
Vapour pressure (mm Hg)[b]	9.03×10^{-6}	5.31×10^{-7}	1.03×10^{-7}	3.25×10^{-14}
Henry's law constant (atm-m^3/mole)[b]	9.63×10^{-9}	1.70×10^{-8}	1.23×10^{-9}	3.99×10^{-19}
Human excretion	100 % unchanged; mainly unchanged	mainly unchanged; inter-individual variations in	mainly unchanged	>92 % unchanged

		conversion to cyclohexylamine		
ADI mg/kg body weight	9 (potassium salt)	7	5 (sodium salt), 3.8 (free acid)	15

[a] From ChemSpider (chemical database).

[b] These values are either experimentally obtained or EPI Suite predicted that are summarized in ChemSpider (ChemSpider, 2014f, 2014b, 2014e, 2014c, 2014a). Other values are summarized in the review paper of artificial sweeteners (Lange et al., 2012).

Despite the growing supply and consumption of artificial sweeteners, it is very controversial to use them as food additives because of their potential toxicological and ecotoxicological properties (Kroger et al., 2006; Zygler et al., 2009). Among these five ASs, sucralose has been drawing the most concern due to potential toxic effects exerted by its chlorine substitutes. A study on Splenda sucralose which was administrated at a dosage of 1.1-11 mg/kg on rats for 12 weeks showed a reduction of beneficial fecal microflora bacteria and enhanced expression levels of intestinal glycoprotein and cytochrome that could affect bioavailability of orally administered drugs (Abou-Donia et al., 2008; Soh et al., 2011). More recently, sucralose and saccharin were suspected to cause inflammatory bowel disease (IBD) by interfering with gut bacteria and digestive enzymes whose incidence changes correlate with their use in different places around the world (Qin, 2002; Qin, 2011). Further research is needed for verification. In terms of ecological effects, for instance, ShSUT1 was shown to be inhibited by sucralose for sucrose transport in sugar canes with an inhibition coefficient of 16.5 mM (Reinders et al., 2006).

Due to limited scientific evidence on the health and ecological impacts, these compounds are usually not regulated by laws. However, daily intakes are usually recommended for various commercial products and guidelines are suggested by governments. For example, a daily intake of SUC is recommended to be no more than 15mg/kg body weight by the European Union Scientific Committee (European Commission Scientific Committee on Food, 2000). The U.S. Food and Drug Administration (FDA) also suggests the following guidelines (Table 2.2).

Table 2.2. Acceptable daily intake guidelines (ADIs) of low and no-calorie sweeteners by

the U.S. FDA (Koelemay, 2014).

Low- and no-calorie Sweeteners	Acesulfame K	Aspartame	Saccharin	Sucralose	Stevia Leaf Extracts
US FDA ADI Guidelines (mg/kg body weight)	15	50	5	5	12

2.1.1. Occurrence and fate in WRPs

The most frequently detected artificial sweeteners are ACE, CYC, SAC and SUC (Lange et al., 2012). The concentrations published in literature are summarized in Table 2.3, focusing on wastewater influent that enters into a WWTP and effluent out from a WWTP. It is easy to observe country-specific differences in the concentrations. For instance, the concentration of SUC in Germany is less than that in USA by two orders of magnitude. This could be due to earlier market introduction and wider application in USA (Lange et al., 2012). Furthermore, based on SUC per capita loads, Lange et al. (2012) emphasized the impact of manufacturers' preferences on AS occurrence for using different ASs in food and beverage products.

Table 2.3. Mean concentrations/Concentration ranges (in μg/L) of artificial sweeteners (ACE, ASP, CYC, SAC, and SUC) in wastewaters (Brorström-Lundén et al., 2008; Buerge et al., 2009; Mead et al., 2009; Scheurer et al., 2009; Neset et al., 2010; Oppenheimer et al., 2011; Scheurer et al., 2011; Torres et al., 2011; Lange et al., 2012; Ordóñez et al., 2012; Gan et al., 2013; Kokotou & Thomaidis, 2013; Loos et al., 2013; Tran et al., 2013).

Country/Region	Year	Sample type	ACE		ASP		CYC		SAC		SUC	
			Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff
Sweden	2006/ 2007	Flow proportional 24-h composite	-	-	-	-	-	-	-	-	3.5-7.9	1.8-10.8
Sweden	2009	Inf: 6-h composite; Eff: 24-h composite[1]	-	-	-	-	-	-	-	-	1.7-3.2	2.3-2.5
Switzerland /Canton of Zurich	2008	24-h composite	12-43	14-46	-	--	10-65	<0.1-0.82	3.9-18	<0.1-3.2	2.0-9.1	2.0-8.8
Germany/Baden-Württemberg	2009	24-h composite	35-47	26-28	n.d.	n.d.	141-195	0.4-1.9	34-43	2.2-2.8	0.82	0.6-0.7
Germany/Baden-Württemberg/ Bavaria	2010	24-h composite	8.2-37	11-39	-	-	-	-	-	-	0.44-1.5	0.44-1.53
USA/NC	2008	not specified	-	-	-	-	-	-	-	-	-	11.93
USA/AZ	2009	-	-	-	-	-	-	-	-	-	-	1.5-4.3
USA/FL, TX, CA, IL & MI	2009/ 2010	grab samples	-	-	-	-	-	-	-	-	-	27
USA/FL	2011	-	-	-	-	-	-	-	-	-	-	5.89-12.08
Spain/NW	2011	grab samples	25.5	32	n.d.	n.d.	31.2	18.3	20.7	7.5	4.2	16.5
Israel[2]	2008/ 2009	grab samples	-	48.7	-	-	-	0.27	-	0.29	-	15.5
China/Tianjin	2011	-	16-17	15-17	0.044-0.053	n.d.	16-21	0.16-0.18	7.2-9.1	0.27-0.28	1.9-2.1	1.5-1.8
EU	2013	Grab/24-h composite	-	76	-	2.6	-	-	-	-	-	-
Greece/Athens	2013	-	11.9 -25.3	12.1-27.2	<LOD	<LOD	6.04-57.8	1.30-4.48	15.0-46.0	<LOD-0.27	15.1-25.4	14.8-26.7
Singapore	2013	grab samples	0.187	-	<MQL	-	0.3-	-	0.5-	-	0.1-	-

	-75.093	-2.262	250.348	135.759	4.719

[1]: Inf=Influent; Eff=Effluent.
[2]: As cited in (Lange et al., 2012).

In Singapore, the concentration ranges of CYC, SAC, ACE and SUC in grab samples of raw wastewater were 300-250348 ng/L, 500-135759 ng/L, 187-75093 ng/L and 100-4719 ng/L respectively using direct injection, as reported by Tran et al. (2013). They further suggested that the detection of ASs in surface water and groundwater indicated sewage leakage and contamination to receiving water bodies since these compounds were highly specific to sewage (Tran et al., 2013).

In general, SUC and ACE are persistent and insignificantly removed in conventional mechanical-biological WWTPs (Lange et al., 2012). Although both these ASs are widely assumed to be excellent anthropogenic wastewater markers due to their stability, high water solubility and wastewater specificity, ACE may be better than SUC because of its much higher environmental concentrations and higher detection sensitivity by LC-ESI(-)-MS/MS (Lange et al., 2012).

In contrast, CYC and SAC are usually of less concern because they are quite biodegradable. The removal rates of CYC and SAC were reported to be >99% and >90% respectively after secondary or tertiary wastewater treatment (Lange et al., 2012). As such, their concentrations in effluents are as low as 1 µg/L or lower, despite their high influent concentrations (Lange et al., 2012).

The concentrations of artificial sweeteners on sludge have only been investigated in a few studies. Subedi et al. (2014) measured AS concentrations on digested sludge from WWTPs in South Korea. For the WWTPs which mainly received domestic water flow, SUC, SAC, ACE and CYC were found to have concentrations between 21.1–122, 7.08–3240, 14.0–166 and 11.2

(mean) ng/g dw respectively. Furthermore, Ordóñez et al. (2013) further investigated AS concentrations on thickened primary (ACE = 50-63 ng/g dw; CYC = 175-326 ng/g dw; SAC = 141-255 ng/g dw; and SUC = 38-59 ng/g dw) and secondary (ACE = 36-151 ng/g dw; CYC = 17-76 ng/g dw; SAC = n.d.-124 ng/g dw; and SUC = 54-628 ng/g dw) sludge in Spain. However, it was proposed that sorption onto sludge was insignificant due to the comparable concentrations in the digested sludge with those in the raw wastewater (Brorström-Lundén et al., 2008; Buerge et al., 2011; Lange et al., 2012).

2.1.2. Bench-scale sorption study

To date, there is no study done on the sorption behavior of artificial sweeteners onto activated sludge, although there are a few studies on sorption onto soil and activated carbon (Scheurer et al., 2010; Soh et al., 2011; Lange et al., 2012).

Soh et al. (2011) conducted sorption isotherms of SUC and ACE-K together with caffeine using loam soil, peat soil and granular activated carbon. In comparison to caffeine as a reference, both SUC and ACE-K showed significantly less sorption affinity to both soils and ACE-K displayed a much higher affinity than SUC in both soil systems (Soh et al., 2011). This was proposed to be caused by their consistently higher hydrophilicity. In addition, both of them demonstrated higher affinity to peat soil which has higher organic content than loam soil (Soh et al., 2011).

GAC is commonly used in water treatment to adsorb chemical contaminants and residues, especially in drinking water treatment. Although SUC showed higher capacity on GAC than ACE at high equilibrium

concentrations in the sorption isotherm study, the capacities were comparable at relatively low environmentally relevant concentrations (Soh et al., 2011). Soh et al. (2011) further concluded that sucralose (K_f = 78.6 (mg/g)(L/mg)$^{1/n}$) sorption to GAC was less likely compared to chlordane, naphthalene and toluene (K_f = 190, 132 and 97 (mg/g)(L/mg)$^{1/n}$) which possessed similar 1/n coefficients. In addition, Lange et al. (2012) summarized that GAC filtration was only possible for SAC and SUC, although SUC was categorized into "drinking water relevance" in a small-scale GAC filter test conducted by Scheurer et al. (2010). In this batch test, SUC exhibited potential removal in full scale plants because of its early but low breakthrough (Scheurer et al., 2010).

2.2. Perfluorinated Compounds

Perfluorinated compounds are organic substances where all of the hydrogen atoms are replaced with fluorine atoms in their hydrocarbon backbones (Stahl et al., 2011). Because of the strength of C-F bond, many PFCs are chemically and thermally stable (Schultz et al., 2003; Stahl et al., 2011). Some of them are biologically persistent with bioaccumulation and biomagnifications potential in food chains (Stahl et al., 2011).

There are diverse classes of PFCs. These can be divided into groups of perfluorinated sulfonic acids (PFSAs), perfluorinated carboxylic acids (PFCAs), fluorotelomer alcohols (FtOHs), fluoropolymers and perfluoroalkanamides (Stahl et al., 2011). The volatile FtOHs and perfluoroalkanamides have a greater mobility by atmospheric transport, which contributes to their global distribution (Martin et al., 2005; D'Eon et al., 2006; Jahnke et al., 2007). PFCAs and PFSAs are also widely spread even though

they are capable of bioaccumulation and adsorption in hydrospheric transportation, which, in addition, is exaggerated by atmospheric transport. They are also possible degradation products of some volatile compounds, such as 8:2 FtOH (Wang et al., 2005a). Among the PFCs, PFOA (C8 of PFCAs) and PFOS (C8 of PFSAs) have drawn the most attention by researchers. They are widely used and persistent in the environment. Their toxicities have been demonstrated via animal and epidemiological studies and they are bioaccumulative on animal tissues and likely to adsorb onto albumins (Sibinski, 1987; Yang et al., 2000; Case et al., 2001; Thomford, 2002; Lau et al., 2003; Butenhoff et al., 2004a; Butenhoff et al., 2004b; Lau et al., 2006; European Food Safety Authority, 2008; Hu & Hu, 2009; Qazi et al., 2009; Zhou et al., 2010; Labadie & Chevreuil, 2011; Nguyen, 2011; Stahl et al., 2011; Zareitalabad et al., 2013).

Due to complexities such as volatile/non-volatile precursors, it is very hard to predict the sources and fate of PFCs which may be present in solid, liquid, aerosol and biomass phases (Nguyen, 2011). Some PFCs have higher affinity to organic carbon, or proteins, which makes sediments and suspended solids an important sink (Zhou et al., 2010; Labadie & Chevreuil, 2011; Nguyen, 2011; Zareitalabad et al., 2013). Limited partitioning data and physical chemical properties with insufficient accuracies pose challenges to predict the fates of various PFCs (Schultz et al., 2003).

With dual hydrophobic and oleophobic nature embedded in their fluorinated alkyl tails, PFCs are applied widely to make products resistant to stain, grease and water in both daily consumer products and various industrial applications (e.g. production of semiconductor and chromium plating

processes) (Schultz et al., 2003; National Institute of Environmental Health Sciences, 2012; Sun et al., 2012; Zareitalabad et al., 2013). For instance, they are used in fluoropolymers (e.g. PTFE), liquid repellants (e.g. for carpets and furniture, coatings of cookware, food packaging, paper, textile, etc.), surfactants in personal-care-products (e.g. shampoo and denture cleaner), industrial surfactants, additives and coatings, and firefighting foams (Schultz et al., 2003; Stahl et al., 2011; National Institute of Environmental Health Sciences, 2012; Sun et al., 2012; Zareitalabad et al., 2013). As such, they have been produced in huge quantities. In 2000, 3M Company in the U.S. reported 3 million kilograms production of perfluorinated sulfonyl fluoride intermediates among which 41% of its American production was coated onto paper and packaging products, 37% in textile, leather and carpet goods, 10% in industrial surfactants, additives and coatings and 3% in firefighting foams (The United States Environmental Protection Agency, 2000; Schultz et al., 2003).

PFCs could be released to the environment by both direct sources via manufacturing process and use of products containing PFCs, and indirect sources such as degradation of precursors (Zareitalabad et al., 2013). The wide applications have resulted in contamination of PFCs everywhere, even in remote areas. Despite detection in blood plasma of human beings, PFCs (PFOS) were even found in the range of 3 to 50 ng/ml in grey and ringed seals from the Canadian and Norwegian Arctic where it is less densely populated with no commercial and industrial sources of PFCs (Giesy & Kannan, 2001; Hansen et al., 2001; Schultz et al., 2003). Human beings are also subject to exposures of PFCs. Stahl et al. (2011) summarized a number of pathways

including diet (e.g. fish consumption), food contact materials, non-food personal items (e.g. jackets, furnitures, cleaning agents, etc.), and indoor and outdoor air with dietary uptake as the largest contributor. The total exposures were estimated as well (Table 2.4) (Stahl et al., 2011). Due to the ubiquity of PFCs and human exposure and uptake, an understanding of their impacts on ecology and human health is important for risk management.

Table 2.4. Estimation of uptake of total PFCs for adults (ng/kg-body weight/day) summarized by Stahl et al. (2011).

Source of Contamination	EFSA (2008) PFOS	Fromme et al. (2009) PFOS	EFSA (2008) PFOA	Fromme et al. (2009) PFOA	Fromme et al. (2009) FTOH	Fromme et al. (2009) FOSE /FOSA
Diet	60 to 200	1.5 to 4.48	2 to 6	2.82 to 11.5	n.r.	0.217-6.87
Fish	45 to 58	n.r.	1.7 to 2.1	n.r.	n.r.	n.r.
Drinking water	0.24	0.023 to 0.130	0.31	0.022 to 0.087	n.r.	n.r.
Indoor air + house dust	0.93	0.0047 + 0.032 to 4.22	0.81	0.0009 + 0.016 to 1.03	0.038 to 0.105 + 0.103 to 1.02	0.460 to 2.05 + 0.983 to 2.03
Outdoor air	0.001 to 0.004	0.0001 to 0.001	0.006 to 0.14	0.001 to 0.012	0.003	0.001 to 0.012
Total uptake	60.9 to 200	1.56 to 8.84	2.82 to 6.95	2.86 to 12.6	0.144 to 1.13	1.66 to 10.9

n.r.: Not reported.

There are numerous biological and toxicological studies on PFCs, particularly for PFOA and PFOS. Their levels in human blood and serum have been rising in the last few decades. Animal experiments have shown modest acute toxicity for these chemicals. For PFOS, LD50 is 251 mg/kg body weight for a single oral dose in rats; while for PFOA, LD50 ranges from 430 to 680 mg/kg body weight (Stahl et al., 2011). In addition, diverse chronic toxic effects were demonstrated including hepatotoxic effects, lipid metabolism,

tumor growth on the liver, Leydig cells and mammary gland tissue, cancerous growth, reproductive and developmental toxic effects and neuro- and immunotoxic effects (Sibinski, 1987; Yang et al., 2000; Case et al., 2001; Thomford, 2002; Lau et al., 2003; Butenhoff et al., 2004a; Butenhoff et al., 2004b; Lau et al., 2006; European Food Safety Authority, 2008; Hu & Hu, 2009; Qazi et al., 2009; Stahl et al., 2011). Epidemiological studies have also been conducted on workers who were occupationally exposed to PFCs (Stahl et al., 2011). Stahl et al. (2011) reviewed and summarized the adverse impacts of PFCs on humans such as glucose, urea, and/or uric acid metabolism, cancer diseases such as bladder and prostrate cancers on pancreas, and potential reproductive and developmental toxic effects . Different PFCs are supposed to pose different toxicities. For instance, the linear isomer of PFOS is expected to be more toxic than the branched-chain PFOA (Stahl et al., 2011). In addition, mixtures of PFCs were revealed to exhibit higher toxicity than single compound dosage (Hu et al., 2003). With the realization of toxicity of longer-chain PFCs, manufacturers are shifting to use short-chain PFCs. However, the studies on short-chain PFCs are limited and fragmented so that it is difficult to draw conclusions (Stahl et al., 2011). Further studies are needed as the background concentrations of short-chain PFCs are building up rapidly (Betts, 2007; Ochoa-Herrera & Sierra-Alvarez, 2008; Eriksen et al., 2010).

Governments have been taking actions to control the use of PFOS and PFOA due to the concerns of toxicological effects. In 2006, USEPA and eight major manufactures reached a voluntary agreement to reduce PFOA emissions from their plants by 95% from a baseline year of 2000 to 2010, while 3M Company ceased usage of PFCs in its famous products (Scotchgard®) in 2002

(The United States Environmental Protection Agency, 2013). The European Union restricted the use of PFOS in a narrow range of specified industrial applications (The European Parliament & The European Council, 2006). PFOS was categorized into POPs (persistent organic pollutants) and restricted in production and use in 2009 by the UN POP Stockholm Convention (Zhou et al., 2010; Sun et al., 2012). However, on a global scale, productions of PFOA and PFOS are still on-going in different regions of the world.

To date, there are no enforceable regulations on PFCs in surface water and drinking water, but guidelines are recommended in drinking water due to health concerns. For instance, the UK Drinking Water Inspectorate (DWI) set drinking water guideline value for both PFOA and PFOS to be 0.3 μg/L (in tier 2 of a multi-tiered approach) (Drinking Water Inspectorate, 2009; Zushi et al., 2012).

2.2.1. Occurrence and fate in WRPs

Many studies on the occurrence of PFCs in wastewater treatment plants showed that there was no consistent concentration profile among all the investigated perfluorinated compounds. Results were highly region and plant specific. The dominant species in influent, effluent and other process units and their respective concentrations were variable. This could be due to the composition of influent wastewater, presence and quantities of precursors, deposition of rainfall, runoff, plant operating parameters, etc (Boulanger et al., 2005; Sinclair & Kannan, 2006; Yu et al., 2009; Kunacheva et al., 2011). To illustrate, industrial wastewater can contribute significant loads of PFCs compared with domestic and commercial wastewater (Sinclair & Kannan, 2006; Yu et al., 2009). Yu et al. (2009) suggested that a large quantity of PFCs

in industrial wastewater could override the dilution effect by rainwater by observing insignificant concentration variations of PFOA and PFOS between wet and dry seasons, thus minimizing seasonal variations of PFCs concentration.

Generally it is concluded that wastewater treatment processes, especially biological process, are not effective at removing PFCs, and higher levels in effluents are observed in some cases (Loganathan et al., 2007; Yu et al., 2009; Pan et al., 2011; Ratola et al., 2012). Families of PFCs may exhibit different behaviors (Ratola et al., 2012). To illustrate, PFOS was reported to decrease after treatment but PFOA behaved inversely (Guo et al., 2010; Pan et al., 2011; Kunacheva et al., 2011; Sun et al., 2012). With higher organic carbon-normalized distribution coefficient compared to its carboxylate analog, PFASs are expected to exhibit higher sorption to sludge, which leads to less mass available in the water phase (Higgins & Luthy, 2006; Yu et al., 2009; Guo et al., 2010).

The increase in effluent concentration has been deemed mainly due to microbial degradation of precursors in aerated activated sludge processes such as N-EtFOSE (N-ethyl perfluorooctane sulfonamidoethanol) (Rhoads et al., 2008). As a result, the control of precursors is necessary to manage PFCs in wastewater. In spite of reduction in sources, the operating parameters can also be adjusted to hinder the degradation of precursors. Based on Yu et al (2009), no biodegradation of precursors could occur when the SRT of activated sludge processes is lower than a critical SRT value.

Overall, sludge disposal is believed to be a major approach for PFCs to leave wastewater treatment plants because of strong sorption onto sludge,

together with effluent discharge into other receiving water environments, volatilization, and so forth (Kunacheva et al., 2011). As such, care should be taken care of the subsequent applications and/or post-treatment of the contaminated sludge.

In Singapore, Yu et al. (2009) monitored PFOA and PFOS in two wastewater treatment plants. The influent concentrations in conventional activated sludge (CAS) ranged from 14.1-82.0 ppt for PFOA and 7.9-25.3 ppt for PFOS in WWTP 1, and 31.8-638.2 ppt for PFOA and 56.3-374.5 ppt for PFOS in WWTP 2, while the effluent concentrations in CAS ranged from 15.8-138.7 ppt for PFOA and 7.3-16.7 ppt for PFOS in WWTP 1 and 77.4-1057.1 ppt for PFOA and 95.6-461.7 ppt in WWTP 2. Obviously, PFOA was dominant in the monitored WWTPs and the industrial influent may vary the concentration significantly. Besides, based on the mass flow calculated, it was found that the primary clarifier was not effective in PFCs removal, while the activated sludge process may actually increase the loads of PFCs by biodegradation of precursors with sufficiently long SRT. Furthermore, based on the concentrations of the sludge samples, the partition coefficients of PFOA and PFOS in the primary sludge were estimated at 188-897 L/kg 897-2237 L/kg, and in the activated sludge at 201-513 L/kg and 720-2324 L/kg accordingly. This supported the observation that higher concentration in aqueous wastewater led to more accumulation on sludge, and the sludge concentration of PFOS with higher K_d was much higher than that of PFOA. Lastly, it was noted that the distribution coefficients in primary sludge and activated sludge were comparable. (Yu et al., 2009)

2.2.2. Bench-scale sorption study

Most fully fluorinated PFCs are confirmed to be non-biodegradable in both experimental and field studies, and they are also likely to adsorb onto organic carbon contents such as sludge. However, recently they have been characterized as “proteinophilic” (Zareitalabad et al., 2013). Since dead and live microorganisms and their extracellular polymeric substances produce polysaccharides and proteins to activated sludge, sorption is an important mechanism for PFCs to be removed with sludge disposal from wastewater treatment plants (Zhou et al., 2010).

The biosorption of PFOA and PFOS reached equilibrium after approximately 11 hours on activated sludge in a test by Zhou et al (2010). Although sorption uptake of PFOS was found to be slower at the beginning than PFOA, its sorption capacity was much higher (Zhou et al., 2010).

Zhou et al (2010) has further looked at the impacts of pH and temperature on sorption capacity. Firstly, sorption is optimum at 25 ^{o}C compared to 15 ^{o}C and 40 ^{o}C at a balance of active sorption (stronger at temperature range of 15-35 ^{o}C that favors bioactivity) and passive sorption (lower at higher temperature because of exothermic reaction in common sorption processes). Also, sorption of both PFOA and PFOS decreases with increasing pH from 2-9. Electrostatic interaction is deemed to be the cause since the surface charge of activated sludge is less negative at lower pH, and the protonation of amino and amide groups and the presence of common cations in the sludge can help the adsorption process of anionic PFCs. At high pH range, other reactions such as hydrophobic reaction are proposed to dominate the sorption process. (Zhou et al., 2010)

Sorption isotherms have been conducted in both single adsorbate system and mixed adsorbates system. Firstly, synergistic effects exist among the tested seven PFCs (PFBA, PFBS, PFHxA, PFHxS, PFOA, PFOS and PFDOA) so that the sorption capacity of the total PFCs is higher in the mixture system compared to a single adsorbate system. In addition, hydrophobic interaction is stronger for compounds with longer C-F chain and/or sulphonic head group compared with corresponding carboxylic compounds, which results in better sorption. Last but not least, based on sorption isotherm experiments, K_d values have been estimated to range from 200 L/kg to 4050 L/kg for PFOS and 150 L/kg to 350 L/kg for PFOA. (Zhou et al., 2010)

2.3. Situation in Singapore

Singapore is a small island-country in Southeast Asia with a land area of only 716.1 km^2 but a population of approximately 5.4 million (Singapore Department of Statistics, 2014). Since Singapore lacks natural water resources, it has been classified by the World Resources Institute (2013) as an "Extremely Highly Stressed" country with a baseline water stress score of 5 (the highest score). This means more than 80% of available annual renewable supply is withdrawn for average water users so that the communities are vulnerable to water scarcity (Reig et al., 2013).

However, its water stress problem is manageable based on its "Four National Taps" – local catchment water (rainwater), imported water from Malaysia, desalinated water and NEWater. Since the first three are currently constrained by limited available land that prevents expansion of reservoir construction, political and economical tension potentially posed by Malaysia, high cost of desalination technologies and climate change, NEWater has drawn

the most attention and research as the most manageable approach (Duerr, 2013). In 2010, it provided 30% of the nation's water needs via 5 plants (The Public Utilities Board, 2010). With economic boom and population growth, water demand will double and NEWater is expected to expand and provide 55% of the nation's water needs in 2060 (The Public Utilities Board, 2013a, 2013b).

NEWater is a result of further purification of reclaimed wastewater using membrane and UV technologies, with a resultant product that is ultra-clean and safe for drinking (The Public Utilities Board, 2010). However, the treatment of secondary effluent quality is challenged by a potential increasing passage of EOCs through membranes.

In addition, potential leakage of sewage into catchments may lead to contamination of EOCs in reservoirs and pose new problems in the drinking water supply. Furthermore, Singapore incinerates sludge wastes from WWTPs, which may possibly produce toxic by-products through combustion of EOCs. As a result, it is important to understand the fate of emerging contaminants in WWTPs in order to better control their removal. These contribute to cost reductions in drinking water and NEWater production and lower the potential negative impacts of EOCs in the environment.

ASs and PFCs has been selected in our study because they are refractory compounds that are ubiquitous in the environment and they are (potentially) toxic to human health and/or ecology.

Chapter 3. Materials and methods

3.1. Occurrence and Fate in the WRP

3.1.1. Materials

3.1.1.1. Artificial sweeteners

Artificial sweeteners in this study included acesulfame K, saccharin, sucralose (Fluka-Sigma-Aldrich, Germany) and cyclamate (N-Cyclohexylsulfamic acid sodium salt, Dr. Ehrenstorfer GmbH) (with reference to Table 2.1 for structures). Four corresponding mass labeled compounds were used as either internal standards or surrogates including ACE-d_4, SAC-$^{13}C_6$, SUC-d_6 and CYC-d_{11} (Toronto Research Chemicals, Inc., Canada).

3.1.1.2. Perfluorinated compounds

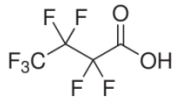
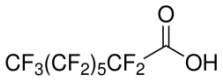
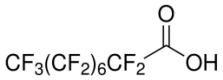
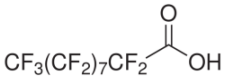
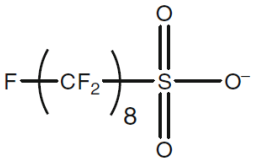
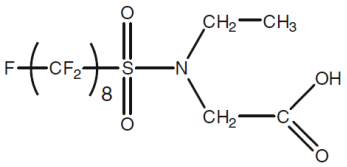
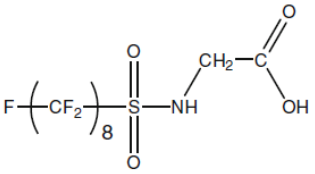
All the perfluorinated analytes were purchased from Wellington Laboratories (Guelph, ON, Canada). They covered typical compounds from families of perfluorinated carboxylic acids (PFBA, PFOA, PFNA and PFDA), perfluorinated sulfonates (PFHxS and PFOS) and perfluorinated derivatives (N-EtFOSAA and FOSAA) (with reference to Table 3.1). All the isotopic PFCs that were used for internal standards or surrogates were purchased from Wellington Laboratories (Guelph, ON, Canada), including [$^{13}C_2$] PFBA (for PFBA), [$^{13}C_2$] PFOA (for PFOA), [$^{13}C_2$] PFNA (for PFNA), [$^{13}C_2$] PFDA (for PFDA), [$^{13}C_2$] PFOS (for PFHxS and PFOS) and [d_5] N-EtFOSAA (for N-EtFOSAA and FOSAA).

3.1.1.3. Other chemicals

HPLC-grade methanol and ammonia acetate (CH_3COONH_4) were

purchased from Fisher Chemical (United States) and Sigma-Aldrich (St. Louis, Missouri, United States) correspondingly.

Table 3.1. The selected PFCs in the monitoring study.

Perfluorinated compounds	Abbreviation	Chemical structure
Perfluorobutyric acid[a]	PFBA	
Perfluorooctanoic acid[a]	PFOA	
Perfluorononanoic acid[a]	PFNA	
Perfluorodecanoic acid[a]	PFDA	
Perfluorohexane sulfonate[a]	PFHxS	
Perfluorooctane sulfonate[b]	PFOS	
N-ethyl perfluorooctane sulfonamidoethanol[b]	N-EtFOSAA	
Perfluorooctane-sulfonamidoacetate[b]	FOSAA	

[a] The chemical structures were from Sigma-Aldrich online catalogue.
[b] The chemical structures were from (Fromel & Knepper, 2010).

3.1.2. Sample collection

The selected wastewater reclamation plant is to reclaim wastewater for seawater discharge and NEWater production. The wastewater received is separated into two streams and directed into two treatment trains which are named Train A (Southworks) and Train B (Northworks) (Figure 3.1). Both streams are settled in primary clarifiers, and then sent through a Modified Ludzack-Ettinger (MLE) process which includes anoxic tanks followed by

aerobic tanks with internal recycling. The major difference between the two trains is the separation units after the MLE biological process. Train A uses the conventional sedimentation tank for solid-liquid separation but Train B uses a membrane bioreactor. Train A is more susceptible to variation while Train B has higher flexibility to yield more stable effluent quality.

Grab wastewater samples were collected monthly from February 2013 to July 2013 for ASs and June and July 2013 for PFCs. For each sampling event, nine sampling points throughout the treatment trains were selected (Figure 3.1). They included influent wastewater (labeled as Inf), primary settled sewages (labeled as A1 and B1), mixed liquor suspended solids (labeled as A2 and B2), effluent and membrane permeate (labeled as A3 and B3 respectively) and return activated sludge (labeled as A4 and B4). All samples were collected in 1L high-density polyethylene (HDPE) bottles (Nalgene, Rochester, USA) which were transported to the lab in an ice box. The samples were stored in a cold room set at 4℃ in dark until analysis which was usually conducted within 10 hours.

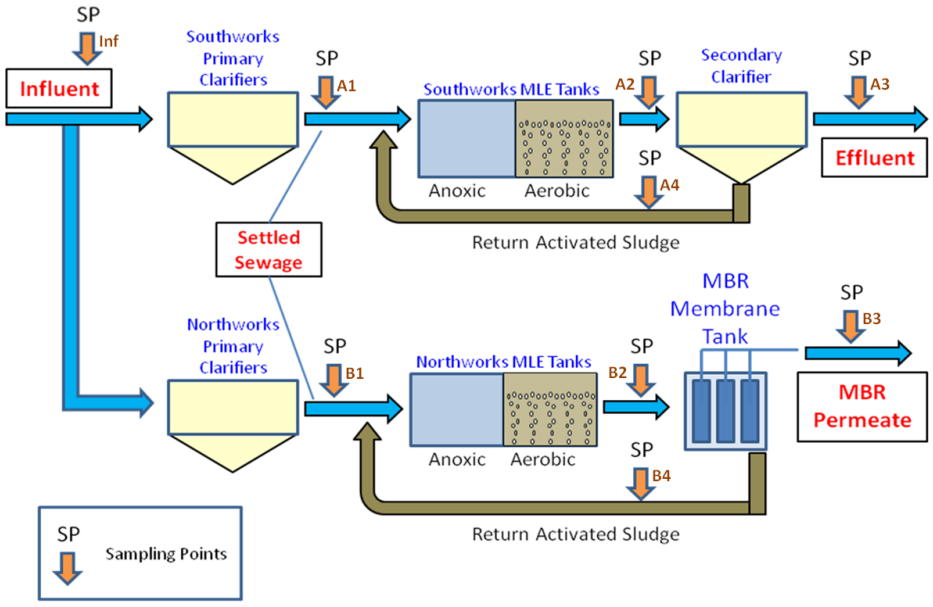


Figure 3.1. Schematic of the wastewater reclamation plant showing the nine sampling points.

3.1.3. HPLC-MS/MS sample preparation

The abundance of ASs and PFCs both in dissolved and suspended solid phases in wastewater samples were measured using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Before instrument injection, wastewater samples were prepared using direct injection method for analytes in dissolved phase and a suspended solid extraction method for those attached on suspended solids. The detailed procedures are listed below. Teflon-made consumables and any potential fluoropolymer materials were avoided throughout the sample preparation and instrumental analysis.

3.1.3.1. Direct injection

Supernatant in the collected settled wastewater samples was centrifuged at 16000 g for 30 minutes in 2 ml polypropylene (PP) micro-centrifuge tubes. Next, the centrifuged supernatant was transferred into 1.5 ml LC

standard vials at a pre-determined volume. For artificial sweeteners, 400 µl of the centrifuged supernatant, 100 µl of the methanol and 20 µl of the mass labeled internal standards mixture were mixed in a LC standard vial, which resulted in 38.5 ppb internal standards and a 400:120 water to solvent ratio in the final solution. For perfluorinated compounds, 150 µl of the centrifuged supernatant, 325 µl of the methanol and 25 µl of the mass labeled internal standards mixture were mixed, which resulted in 1.2 ppb internal standards and a 150:350 water to solvent ratio in the final solution. All the samples were prepared in triplicates.

3.1.3.2. Suspended solid extraction

5 ml of well-mixed wastewater sample was filtered through a nylon syringe filter tip (25 mm in diameter, 0.2 µm in pore size, Environmental Express) using a vacuum manifold (Visiprep^{TM} SPE vacuum manifold with standard lid, Supelco). The filtration process lasted for one hour in order to get out as much water trapped in the suspended solids as possible. Next, each filter tip was eluted using 2 ml methanol by gravity drip 3 times, and then the elution was vortexed with 20 µl mixture of the mass labeled artificial sweeteners (1ppm) as surrogates or 25 µl for that of perfluorinated compounds (24ppb). The elution was nitrogen-dried subsequently and reconstituted with 0.5 ml (1:4) (methanol:1mM ammonium acetate (NH_4CH_3COO) in DI solution) for artificial sweeteners or 0.5 ml (3:7) (DI:methanol) for perfluorinated compounds. The final reconstitute was filtered through a 0.2µm nylon membrane syringe filter tip (13 mm in diameter, 0.2 µm in pore size, Cronus) to remove potential residues before transferring into a 1.5 ml LC standard amber vial. All the samples were

prepared in triplicates.

3.1.3.3. Total suspended solids (TSS)

Concentration of total suspended solids was measured for all the samples following the procedure in Section 2540-D in Standard Methods for the Examination of Water and Wastewater (American Public Health Association et al., 1989). A well-mixed sample of a pre-determined volume was filtered through a pre-conditioned and weighed TCLP glass-fiber filter (0.7 μm in pore size, 47 mm in diameter, Environmental Express, USA) and the deposit retained on the filter was dried over night at 105 ℃ until constant weight. The weight difference before filtration and after dryness was used to compute the mass of the total suspended solids, as shown below. The pre-determined volumes were selected to yield dried residues between 2.5 mg and 200 mg.

$$mg\ TSS/L = \frac{(A - B) \times 1000}{sample\ volume, mL}$$

$$A = weight\ of\ filter + dried\ residue, mg$$

$$B = weight\ of\ filter, mg$$

The TSS concentrations in effluents from Train A and Train B were detected at <15 mg/L and <3 mg/L respectively. Because of the low concentrations, analytes on suspended solids in effluents were not measured.

3.1.4. HPLC-MS/MS

Ultra high performance liquid chromatograph (UHPLC) (UltiMate® 3000 Standard LC systems, Dionex, U.S.A.) interfaced with a triple quadrupole

tandem mass spectrometry (MS/MS) (AB SCIEX QTRAP® 5500, Toronto, Canada) was used for analytes detection in the electrospray negative ionization mode for both ASs and PFCs (Table 3.2).

3.1.4.1. Artificial sweeteners

Separation of analytes was performed by injecting aliquots of 5 μL into a ZORBAX C18 column (Eclipse Plus, 3.5 μm (particle size), 2.1×100 mm (internal diameter×length), Agilent, USA). The mobile phases included aqueous phase of Milli-Q water with 2 mM ammonium acetate (AAc) and organic phase of methanol with 10 mM AAc which were degassed and delivered at a flow rate of 0.600 mL/min. The solvent gradient mode started at 20% methanol, ramped up to 80% from 0.9 minutes to 1.3 minutes and held to 2.6 minutes, ramped down to 20% at 3 minutes and then continued until 3.5 minutes. The total run time was 3.5 minutes.

The analytes were measured in the multiple reaction monitoring (MRM) MS/MS mode. The mass spectrometer was operated with electrospray ionization (ESI) negative mode. The spray voltage was -4500 eV. Instrumental parameters were optimized and summarized in Table 3.2. The dwell time was 80 msec. The source temperature was 450 ℃. The collision energy was optimized for each analyte. For all compounds, two transition daughter ions were monitored with one for quantification and the other for qualification/confirmation.

The instrument detection limit (IDL) (defined as the concentration corresponding to a S/N ratio equal to or higher than 3) was determined by calibration standards which is summarized in Table 3.2 (Yu et al., 2009). In addition, the method detection limit (MDL) (defined as the concentration

corresponding to a S/N ratio equal to or higher than 3) was determined by extrapolating the S/N of the lowest measured concentrations in water samples to S/N values of 3 (Tran et al., 2013). Procedural blanks for all analytes were below the IDL, which implied that no significant contamination occurred in the analytical process including the instrument itself. Recoveries were determined to verify the feasibility of the sample preparation methods and matrix effects. It was performed by spiking analytes before sample extraction and comparing the result with non-spiked samples after the same analytical process. In the direct injection for the dissolved phase samples, surrogates were spiked as internal standards; while in the suspended solid extraction, surrogates were spiked after elution. As a result, the recovery only covered part of the suspended solid extraction method. Repeatability of the instrument was performed by injecting a spiked influent wastewater sample 6 times continuously in one day and reproducibility was conducted by injecting the same spiked influent sample once per day on 5 different days. The relative standard deviation values were reported accordingly. 16 calibration standards (0.01-1000 ppb) were prepared for the calibration curve and an internal standard method was used for quantification. The correlation coefficients were over 0.99 indicating good linearity.

Table 3.2. Analytical parameters of each AS and PFC in UHPLC-MS/MS analysis.

Compound	Parent ion (m/z)	Q/q[a] (m/z)	CE (eV)	Retention time (min)	IDL (ppb)	Wastewater				Sludge			
						Recovery (%)	MDL (ppb)	Repeat (%)	Reproduce (%)	Recovery (%)	MDL (ppb)	Repeat (%)	Reproduce (%)
Artificial sweeteners													
ACE	161.44	81.9/77.9	-18/ -42	0.494	0.005	119.5±12.0	0.01	5.1	9.2	83.1±9.7	0.33	3.0	3.2
CYC	177.9	79.8/80.4	-34/ -28	1.038	0.005	120.4±7.2	0.02	1.1	2.9	92.9±16.6	0.10	1.2	1.5
SAC	182.257	42/105.9	-52/ -24	0.641	0.01	122.8±10.1	0.05	7.8	13.4	108.8±12.4	0.15	9.1	23.3
SUC	394.936	35/358.8	-52/ -14	2.104	0.05	134.4±16.9	0.2	3.0	3.0	107.7±24.5	0.16	2.0	5.4
Perfluorinated compounds													
PFBA	212.93	169/96.9	-14/ -24	1.05	0.0005	167.2±27.1	0.0004	2.1	3.4	101.6±7.2	0.0007	3.7	2.4
PFOA	412.95	368.9/168.9	-14/ -22	7.03	0.0005	122.6±0.8	0.0006	5.4	5.1	21.4±6.4	0.0033	2.4	3.2
PFNA	462.88	418.8/218.8	-16/ -22	7.41	0.0005	84.0±18.8	0.0008	1.6	2.7	16.7±2.0	0.0043	2.7	3.6
PFDA	512.82	468.7/218.8	-14/ -24	7.72	0.0005	127.5±1.4	0.0006	10.2	5.2	36.1±5.6	0.0020	6.2	2.7
PFHxS	398.851	79.916/98.866	-82/ -44	6.59	0.0005	169.3±7.3	0.0004	2.9	1.8	110.3±55.5	0.0006	4.1	3.6
PFOS	498.8	79.918/98.873	-108/ -86	7.37	0.0005	105.3±15.1	0.0007	1.8	1.9	100.3±16.5	0.0007	3.1	3.7
N-EtFOSAA	583.899	418.9/525.9	-28/-30	7.99	0.001	162.9±10.3	0.0009	3.8	3.2	122.1±50.0	0.0012	4.6	2.0
FOSAA	555.86	497.8/418.7	-38/-34	7.71	0.001	-	0.0014	4.1	6.8	-	0.0014	5.3	7.2

[a] Q refers to main product ion for quantification (quantifier), and q refers to secondary product ion for confirmation (qualifier).

3.1.4.2. Perfluorinated compounds

Separation of analytes was performed by injecting aliquots of 10 μL into a TARGA C18 column (Sprite, 5 μm (particle size), 2.1×40 mm (internal diameter×length), Higgins, USA) with a Luna C18(2) guard column (3 μm (particle size), 2×100 mm (internal diameter×length), Phenomenex, USA). The mobile phases included aqueous phase of Milli-Q water with 2 mM ammonium acetate (AAc) and organic phase of methanol with 10 mM AAc which were degassed and delivered at a flow rate of 0.400 mL/min. The solvent gradient mode started at 30% methanol, ramped up to 100% from 2 minutes to 8 minutes and held to 12 minutes, ramped down to 30% at 13 minutes and then held and stopped at 15 minutes. The total run time was 15 minutes.

The analytes were measured in the multiple reaction monitoring (MRM) MS/MS mode. The mass spectrometer was operated with electrospray ionization (ESI) negative mode. The spray voltage was -4500 eV. Instrumental parameters were optimized and summarized in Table 3.2. The dwell time was 30 msec. The source temperature was 450 ℃. The collision energy was optimized for each analyte. For all compounds, two transition daughter ions were monitored with one for quantification and the other for qualification/confirmation.

Similar procedures as in Section 3.1.4.1 were applied, with the IDLs, the MDLs and the recoveries for PFCs summarized in Table 3.2. Procedural blanks for all analytes were below the IDL, which implied that no significant contamination occurred in the analytical process including the instrument itself. 15 calibration standards (0.0001-30 ppb) were prepared for the

calibration curve and an internal standard method was used for quantification. The correlation coefficients were mostly over 0.999 indicating good linearity.

3.1.5. Data analysis

In data reporting, data below either IDL or MDL were reported as <LOD (limit of detection). They were assigned with half of the corresponding MDL in calculation.

All the concentrations reported from Analyst software after correcting with dilution factors were in ppb unit. As a result, the dry-weight concentrations on suspended solids were obtained by dividing the suspended solid concentrations in ppb with TSS concentrations in wastewater samples. This is important to report sludge concentration in different units. “ppb” focuses on the relative abundance of analytes on suspended solids in the bulk wastewater samples while “ng/g-dry weight” reflects the sorption affinity onto suspended solids.

The one-way ANOVA (ANOVA-Prism 6.1) test was performed to evaluate whether there was any significant difference between the mean concentrations in all various independent unit processes. In addition, to enhance understanding of sorption capacity of each analyte, the solid to liquid ratio was calculated as an indication.

3.2. Bench-scale sorption study

3.2.1. Materials

3.2.1.1. Artificial sweeteners

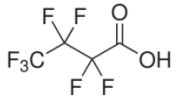
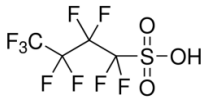
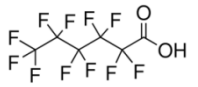
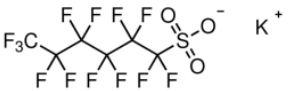
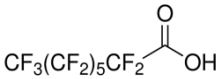
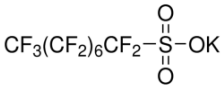
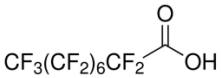
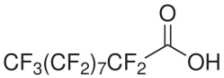
Acesulfame and sucralose were of the most concern since they were found to be persistent throughout the WRP. In contrast, the other ASs were degraded

in biological processes. Acesulfame K and sucralose were purchased from Fluka Analytical, Sigma-Aldrich. The internal standards/surrogates contained the mixture of the mass labeled compounds, i.e. ACE-d_4 and SUC-d_6.

3.2.1.2. Perfluorinated compounds

All the perfluorinated analytes were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). They covered linear sulphonates and carboxylic acids with the number of carbon from 4 to 10 which are summarized in Table 3.3. All the isotopic PFCs that were used for internal standards or surrogates were purchased from Wellington Laboratories (Guelph, ON, Canada), i.e. [$^{13}C_2$] PFBA (for PFBA), [$^{13}C_2$] PFHxA (for PFHxA), [$^{13}C_2$] PFOA (for PFOA), [$^{13}C_2$] PFNA (for PFNA), [$^{13}C_2$] PFDA (for PFDA), [$^{13}C_2$] PFBS (for PFBS) and [$^{13}C_2$] PFOS (for PFHxS and PFOS).

Table 3.3. The selected PFCs in the sorption study.

Perfluorinated compounds	Abbreviation	CAS-No.	Molecular weight (g/mol)	Linear structure	Chemical structure[a]
Perfluorobutyric acid	PFBA	375-22-4	214.04	$CF_3CF_2CF_2COOH$	
Nonafluorobutane-1-sulfonic acid	PFBS	375-73-5	300.1	$CF_3(CF_2)_3SO_3H$	
Undecafluorohexanoic acid	PFHxA	307-24-4	314.05	$CF_3(CF_2)_4COOH$	
Tridecafluorohexane-1-sulfonic acid potassium salt	PFHxS	3871-99-6	438.2	$CF_3(CF_2)_5SO_3K$	
Pentadecafluorooctanoic acid	PFOA	335-67-1	414.07	$CF_3(CF_2)_6COOH$	
Heptadecafluorooctanesulfonic acid potassium salt	PFOS	2795-39-3	538.22	$CF_3(CF_2)_7SO_3K$	
Perfluorononanoic acid	PFNA	375-95-1	464.08	$CF_3(CF_2)_7COOH$	
Perfluorodecanoic acid	PFDA	335-76-2	514.08	$CF_3(CF_2)_8CO_2H$	

[a] From Sigma-Aldrich online catalogue.

3.2.1.3. Adsorbent

Activated sludge biomass, treated as the adsorbent, was collected from the most concentrated stream in the monitored WRP in Singapore, i.e. the return activated sludge (RAS) stream in Train B.

3.2.1.4. Other chemicals

HPLC-grade methanol and sodium azide (NaN_3) were purchased from Fisher Chemical (United States) and ACROS Organics respectively. Besides, ammonia acetate (CH_3COONH_4) and phosphates (K_2HPO_4 and KH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, Missouri, United States).

3.2.2. Preparation of biomass solids for sorption

Activated sludge biomass solids were obtained by centrifugation of the collected wastewater samples. The solids were washed using phosphate buffer solution (for PFCs) or DI water (for ASs) for three times via resuspension-centrifugation-supernatant decantation process in order to remove matrix and colored materials. The tabletop centrifugation vessel (Thermo Scientific Sorvall® Legend Mach 1.6) was set at 4600 g for 10 minutes in each cycle. Care was taken to avoid biomass loss as much as possible during decantation.

For the sorption study of ASs, the washed wet biomass was used as adsorbent directly. The solids were weight immediately and the moisture content was measured by oven-dryness with known amount of wet sludge at a temperature of 105 ℃. The mass of biomass solids was estimated accordingly. In comparison, the biomass was also inhibited by NaN_3 to prevent potential biodegradation effects in the sorption study.

For the sorption study of PFCs, the washed wet biomass was prepared using two different methods for comparison. The first method was to prepare dried biomass. The wet solids were oven-dried under 40 ℃ for 24 hours so that the weight became relatively stable for measurement. This process was deemed to have minimum change in solids characteristics. After this, the biomass was crushed and sieved through a mesh. In addition, the washed wet sludge was also treated following the USEPA OPPTS 835.1110 method (The Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency, 1998). This method included lyophilization at -110 ^{o}C and desiccation in an oven at 103 ^{o}C for 8 hours. Before desiccation, the lyophilized dried biomass cake was crushed into powder easily. After the series of processes, the microorganisms in the biomass were expected to be inactivated and as such, the sludge could be used as sorbents (The Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency, 1998). All the prepared sludge solids were stored in a 4 ^{o}C cold room for less than 24 hours before sorption experiments.

3.2.3. Sorption studies

Sorption experiments were conducted in either duplicates or triplicates and there were three sets of tests under distinguished experimental conditions including set 1 – sorption test, set 2 – control test and set 3 – blank test (Table 3.4). The flow charts of experimental procedures are shown in Figure 3.2 and Figure 3.3 which are explained below.

Table 3.4. Compositions in the bottles of the three sets of tests in sorption studies which include Set 1 - sorption test, Set 2 - control test and Set 3 - blank test.

Composition	Set 1 (Sorption)	Set 2 (Control)	Set 3 (Blank)
(Treated) sludge solids	√	√	-
Test solution (DI water with or without NaN_3 and phosphate buffer)	√	√	√
Analytes spiked (PFCs/AS)	√	-	√

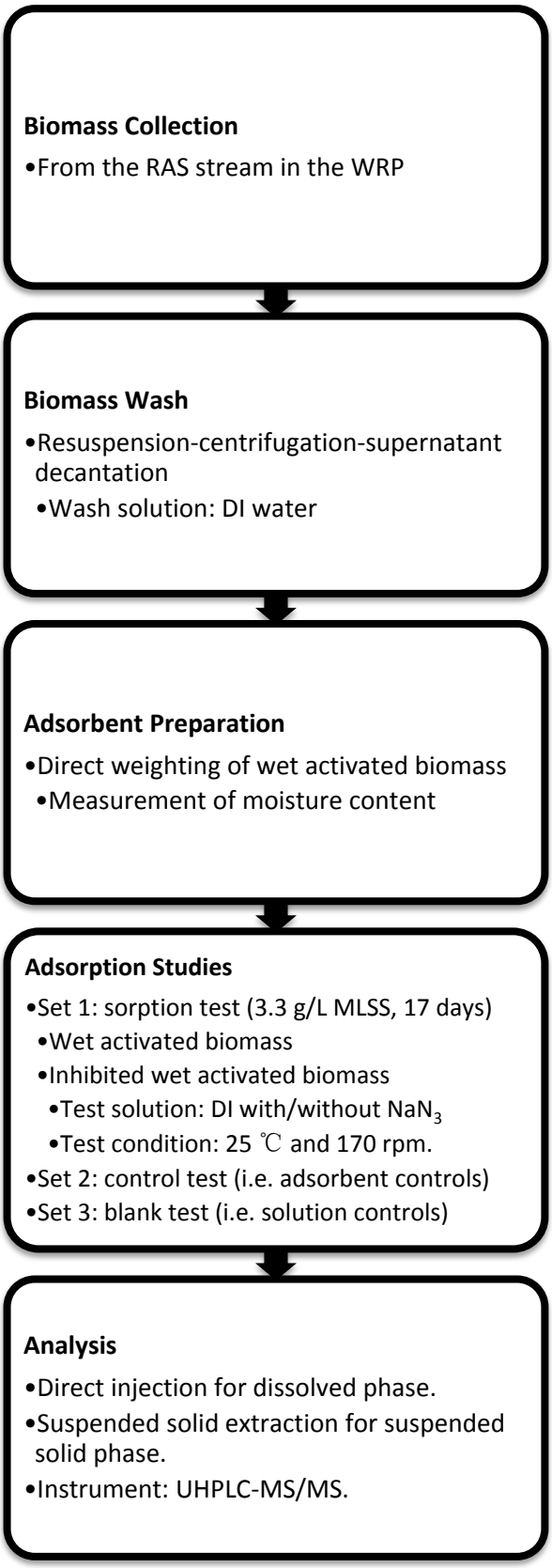


Figure 3.2. Flowchart of bench-scale study on ASs.

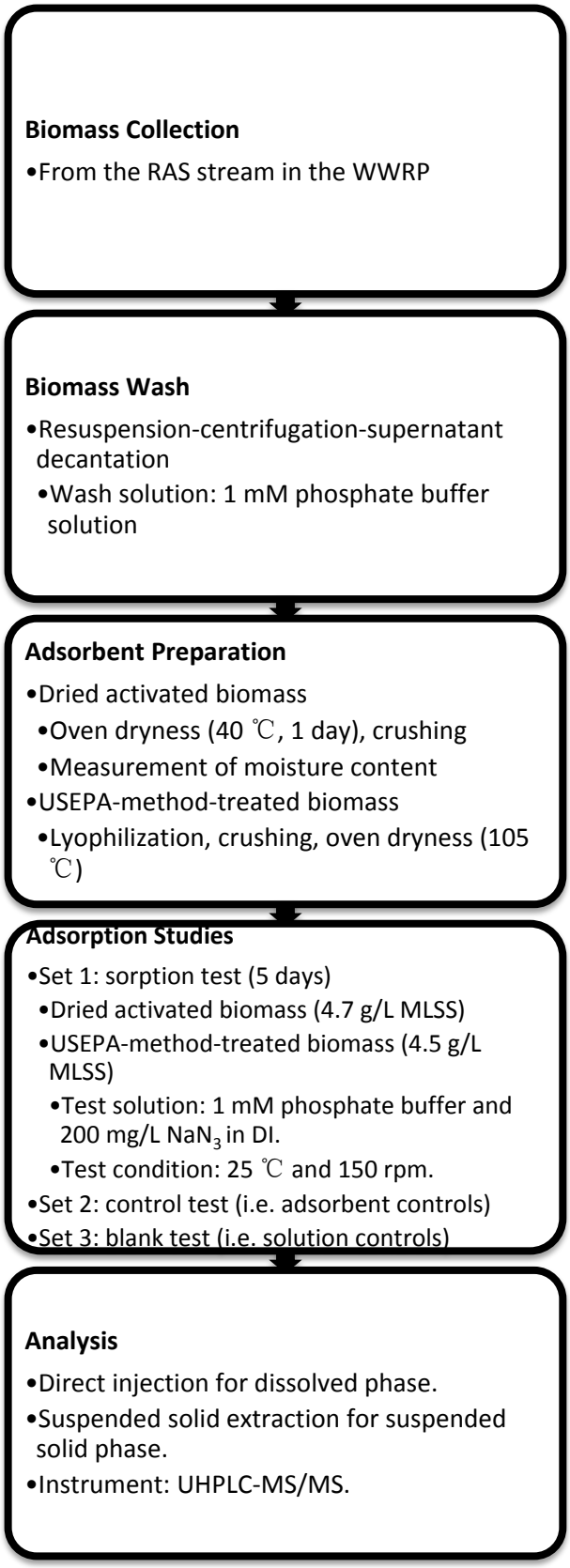


Figure 3.3. Flowchart of bench-scale study on PFCs.

The sorption test (set 1) is to investigate sorption uptake. It was conducted in 1 L high-density polyethylene (HDPE) bottles (Thermo Scientific™ Nalgene™, New York, United States) using 900 ml of biomass-analytes solutions. Wet sludge was diluted to 3300 mg/L MLSS for ASs, while dried biomass and lyophilization-heat treated biomass were weight to yield MLSS concentration at 4700 mg/L and 4500 mg/L respectively for PFCs. The biomass solids were rehydrated for 1 hour before injection of PFCs analytes in the corresponding sorption studies.

For sorption uptake of ASs, the initial concentrations of ACE and SUC were approximately 70 ppb in ultrapure water. The pH fluctuated around 5.6 for inhibited biomass, but it ranged from 5.4 to 4.8 for the original wet biomass, which may indicate some biodegradation. The test bottles were agitated at 170 rpm and 25 ^{o}C in an incubator shaker (IKA® KS 4000) for 17 days. For the analysis of aqueous ASs residues, samples were taken at pre-determined time intervals. The AS-biomass mixtures were centrifuged at 16000 g for 5 minutes and then the supernatant was stored under -30 ^{o}C (SANYO biomedical freezer) for future analysis. HDPE bottles were covered with aluminum foil to prevent potential photodegradation of ASs.

For sorption uptake of PFCs, the initial concentrations were approximately 50 ppb and the test solutions contained 1mM phosphate buffer and 200 mg/L NaN_3. The pH ranged from 6.1 to 6.4 and the addition of NaN_3 was to maintain abiotic conditions during the sorption comparison experiments. The test bottles were shaken at 150 rpm and 25 ^{o}C (IKA® KS 4000) for 5 days. Samples were taken by filtration of PFCs-biomass

mixtures through nylon syringe filter tips (13 mm in diameter, 0.2 μm in pore size, Cronus) at pre-determined time intervals and the filtrates were stored under -30 ^{o}C (SANYO biomedical freezer) until analysis.

The control test (set 2) acted as an adsorbent control such that pre-treated adsorbent was mixed in the test solution without addition of analytes, i.e. without ASs and PFCs. The controls were to determine if there was any contamination and any color/matrix interferences.

Last but not least, the blank test (set 3) refers to a solution control such that analytes were added into the test solution without addition of adsorbent. This blank aims to verify the initial concentrations of analytes and demonstrate whether there was any abiotic reduction of analytes in the test solution during the test period, e.g. sorption to test vessels, interaction with phosphate buffer and/or NaN_3, hydrolysis and photodegradation.

3.2.4. Data analysis

The analytical detection of ASs and PFCs in the collected samples followed the same method described in Section 3.1.3 and 3.1.4 using UHPLC-MS/MS with respective sample preparations.

The sorption behavior can be monitored by both direct and indirect measurements of mass change. The direct method measures the adsorbed mass directly by extraction of biomass. In contrast, the indirect method quantifies the residual mass in the test solution using direct injection so that the adsorbed mass is reflected indirectly by mass balance. Since the accuracy and recovery of the direct injection is higher than the extraction of biomass, the indirect method was applied in this study.

In data analysis, the percentage of residual analytes in aqueous solution

was monitored with time, i.e. the time variation of $\frac{C_t}{C_0}$ (the average relative aqueous concentration at time t normalized to the initial aqueous concentration at time t=0). The time variant percentages depicted the equilibration time and the relative extents of adsorption/biodegradation. Means of the duplicates/triplicates were presented with ± 1 standard deviation (SD). For triplicates, outliers were identified using Dixon's Q test (Dol & Verhoog, 2010).

Chapter 4. Results and discussions

4.1. Occurrence and fate of ASs and PFCs in the WRP

4.1.1. Artificial sweeteners

4.1.1.1. Occurrence of ASs in the WRP

Four artificial sweeteners were monitored in the dissolved and suspended solid phases of the wastewater samples. They included ACE, SUC, CYC and SAC. With reference to Figure 4.1 (a) which depicts the monthly aqueous concentrations during the monitoring period of 6 months (i.e. the dissolved phase concentration ranges of each compound for each treatment process), all of the compounds were detected in 100% of the collected influents (INF) and primary settled sewages (A1 and B1). While ACE and SUC were detected in all of the aqueous samples, CYC and SAC were not detected in most of the samples after biological treatment, including the mixed liquor suspended solids (A2/B2), effluents (A3/B3) and return activated sludge (A4/B4).

Overall, with reference to Figure 4.1 (a), the aqueous concentrations ranged up to tens of ppb level. Aqueous concentrations of ACE, SUC, CYC and SAC were in the ranges of 5.63-10.91 ppb, 1.30-6.50 ppb, n.d.-41.88 ppb and n.d.-18.80 ppb respectively throughout the whole WWTP (please refer to Table B and Table D in Appendix B and Appendix C which summarize the mean concentrations, median values and concentration ranges of ASs in the dissolved phase over the 6 months.).

Many studies have investigated occurrences of ASs in wastewater influents and effluents in the WWTPs, which are summarized in Table 2.3. Average concentrations of ACE, SUC, CYC and SAC occurring in the wastewater influents in this study were 8.19 ppb (6.32-10.51 ppb), 3.96 ppb (2.11-6.50

ppb), 36.60 ppb (29.57-41.88 ppb) and 14.93 ppb (9.31-18.80 ppb) respectively (Table D in Appendix C). These values have more or less the same order of magnitude as the literature data (see Table 2.3), except that Germany and Greece showed an obviously higher level of CYC (Scheurer et al., 2009) and SUC (Kokotou & Thomaidis, 2013) respectively. Moreover, ACE concentration was generally slightly lower than values reported around the world (Buerge et al., 2009; Scheurer et al., 2009; Scheurer et al., 2011; Ordóñez et al., 2012; Gan et al., 2013; Kokotou & Thomaidis, 2013). Note that, for each compound, the influent concentrations have different magnitudes in different countries. This implies that a variety of ASs are being used and released to the sewage and the observed concentrations are different around the world based on diverse applications.

Furthermore, average concentrations of ACE, SUC, CYC and SAC occurring in the wastewater effluents (A3/B3) in this study were 7.39/7.48 ppb (5.84-9.00/6.00-9.15 ppb), 3.11/3.07 ppb (1.66-5.26/1.30-5.94 ppb), 0.017/0.046 ppb (n.d.-0.05/n.d.-0.16 ppb) and n.d. respectively (Table D in Appendix C). Similarly, concentration of ACE was lower than values reported around the world (Buerge et al., 2009; Scheurer et al., 2009; Scheurer et al., 2011; Ordóñez et al., 2012; Gan et al., 2013; Kokotou & Thomaidis, 2013). SUC was in the lower range of effluents around the world such as the US (Buerge et al., 2009; Mead et al., 2009; Oppenheimer et al., 2011; Torres et al., 2011; Ordóñez et al., 2012; Gan et al., 2013; Kokotou & Thomaidis, 2013). CYC and SAC were under instrument detection limits which were lower than values reported in all the studies summarized in Table 2.3. It could be observed that compared to CYC and SAC which can be removed efficiently

from aqueous phase of wastewater, SUC and ACE are persistent and are continuously discharged in effluents. As such, SUC and ACE may be more likely to pose challenges in subsequent industrial water reclamation and cause risks to the aquatic environment in water discharge (Gan et al., 2013).

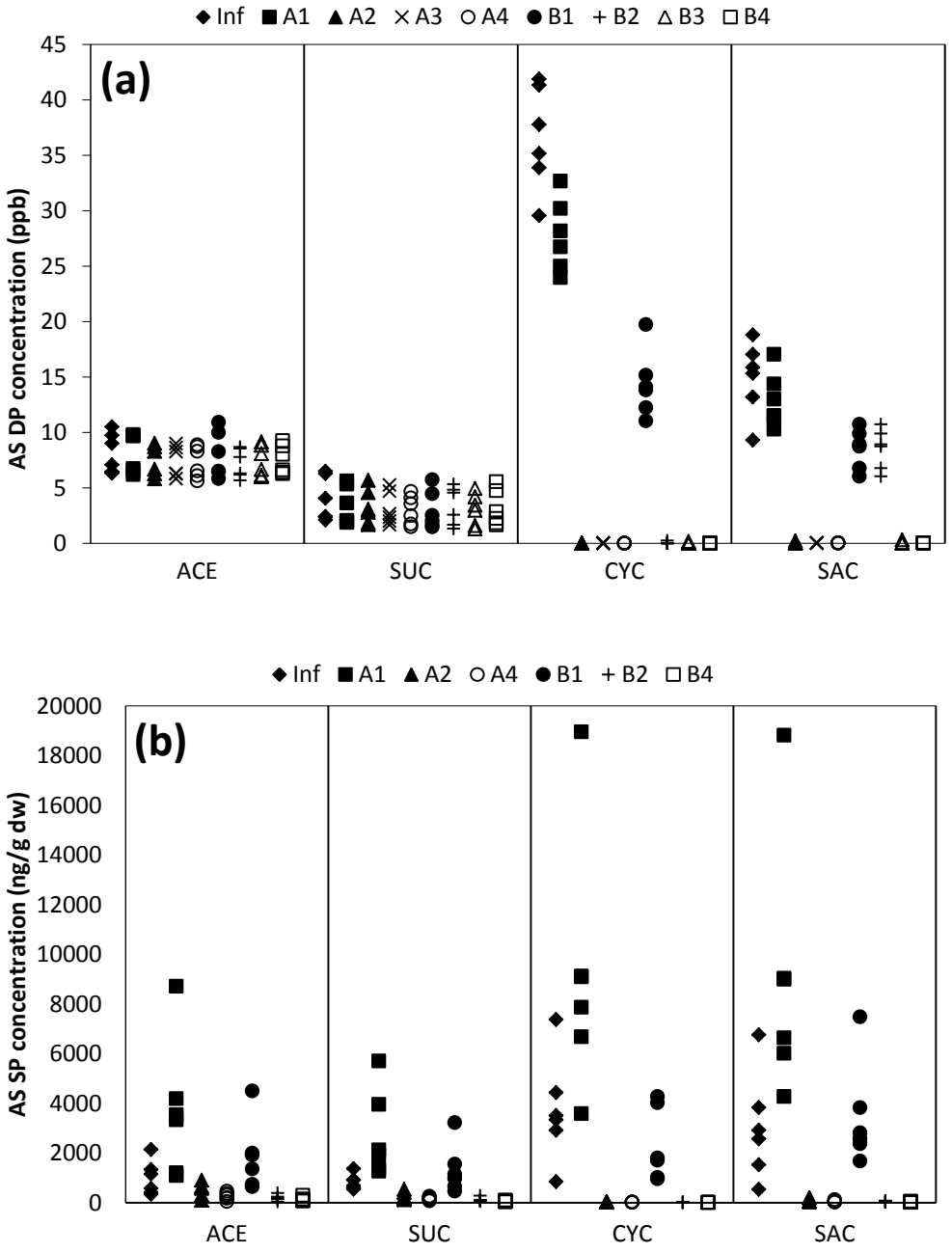


Figure 4.1. Monthly concentrations of each AS in (a) the dissolved phase (ppb) and (b) the suspended solid phase (ng/g dw) of the collected wastewater samples in the local WRP from February 2013 to July 2013.

With reference to Figure 4.1 (b), the suspended solid phase concentrations

ranged from tens to ten thousands of ng/g dw level (except those samples under detection limit). Suspended solid phase concentrations of ACE, SUC, CYC and SAC were in the ranges of n.d.-8709 ng/g dw, 33.6-5702.6 ng/g dw, n.d.- 18951.5 ng/g dw and n.d.-18818.2 ng/g dw respectively throughout the whole WWTP trains (please refer to Table C and Table E in Appendix B and Appendix C which summarize the mean concentrations, median values and concentration ranges of ASs in the suspended solid phase over the 6 months.). Even though ASs are known as polar compounds, their concentrations in the suspended solids in wastewater were easily detected. Sorption of ASs onto suspended solids has been of concern because this process directly immobilizes ASs and reduces their discharge in effluents. Especially in the aeration tank, a high concentration of MLSS could serve as one of the important sinks to accumulate AS mass, and consequently, daily sludge disposal would be one of the important sinks for ASs.

Average suspended solid phase concentrations of ACE, SUC, CYC and SAC occurring in wastewater influents in this study were 990.1 ng/g dw (n.d.-2141.3 ng/g dw), 835.6 ng/g dw (540.3-1367.5 ng/g dw), 3733.2 ng/g dw (842.6-7370.2 ng/g dw) and 3022.4 ng/g dw (536.3-6757.4 ng/g dw) respectively (Table E in Appendix C). Since TSS concentration was very low in the discharge (see Table A in Appendix A), AS concentrations in the suspended solid phase were not quantified. Furthermore, no literature has been found where the concentrations in suspended solids were measured, although Ordóñez et al. (2013) measured concentrations in thickened primary sludge. In this study, concentration ranges of ACE, SUC, CYC and SAC in the primary suspended solids (A1/B1) were (n.d.-8709.1)/(n.d.-4495.4),

(1260.3-5702.6)/(462.3-3223.2), (3581.1-18951.5)/(956.1-4273.8) and (4267.6-18818.2)/(1672.3-7476.9) ng/g dw respectively which were 10-100 times larger than values reported by Ordóñez et al. (2013). This could be attributed to many factors such as different aqueous concentrations in influents, different characteristics between suspended solids and settleable primary sludge, etc.

4.1.1.2. Relative abundance of ASs in different treatment processes

Figure 4.2 shows the relative abundance of ASs in (a) the dissolved phase and (b) the suspended solid phase of samples for the different treatment processes. The AS compositions were similar for influent and primary settled sewage in both the dissolved and the suspended solid phases, based on the comparable percentages for each compound in the respective total ASs by mass (see Table 4.1 for the dissolved phase and Table 4.2 for the suspended solid phase). CYC and SAC took up the two highest proportions in both aqueous phase and suspended solid phase, which indicates that these two compounds are the predominant artificial sweeteners in the sewer system (Scheurer et al., 2009). In comparison, ACE and SUC may be less widely consumed since their total proportion was only around 20% in the INF. This may be due to the lower sugar equivalents of CYC and SAC, which may introduce more addition into food and beverages for the same intensity of sweetness (Lange et al., 2012). In comparison, the consumption pattern may be different in Tianjin, China, where SAC and ACE were found to be the two most abundant sweeteners in influents (Gan et al., 2013). Furthermore, removal of settleable suspended solids from influent wastewater in primary clarifiers did not alter the AS compositions significantly.

After biological treatment, the composition changed significantly with disappearance of CYC and SAC in the aqueous phase (Figure 4.2 (a)). This resulted in 70% and 30% of ACE and SUC in the MLSS (A2/B2), effluents (A3/B3) and RAS (A4/B4) respectively (Table 4.1), consistent with findings summarized by Lange et al. (2012) where CYC and SAC were biodegraded efficiently in the aqueous phase of wastewaters by microbes in municipal WWTPs. Because of their relative persistence, ACE and SUC almost occupied the whole proportion of ASs (Gan et al., 2013; Lange et al., 2012). Concurrently, the AS composition behaved in a similar pattern in the suspended solid phase (Figure 4.2 (b)). The dramatic decrease in the portions of CYC and SAC in both dissolved and suspended solid phases of the wastewater in the biological aeration tanks and afterwards reconfirmed their biodegradability in municipal WWTPs. However, unlike the aqueous phase, there were still approximately 14% of CYC and SAC adsorbed on the suspended solids (Table 4.2). Although different sorption affinities may contribute to various percentages, it is suggested that biodegradation of ASs was more efficient in the aqueous phase than the suspended solid phase. As such, sludge could be another sink for ASs due to potential sorption and incomplete biodegradation. Hence, the treatment and then disposal of sewage sludge should be properly addressed.

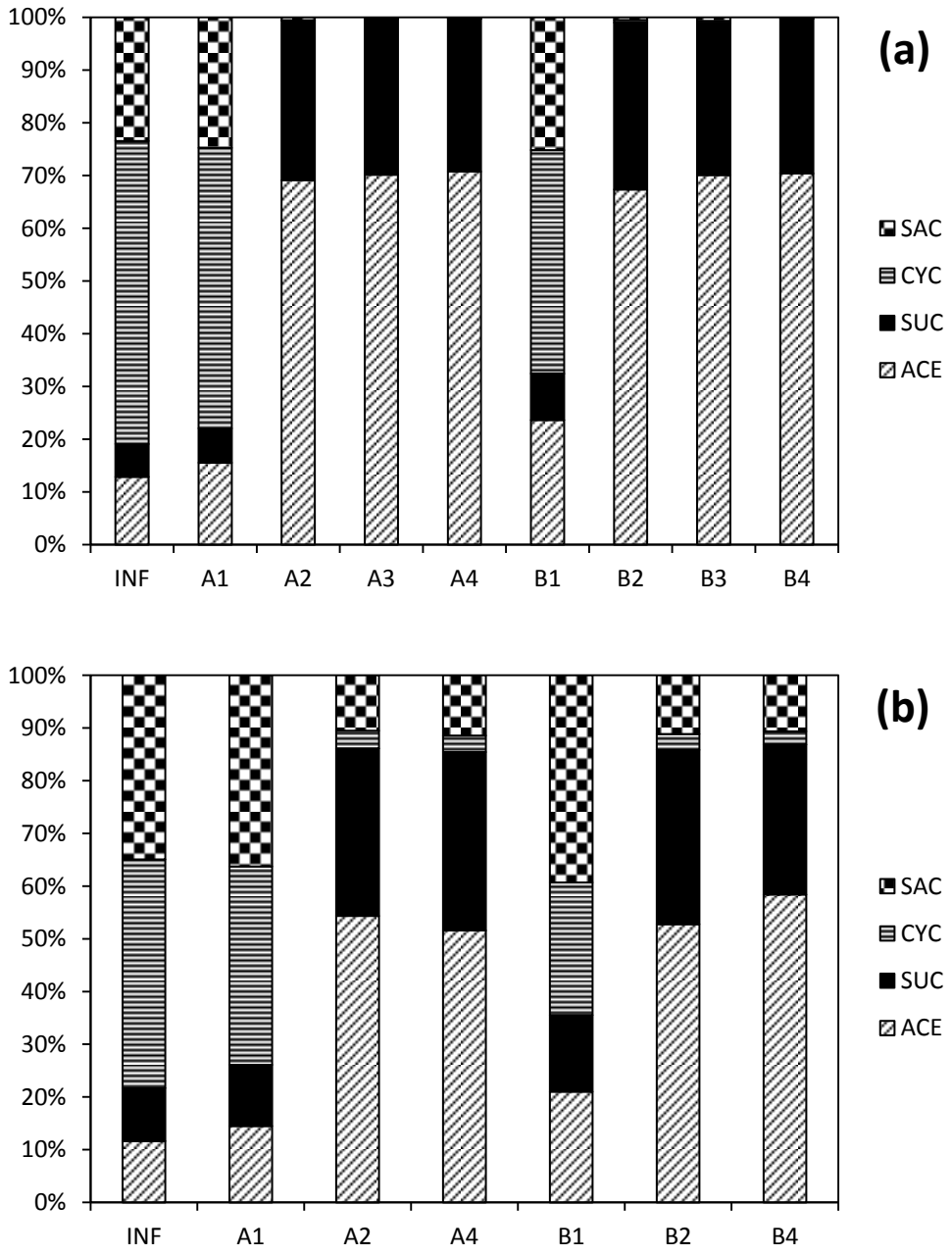


Figure 4.2. Compositions of total ASs in (a) the dissolved phase and (b) the suspended solid phase of wastewater samples collected for each treatment process over the monitoring period of 6 months.

Table 4.1. Percentages of mean concentrations of each AS in the dissolved phase of wastewater samples collected for each treatment process over the monitoring period of 6 months. Unit: %.

Sample name	Sample label	ACE	SUC	CYC	SAC	Total
Influent	INF	13	6	57	23	100
Southworks (A)						
Primary settled sewage	A1	16	7	53	25	100

MLSS	A2	69	30	0	1	100
Effluent	A3	70	30	0	0	100
RAS	A4	71	29	0	0	100
Northworks (B)						
Primary settled sewage	B1	24	9	42	25	100
MLSS	B2	67	32	0	1	100
Permeate	B3	70	29	0	1	100
RAS	B4	70	29	0	0	100

Table 4.2. Percentages of mean concentrations of each AS in the suspended solid phase of wastewater samples collected for each treatment process over the monitoring period of 6 months. Unit: %.

Sample name	Sample label	ACE	SUC	CYC	SAC	Total
Influent	INF	12	10	43	35	100
Southworks (A)						
Primary settled sewage	A1	14	12	38	36	100
MLSS	A2	54	32	3	10	100
RAS	A4	52	34	3	11	100
Northworks (B)						
Primary settled sewage	B1	21	15	25	39	100
MLSS	B2	53	33	3	11	100
RAS	B4	58	28	3	11	100

Figure 4.3 shows the mass distribution of total ASs between dissolved and suspended solid phases. It is clear that ASs were dominant in the dissolved phase with proportions ranging from 84% to 95% for various treatment processes. This is consistent with their low $\log K_{ow}$ values and high solubilities which indicate relatively weak sorption. As such, the majority of AS mass tended to stay in the aqueous phase and discharge of effluents could be more likely to cause risks to the receiving aquatic environment. Note that the mass percentages of total ASs in the suspended solid phase from outlets of the biological processes (A2, B2, A4 and B4) were relatively higher than those in the other stages, although the reverse trend was observed for dried mass concentrations (discussed later). This could be attributed to higher

concentrations of suspended solid in biological tanks and RAS (Shivakoti et al., 2010).

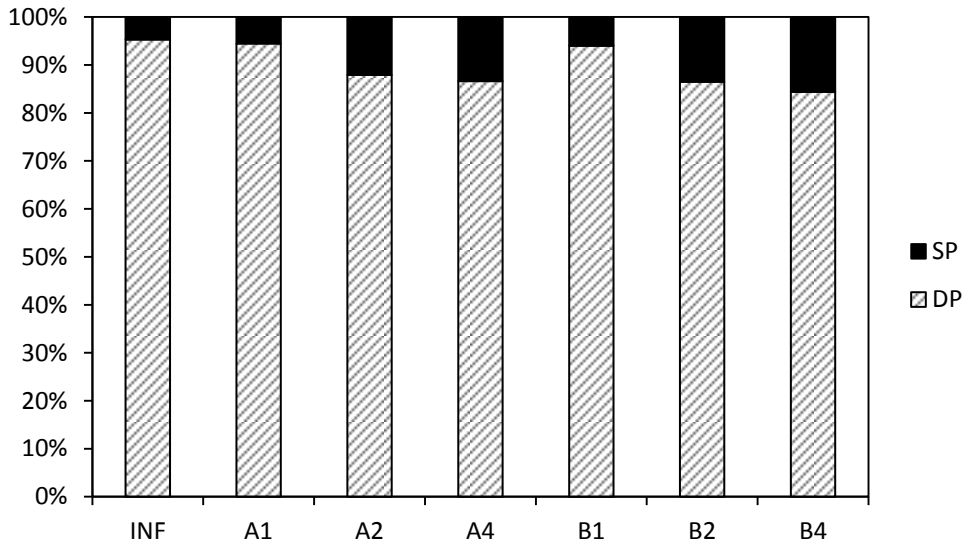


Figure 4.3. Percentages of the average dissolved phase concentration and the average suspended solid phase concentration of total ASs in entire wastewater samples for each treatment process over the monitoring period of 6 months.

4.1.1.3. Behavior of ASs in the WRP

Figure 4.4 shows the average combined concentrations of ACE (a), SUC (b), CYC (c) and SAC (d) which were separated into dissolved phase and suspended solid phase in wastewaters for the different treatment processes. The behavior and removal of ASs were investigated by comparing different concentrations in the different treatment processes. Note that the suspended solid phase concentrations of ASs in A3 and B3 were not measured due to minor suspended solids in these samples. Due to lack of flow data which could change the concentration, wastewater volume was assumed to remain relatively constant in all treatment processes (Shivakoti et al., 2010).

The behaviors of ACE and SUC were similar based on Figure 4.4 (a) and (b). The combined concentrations in the entire wastewater samples were relatively stable throughout the WRP, which indicates the persistence and

recalcitrant nature of these two compounds in WWTPs. This observation is consistent with the literature (Scheurer et al., 2009; Scheurer et al., 2011; Gan et al., 2013; Kokotou & Thomaidis, 2013). The total removal was approximately 13% and 28% for ACE and SUC respectively with an aqueous phase elimination of 10% and 22%. ACE removal efficiency was found to be lower than the value reported by Scheurer et al. (2009) (up to 41%) but elimination of SUC was higher than values reported by Brorström-Lundén E et al.(2008) (<10%) and Scheurer et al. (2009) (around 20%). The difference could be caused by different treatment units and operational parameters in different WWTPs.

A more detailed analysis shows that, when the influent flowed through the primary clarifiers, aeration tanks (AT or RAS) and secondary clarifiers or MBR to become effluent, the aqueous concentrations kept decreasing while the particulate concentrations kept increasing for ACE and SUC, with a small decrease in the combined concentrations. First, ACE concentrations in INF and A1/B1 were comparable to each other while there was a reduction of SUC concentration in settled sewage. The decrease after the primary clarifiers could be caused by removal of ASs in settled suspended solids (Shivakoti et al., 2010). The small increase in solid to liquid ratios could be due to higher dried mass concentrations of ACE and SUC on primary suspended solids compared to those on influent suspended solids (shown in Figure 4.1 and discussed later), even though the concentration of total suspended solids decreased in primary settled sewage compared to the influent (see Table A). Second, when wastewater reached the aeration tanks (A2/B2) or RAS (A4/B4), the combined concentrations did not change much but the fraction in the suspended solid

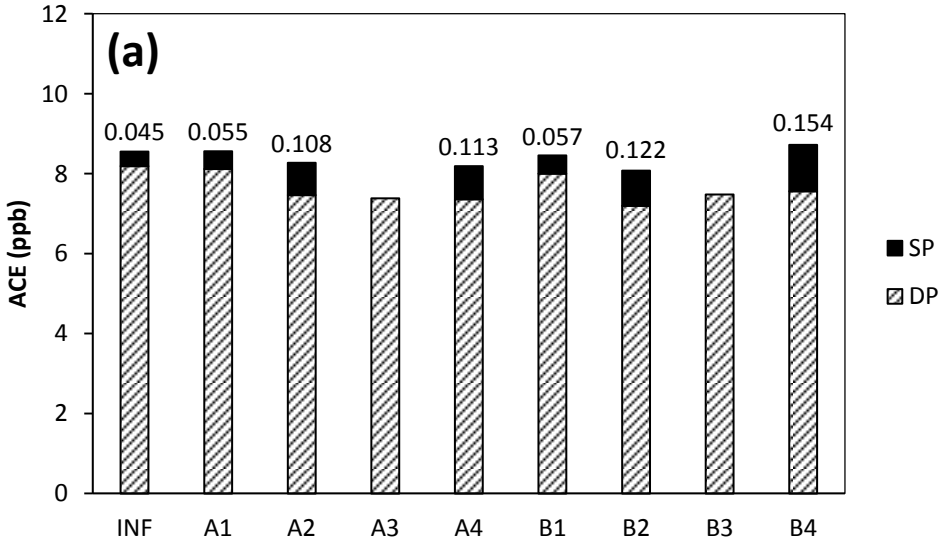
phase increased obviously with larger solid to liquid ratios. This may be due to the presence of much higher concentration of mixed liquor suspended solids in the aeration tanks which provided more solid mass for sorption (Table A). As a result, a greater mass of ACE and SUC in the aqueous phase could partition onto the abundant MLSS, although the sorption capacity may be relatively limited. Last but not least, after the last solid-liquid separation units (A3: secondary clarifier; B3: membrane bioreactor), the aqueous phase concentrations in effluents did not change significantly compared to those in the previous aeration tanks, but the MLSS concentration decreased to a negligible amount. On the one hand, this indicates that the settling/separation mechanisms of MLSS in the last step may not re-suspend the MLSS-sorbed ACE and SUC significantly. On the other hand, the discharge loadings of ACE and SUC in the WRP effluent could be reduced with sludge removal, which can particularly contribute to the overall removal efficiency. This point emphasizes the significance of sorption of ACE and SUC on MLSS. It may be the only or the most effective process that can reduce persistent ACE and SUC discharge in the effluent of the WRP, although their sorption affinity may be weak and sorption capacity of MLSS for ASs may be limited, as mentioned in the literature (Brorström-Lundén E et al., 2008; Lange et al., 2012). Hence, a lab-scale sorption study was conducted in this research to confirm the sorption potential of ACE and SUC. Care should be taken with sludge disposal, recycling and reuse, since sorption may only transform the ASs problem in aqueous wastewater discharge to a sludge problem without chemical destruction of ACE and SUC. Measurement of ACE and SUC on sludge wastes could help confirm this hypothesis.

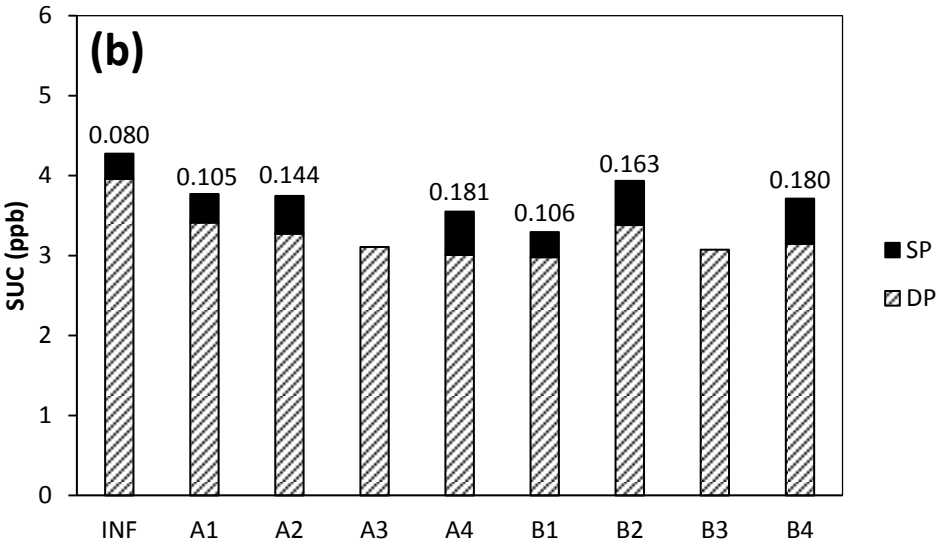
The behaviors of CYC and SAC were similar based on Figure 4.4 (c) and (d). They were almost completely eliminated with little residue of SAC left on suspended solids after biological processes in the aeration tanks. This observation is consistent with the literature (Gan et al., 2013; Kokotou & Thomaidis, 2013; Scheurer et al., 2009; Scheurer et al., 2011). The total removal was approximately >99.9% and ~99.7% for CYC and SAC respectively. These values were found to be higher than values reported by Scheurer et al. (2009) (>90% for both CYC and SAC) and Kokotou & Thomaidis (2013) (70% for CYC and >99.5% for SAC).

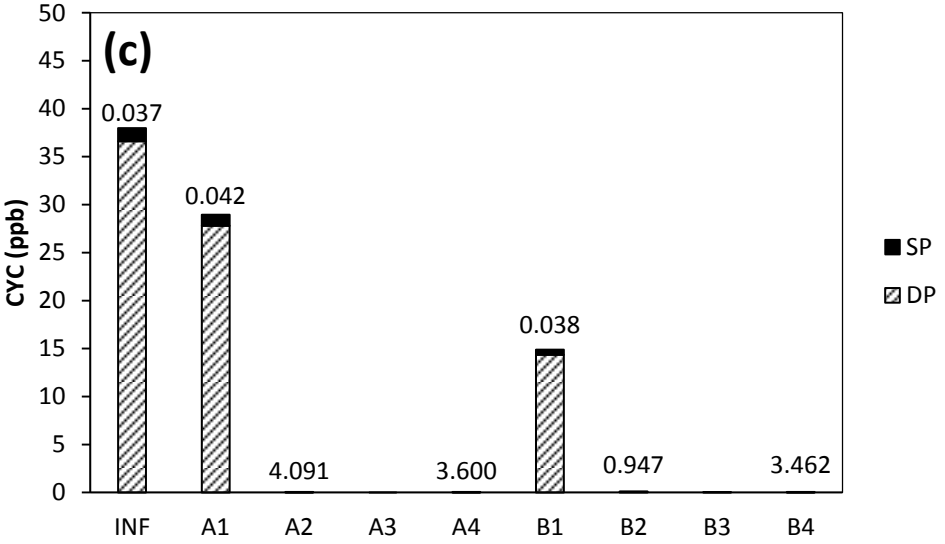
When the influent flowed through the WRP, both the aqueous phase concentration and the suspended solid phase concentration kept decreasing, especially in the biological aeration tanks. This implies good biodegradability of CYC and SAC in the WWTP (Gan et al., 2013; Kokotou & Thomaidis, 2013; Scheurer et al., 2009; Scheurer et al., 2011). The removal in the primary clarifiers was limited while there was a dramatic reduction in the biological aeration tanks. This is not surprising since the biological activity in the aeration tank is much higher than that in the primary clarifiers, which emphasizes the importance of biological degradation in determining the fate of CYC and SAC in the WWTP. In addition, the removal with settlable suspended solids seemed to only play a minor role compared to biodegradation.

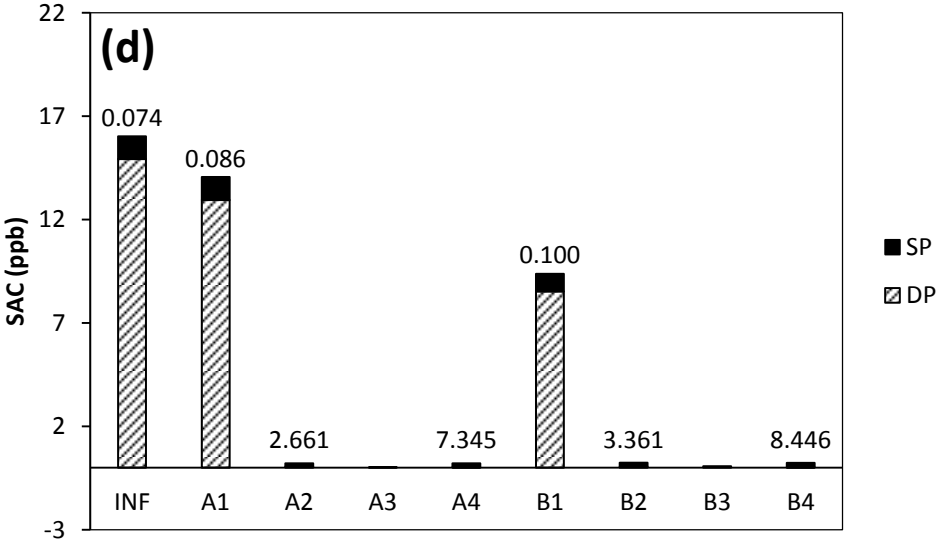
Figure 4.4 (e) illustrates the average concentrations of the total ASs in both dissolved and suspended solid phases of wastewater samples for various treatment processes. The entire wastewater influent carried approximately 66.82 ppb of total ASs and their combined removal was approximately 84%

throughout both treatment trains in the WRP, which resulted in around 10.5 ppb total ASs in the aqueous phase of effluent discharge. The removal efficiency was mainly in the aqueous phase and contributed by biodegradation of CYC and SAC, as mentioned earlier. Statistically speaking, the results of ANOVA test (ANOVA-Prism 6.1) (Table F in Appendix E) showed that SUC and ACE appeared to be persistent to all types of treatment throughout the WWTP with relatively stable aqueous concentrations, while CYC and SAC presented significant elimination after biological treatment stages in both trains. Lastly, with reference to Figure A in Appendix D, the consistent trends in the combined (dissolved phase and suspended solid phase) concentration of ASs throughout the treatment trains for all 6 months, imply a stable performance of the conventional MLE biological process for the removal of ASs.




(b)
SUC (ppb)
6
5
4
3
2
1
0
0.080
0.105
0.144
0.181
0.106
0.163
0.180
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(c)
CYC (ppb)
50
45
40
35
30
25
20
15
10
5
0
0.037
0.042
4.091
3.600
0.038
0.947
3.462
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(d)
SAC (ppb)
22
17
12
7
2
-3
0.074
0.086
2.661
7.345
0.100
3.361
8.446
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP

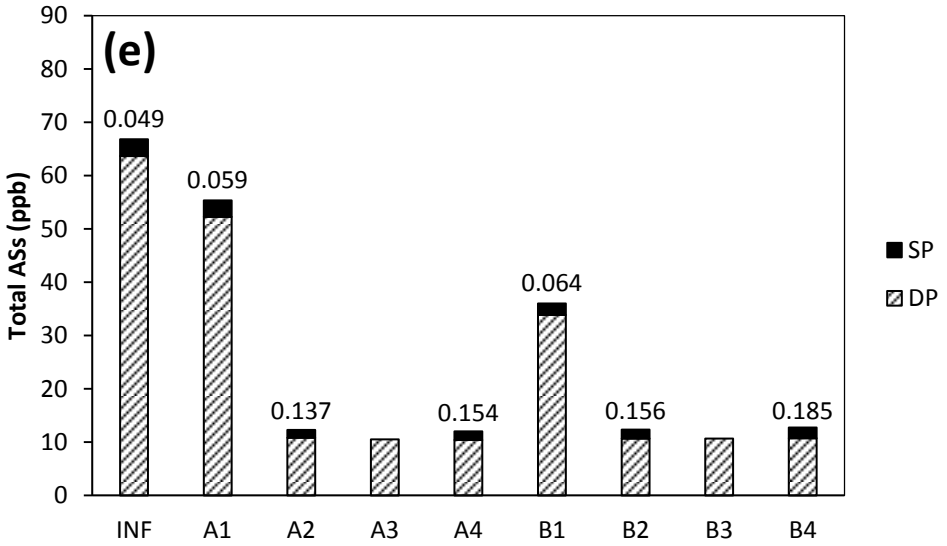


Figure 4.4. Mean concentrations (ppb) of (a) ACE, (b) SUC, (c) CYC, (d) SAC and (e) total ASs in the dissolved phase and the suspended solid phase (volume unit) of the wastewater samples collected for each treatment process in the WRP over the monitoring period of 6 months. The values for bars inside each figure are solid to liquid ratios (unitless) by dividing dissolved phase concentrations (ppb) by corresponding suspended solid phase concentrations (ppb).

Last but not least, Table 4.3 shows the ratios (10^3L/g) of the suspended solid phase concentrations (ng/g dw) to the corresponding dissolved phase concentrations (ppb) of the four ASs in the various treatment stages. There were no estimated ratios for CYC and SAC in biological aeration tanks and RAS since their concentrations were under the detection limit. It should be noted that the solid to liquid ratio is more appropriate to use in this study instead of the distribution coefficient. This is because the distribution coefficient requires the equilibrium condition which may not necessarily be obtained in dynamic systems in real WWTPs due to the low HRTs.

In general, SUC showed the highest ratios followed by SAC, ACE and CYC (Table 4.3). Part of this observation is consistent with findings in the subsequent bench-scale sorption study where SUC showed higher sorption affinity than ACE (see Chapter 4.2.1).

Furthermore, ratios were higher in the primary clarifiers, which may be attributed to the higher AS concentrations in the suspended solid phase in terms of dry weight (Figure 4.1 (b)). However, care should be taken when comparing ratios between various treatment units since many factors could affect the values such as mixing strength, composition and characteristics of suspended solids, dynamics and hydraulics of flow (e.g. nonequilibrium condition) and variability in loadings of ASs in wastewaters. The large ranges shown in Table 4.3 verify the large variations in the ratios affected by these factors. For example, the increase in ratios in the primary clarifiers may be possibly due to quiescent settling conditions which may favor labile uptake of ASs on primary suspended solids (Labile uptake is treated as a fast and instantaneous adsorption process which is operationally described as the amount of sorbed species desorbed promptly in a standard experiment (Li et al., 1996). In contrast, nonlabile uptake involves chemisorption or slow intraparticle diffusion mechanisms (Li et al., 1996).). In contrast, although mixing conditions in aeration tanks may favor surface contact for adsorption, the agitation may both resuspend the labile portion and reduce the labile fraction on solids. Considering another factor, i.e. different characteristics of suspended solids such as porosity, this may affect moisture content and consequently concentration measurement on the suspended solid phase. As suggested by Ordóñez et al. (2013), suspended solids with higher moisture content may have more ASs left over from pore water during LC sample preparation, so the suspended solid phase concentration would be measured higher. Since the characteristics of the suspended solids in different units vary, it is difficult to draw precise conclusions about the accumulation. Bench-scale

studies are recommended to investigate the difficult factors sequentially to determine how they can affect the adsorption and accumulation of ASs in the suspended solid phase.

Table 4.3. Average solid (ng/g dw) to liquid (ppb) ratios of each AS. The values in parenthesis show the range of S/L ratios. Unit: 10^3L/g.

	Influent	A1	A2	A4	B1	B2	B4
ACE	114 (53-238)	425 (176-896)	59 (16-109)	33 (7-55)	231 (98-544)	26 (6-50)	17 (9-37)
SUC	239 (146-387)	756 (377-1069)	91 (66-133)	53 (33-73)	438 (198-721)	36 (17-61)	20 (10-30)
CYC	101 (28-217)	332 (134-673)	-	-	155 (73-285)	-	-
SAC	188 (58-396)	678 (373-1309)	-	-	406 (239-2784)	-	-

4.1.2. Perfluorinated compounds

4.1.2.1. Occurrence of PFCs in the WRP

Eight perfluorinated compounds and derivatives were monitored in the dissolved and suspended solid phases of the wastewater samples. They included PFCAs (PFBA, PFOA, PFNA and PFDA), PFSAs (PFHxS and PFOS) and PFC derivatives (N-EtFOSAA and FOSAA). With reference to Table G and Table H in Appendix F which shows the two monthly dissolved phase and suspended solid phase concentrations of each compound for each treatment process, all of the compounds were detected in 100% of the wastewater samples. Concentrations of total PFCs ranged from 82.4 ppt to 148.8 ppt in the dissolved phase and from 226.2 ng/g dw to 1390.3 ng/g dw in the suspended solid phase in the WRP. Overall, the aqueous concentrations were at ppt level for all the PFCs (except N-EtFOSAA in one MBR permeate (B3) in July which was under detection limit) and the suspended solid phase concentrations ranged from several to hundreds of ng/g dw (Table G and Table H in Appendix F). Similar to ASs, since TSS concentration was very low in

the effluent (Table A), concentrations of PFCs in the suspended solid phase were not quantified.

Table 4.4 summarizes the mean concentrations of each PFC in the dissolved and the suspended solid phases of influent and effluent samples with respective ranges throughout the WRP. In terms of the dissolved phase concentrations, PFOA and PFNA showed similar or lower concentrations compared to the values reported in most Asian countries such as Korea (Guo et al., 2010), Japan (Shivakoti et al., 2010) and China (Pan et al., 2011) (see Table 4.5). In contrast, PFDA, PFHxS and PFOS showed higher concentrations. However, Thailand, whose WWTP receives wastewater in the industrial zone, showed much higher concentrations than those found in our study for all the selected compounds. This could be attributed to high loadings of PFCs in the industrial wastewater, which suggests that industrial wastewater is one of the most important sources of PFCs into WWTPs. Furthermore, compared to these five compounds, PFBA, N-EtFOSAA and FOSAA have drawn less attention in the monitoring studies in WWTPs. Boulanger et al. (2005) studied the biotransformation fate of N-EtFOSE in WWTPs in Iowa, U.S., and showed similar N-EtFOSAA concentrations, as one of the metabolites, with those found in our study. However, FOSAA was not detected by them. In addition, PFBA in our study showed higher concentrations than those reported in Hong Kong, China (which were all under detection limit) (Ma & Shih, 2010); but was in the middle range of values from Tianjin, China (Sun et al., 2012).

It should be noted that in this study, grab samplings may bias the measured concentrations via factors such as weather and flow. To acquire a set of more

representative concentrations, more sampling events and composite sampling methods are recommended. In addition, the high effluent concentrations of PFCs indicate the general removal inefficiency of conventional WWTPs. Residual PFCs are likely to be discharged to receiving water bodies and may cause possible risks to the aquatic environment and/or pose challenges in subsequent industrial water recycling.

Table 4.4. Summary of mean concentrations of PFCs in the influent and effluent, and the respective concentration ranges throughout the WRP in both the dissolved and the suspended solid phases of the collected wastewater samples.

Analyte	Dissolved phase			Suspended solid phase	
	Influent (ppt)	Effluent (ppt)	Range (ppt)	Influent (ng/g dw)	Range (ng/g dw)
PFBA	29.20	39.13/50.76	18.47-57.33	17.90	1.97-63.27
PFOA	10.47	12.69/13.44	8.04-19.64	43.27	10.72-158.30
PFNA	2.02	4.51/3.69	1.55-6.38	25.13	7.26-105.58
PFDA	17.40	26.26/21.89	5.10-47.43	23.32	21.00-104.50
PFHxS	14.48	13.93/13.23	12.67-16.52	35.27	2.74-100.49
PFOS	6.51	7.92/17.2 7	4.94-20.25	88.62	75.08-638.03
N-EtFOSAA	8.19	4.47/3.90	n.d.-17.85	30.80	23.86-165.58
FOSAA	4.06	4.41/5.33	3.43-6.52	21.94	7.52-57.33

Few papers have published suspended solid phase concentrations, but many have measured sludge concentrations. Shivakoti et al. (2010) measured suspended solid phase concentrations of PFOA, PFNA, PFDA, PFHxS and PFOS in ng/L in the WWTPs in Japan and Thailand (Table 4.5). Their values were lower than those found in our study, except that PFOS showed several comparable values (Shivakoti et al., 2010). It should be noted that the volumetric concentration depends on many factors especially the TSS concentrations in wastewater samples. More TSS content can provide more solids for sorption and accumulation of PFCs. In Korea, most of the sludge samples were below the detection limit except that PFDA and PFOS showed lower concentrations compared to our study (Guo et al., 2010). Furthermore, PFBA in activated sludge showed comparable dry weight concentrations to

those reported in thickened sludge in Tianjin China (Sun et al., 2012), but lower than Hong Kong (Ma & Shih, 2010). No studies were found that reported suspended solid phase concentrations of the (intermediate) metabolites, N-EtFOSAA and FOSAA, from the literature review.

Table 4.5. Concentrations of PFOA, PFNA, PFDA, PFHxS and PFOS in WWTPs in Asian countries, including Korea (Guo et al., 2010), Japan (Shivakoti et al., 2010), Thailand (Kunacheva et al., 2011), China (Pan et al., 2011) and Singapore (this study). Units: ppt for inf, eff, inf liq., eff liq., inf pr., and eff pr.; ng/g dw for sludge and activated sludge.

	Korea[a]	Japan[b]	Thailand[c]	Beijing, China[d]	Singapore
PFOA	**CAS**: 8.2 (inf); 9.1 (eff); <LOQ (sludge). **MLE**: 5.5 (inf); 7.4 (eff); n.d. (sludge).	2.1-26.7 (inf liq.); 11.6-139.4 (eff liq.); 1.3-14.3 (inf pr.); 1.3-4.0 (eff pr.).	142.1 ±7.2 (inf); 49.8 ±7.8 (eff); 136.0 ±32.4 (sludge).	1.33-135 (inf); 2.37-104 (eff); <1-12.6 (activated sludge).	10.47 (inf liq.); 12.69/13.44 (eff liq.); 15.89 (inf pr.).
PFNA	**CAS**: 0.7 (inf); <LOQ (eff); n.d. (sludge). **MLE**: <LOQ (inf); 0.7 (eff); n.d. (sludge).	n.d.-10.7 (inf liq.); 9.9-61.9 (eff liq.); 0.2-3.4 (inf pr.); 1.7-4.2 (eff pr.).	15.3 ±1.8 (inf); 21.4 ±2.6 (eff); 5.1 ±7.2 (sludge).	<0.15-59.5 (inf); <0.15-81 (eff); <0.3-2.66 (activated sludge).	2.02 (inf liq.); 4.51/3.69 (eff liq.); 9.23 (inf pr.).
PFDA	**CAS**: n.d. (inf); 0.7 (eff); 3.8 (sludge). **MLE**: n.d. (inf); 0.6 (eff); <LOQ (sludge).	n.d.-1.1 (inf liq.); 0.4-10.6 (eff liq.); 0.2-2.8 (inf pr.); 0.1-3.8 (eff pr.).	63.1 ±7.6 (inf); 81.4 ±17.0 (eff); 327.7 ±0.0 (sludge).	<0.15-2.66 (inf); <0.15-10.6 (eff); <0.3-3.32 (activated sludge).	17.40 (inf liq.); 26.26/21.89 (eff liq.); 8.54 (inf pr.).
PFHxS	**CAS**: n.d. (inf); 2 (eff); n.d. (sludge). **MLE**: 23 (inf); 5.6 (eff); n.d. (sludge).	n.d.-4.8 (inf liq.); n.d.-4.5 (eff liq.); n.d. (inf pr.); n.d. (eff pr.).	31.7 ±8.3 (inf); 28.8 ±6.9 (eff); 157.7 ±1.9 (sludge).	<0.07-2.06 (inf); <0.07-3.49 (eff).	14.48 (inf liq.); 13.93/13.23 (eff liq.); 12.78 (inf pr.).
PFOS	**CAS**: 1.6 (inf); 1.3 (eff); 4.2 (sludge). **MLE**: 13.3 (inf); 4.8 (eff); 13.2 (sludge).	n.d. in seperated sewage systems.	465.4 ±55.9 (inf); 296.2 ±38.2 (eff); 396.9 ±82.3 (sludge).	<0.07-29.9 (inf); 0.51-12.1 (eff); 0.69-16.7 (activated sludge).	6.51 (inf liq.); 7.92/17.2 7 (eff liq.); 32.53 (inf pr.)

[a] Water sources: combined domestic wastewater and landfill leachate for CAS (conventional activated sludge) M-WWTP (municipal wastewater treatment plants); and combined domestic and industrial wastewater for MLE (Modified Ludzack-Ettinger) M-WWTP.
[b] Water source: mixture of domestic and industrial wastewater. Concentrations of influent and secondary clarifier effluent from CAS-WWTPs are summarized.
[c] Concentrations of influent and secondary clarifier effluent from one CAS-WWTP in the central industrial zone in Thailand are summarized.
[d] Concentrations of influent and effluent for seven main M-WWTPs in Beijing, China are summarized.
Inf: influent; eff: effluent; liq.: liquid; pr. Particulate; n.d.: not detected.

Many factors could contribute to variations in solid phase concentrations, such as solid characteristics and operational parameters. The high PFC concentrations in the suspended solids reconfirmed the hydrophobicity of these PFCs with high $\log K_{oc}$ values (Arvaniti et al., 2012; Zareitalabad et al., 2013). Sorption of PFCs onto suspended solids and sludge in WWTPs have been emphasized by many studies, in addition to the sludge disposal problem since sludge is one of the major sinks for accommodating and immobilizing PFCs (Yu et al., 2009; Kunacheva et al., 2011). As a result, care should be taken to post-treat and recycle sludge after WWTPs, and a monitoring program for PFCs during sludge postreatment (e.g. incineration in Singapore) is recommended for better control of EOCs.

A previous study on the occurrence of PFCs has been conducted in two sewage treatment plants in 2006/2007 by Yu et al. (2009) in Singapore, one of which includes a CAS treatment train and an MBR treatment train treating mainly domestic wastewater. Compared to the values in our study, the dissolved phase concentrations of PFOA and PFOS were slightly higher for influent and effluent samples in both trains, but suspended solid phase concentrations were generally lower in influent samples for PFOA and similar for PFOS. This indicates variability in the characteristics of influent wastewater and influent concentrations of PFCs with time, which is a challenge for WWTPs to achieve a stable performance for the persistent emerging contaminants.

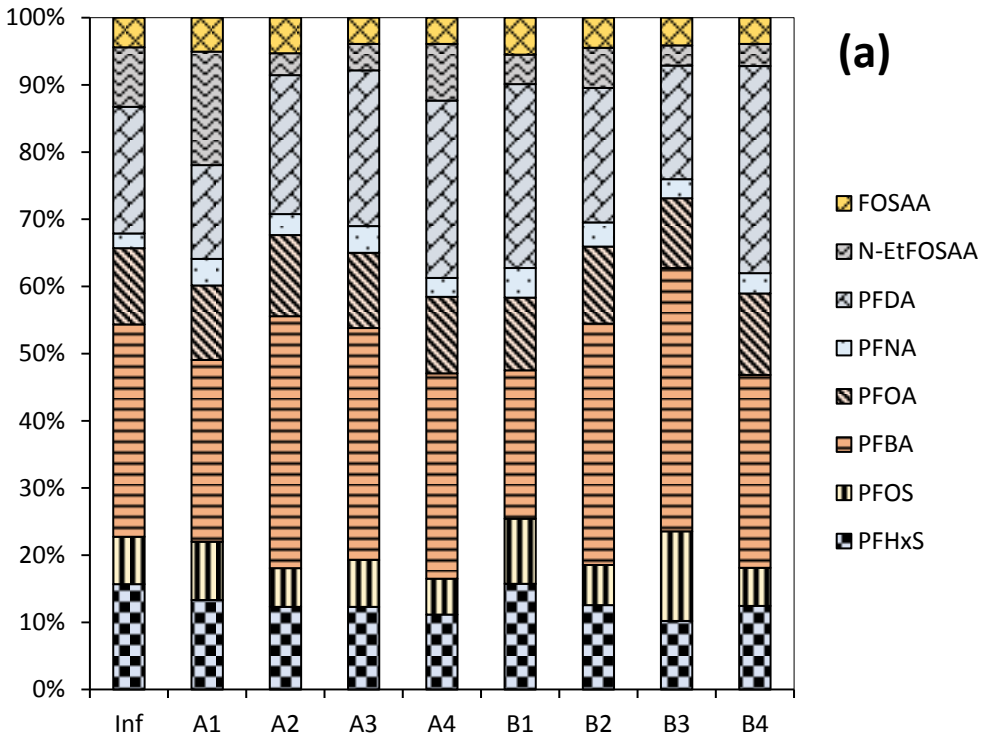
4.1.2.2. Relative abundance of PFCs in different treatment processes

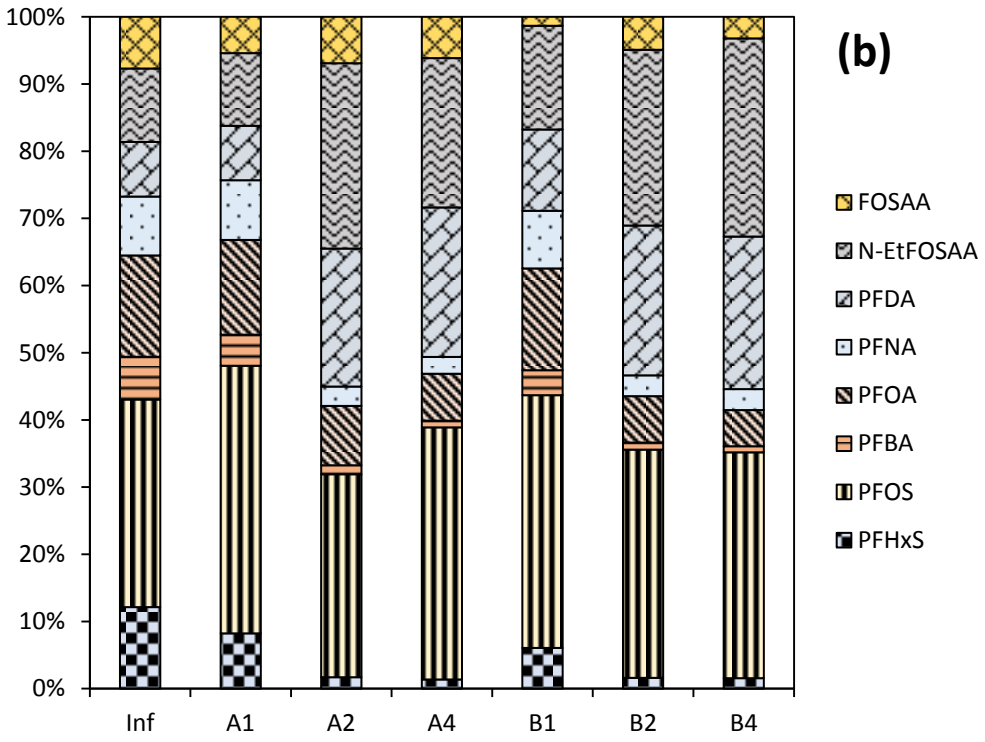
Figure 4.5 shows the relative abundance of PFCs in the dissolved phase, the suspended solid phase and the entire wastewater sample for various

treatment processes. With reference to Figure 4.5 (c), PFOS and PFBA were the two most abundant PFCs in the influent which were around 20% and 18% respectively. This indicates that these two compounds may be the predominant PFCs applied in industrial and domestic products, assuming no losses in the sewer system. As PFOS was listed as POPs in the Stockholm convention (2009), the release of PFOS could be of significant concern for environmental discharge and further industrial water recycling (Shivakoti et al., 2010; Zhou et al., 2010; Sun et al., 2012). In addition, it is not surprising to observe elevated concentrations of PFCs with short C chains in wastewater influents, since there has been an increasing trend to substitute longer C-chain PFCs with shorter C-chain compounds due to potential risks associated with longer C-chains PFC, especially PFOA and PFOS (Betts, 2007; Ochoa-Herrera & Sierra-Alvarez, 2008; Eriksen et al., 2010). Overall, PFBA, PFDA, PFHxS and PFOA were the four most dominant PFCs in the dissolved phase of the influent, with approximately 32%, 19%, 16% and 11% distribution respectively, while the others took up 22% in total (Figure 4.5 (a)). In the suspended solid phase of the influent, PFOS, PFOA, PFHxS and N-EtFOSAA were the four most dominant PFCs with an approximate distribution of 31%, 15%, 12% and 11% respectively, while the others took up 31% in total (Figure 4.5 (b)). PFHxS showed relatively elevated levels. It is believed to be one of the major components in firefighting materials.

When wastewater flowed through the WRP as influents to effluents via clarifiers and biological aeration tanks, the relative abundance of the 8 PFCs was relatively stable in the dissolved phase. This suggests insignificant removal of dissolved PFCs through the treatment stages. On the other hand, in

terms of the relative abundance of PFCs in the suspended solid phase, although it was comparable between influents and settled sewages (A1/B1), there was an obviously consistent change after wastewater entered the biological aeration tanks and RAS. The percentages of PFDA and N-EtFOSAA increased dramatically while the percentages of PFOA, PFNA, PFHxS and PFBA decreased accordingly in the aeration tanks and RAS, with the result that PFOS, N-EtFOSAA and PFDA were the three dominant PFCs (>80%) on MLSS in biological tanks. In addition to a rapid increase in TSS concentrations in biological tanks, this redistribution could be mainly related to SRT and relative sorption affinities of different PFCs due to different carbon chain lengths and functional groups in the structures (see Table 3.1). PFDA (9 CF_2 units), N-EtFOSAA (8 CF_2 units) and PFOS (8 CF_2 units) with longer carbon chains could exhibit higher hydrophobicity, which favors PFCs sorption onto activated sludge. Compared to FOSAA, N-EtFOSAA has one more ethyl group ($-CH_2CH_3$) and may be more hydrophobic consequently. As a result, these three compounds tended to accumulate onto the MLSS better than the others within the same time period.


(a)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Inf
A1
A2
A3
A4
B1
B2
B3
B4
FOSAA
N-EtFOSAA
PFDA
PFNA
PFOA
PFBA
PFOS
PFHxS


(b)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Inf
A1
A2
A4
B1
B2
B4
FOSAA
N-EtFOSAA
PFDA
PFNA
PFOA
PFBA
PFOS
PFHxS

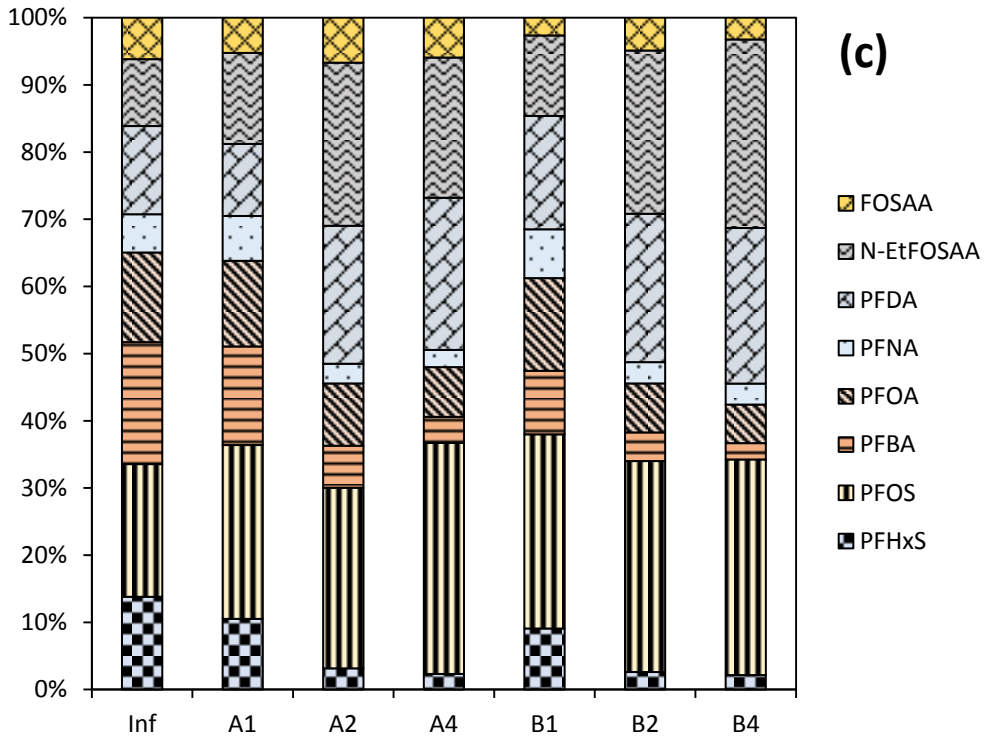


Figure 4.5. Compositions of total PFCs in (a) the dissolved phase, (b) the suspended solid phase and (c) the entire wastewater, of wastewater samples collected for each treatment process in June and July 2013.

Figure 4.6 shows the mass distribution of total PFCs between dissolved and suspended solid phases. It can be observed that compared to the dissolved phase, there was a more significant mass accumulation on the suspended solid phase (the percentages on the suspended solid phase were from 53% to 68% in INF and A1/B1, and even increased to around 90% in the biological tanks, i.e. A2/B2 and A4/B4). This is consistent with high sorption tendency and bioaccumulation of most PFCs with relatively large $\log K_{oc}$ values (Zhou et al., 2010; Liu et al., 2011; Stahl et al., 2011). Furthermore, higher concentrations of PFCs were detected in the suspended solid phase in A2, A4, B2 and B4, which could be due to higher TSS (i.e. MLSS) concentrations and more organic contents in activated sludge. In fact, PFCs are suggested to be more "proteinophilic" in which protein content in activated sludge would strongly

influence sorption capacity (Zhou et al., 2010; Labadie & Chevreuil, 2011; Zareitalabad et al., 2013). These distribution results for total PFCs emphasize the importance of sludge disposal and postreatment where sludge is the most important sink for total PFCs in this WTP.

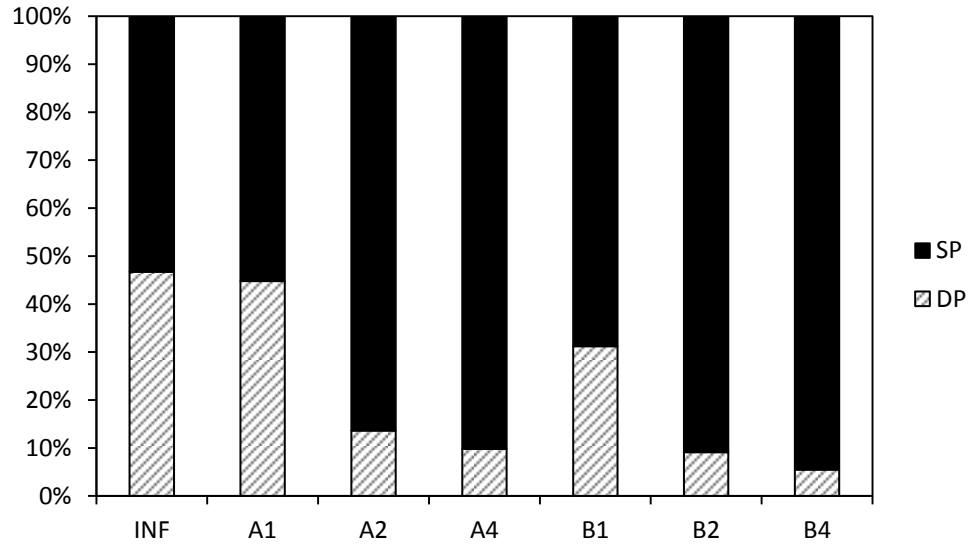


Figure 4.6. Percentages of the average dissolved phase concentration and the average suspended solid phase concentration of total PFCs in wastewater samples for each treatment process in June and July 2013.

4.1.2.3. Behavior of PFCs in the WRP

Figure 4.7 shows the average combined concentrations of PFBA, PFOA, PFNA, PFDA, PFHxS, PFOS, N-EtFOSAA and FOSAA separated into dissolved phase and suspended solid phase in wastewaters in the different treatment processes. The behavior and removal of PFCs were investigated by comparing different concentrations in different treatment processes. Note that the suspended solid concentrations of PFCs in A3 and B3 were not measured due to minor suspended solids in these samples. Similar to the discussion of ASs, wastewater volume was assumed to remain relatively constant in all treatment processes due to lack of flow data which could potentially change the concentration (Shivakoti et al., 2010).

Overall, the behavior of most PFCs was similar, especially for PFOA (Figure 4.7 (b)), PFNA (Figure 4.7 (c)), PFDA (Figure 4.7 (d)), PFOS (Figure 4.7 (f)), N-EtFOSAA (Figure 4.7 (g)) and FOSAA (Figure 4.7 (h)). The consistently high and increasing solid to liquid ratios throughout the WRP confirm the high sorption uptake of these six hydrophobic compounds on suspended solids. The total removal efficiencies by comparing effluent concentrations against influent concentrations were 51.8%/49%, 59.9%/67.2%, -1.3%/15.6%, 79.7%/55.8%, 77.3%/80.2%, and 63.7%/56.2% (A3/B3) respectively throughout the WRP, assuming no TSS in the effluents. This incomplete removal of PFCs in the WRP reflects the nonbiodegradability of the (fully fluorinated) PFCs (Schröder, 2003; Sáez et al., 2008; Fromel & Knepper, 2010).

Despite the fact that the combined PFC concentrations were comparable in influents (INF) and primary settled sewages (A1/B1), these compounds were dramatically increased in biological units but decreased in effluents with relatively high solid to liquid ratios throughout the WRP. This observation is consistent with some literature (Yu et al., 2009; Shivakoti et al., 2010). The increase in combined concentrations in biological tanks could be due to several factors. Firstly, the presence of much higher concentration of MLSS in the aeration tanks could provide more solid mass for sorption (see Table A). This could also explain why the suspended solid phase concentration in MBRs was higher than that in CAS reactors since MBRs usually contain a higher content of MLSS (Table A). As mentioned earlier, high organic and protein contents in activated sludge biomass could possibly favor uptake of PFCs (Zhou et al., 2010; Labadie & Chevreuil, 2011; Zareitalabad et al., 2013).

Furthermore, accumulation of PFCs on MLSS could be promoted with recirculation of sludge by RAS. Although a small HRT (~7.1 hours) may limit the fresh batch of wastewater flow from contacting the MLSS for sufficient time before equilibrium is reached, a long SRT (=~6.6 days which is approximately 22 times longer than HRT) with recirculation of sludge provides an extended time for sludge to adsorb PFCs in fresh wastewater flows. This could enhance the accumulation and increase the concentration of PFCs on MLSS. Lastly, another important factor that may contribute to the mass increase in biological tanks could be the existence of potential precursors in the wastewater influents, such as some fluorotelomer alcohols and perfluoroalkane sulfonamide derivatives, etc (Liu & Mejia Avendaño, 2013). For example, PFOA has been detected as one of the metabolites in all biological degradation studies of 8:2 FTOH so far and other PFCAs such as PFHxA, PFHpA and even PFBA have been detected as well in some literature (Wang et al., 2005b; Wang et al., 2005a; Wang et al., 2009; Kim et al., 2012; Liu & Mejia Avendaño, 2013). In addition, N-EtFOSAA and FOSAA were both found in the biodegradation of N-EtFOSE as metabolites, which could be further biodegraded into PFOS (Lange, 2000; Boulanger et al., 2005; Rhoads et al., 2008; Fromel & Knepper, 2010; Liu & Mejia Avendaño, 2013). Although N-EtFOSAA could be further degraded to N-EtFOSA, the transformation rate was much slower than from degradation of N-EtFOSE, which may contribute to the accumulation of N-EtFOSAA in the biological tanks in this study (Rhoads et al., 2008). As a result, selected PFCs could be increased via biodegradation of corresponding precursors. Monitoring the fate of precursors in WWTPs can further enhance our understanding. This

hypothesis reminds us that although the use of PFOA and PFOS follow a decreasing trend due to concerns of potential risks, the degradation of precursors is still a source for them to be present in the environment. Biodegradation studies are recommended to cover a wider range of precursors and metabolites and elucidate the metabolic pathways more clearly. A review of the monitoring results showed that there was a slight increase in the total concentrations in the primary clarifiers for PFOS and N-EtFOSAA. Furthermore, since inefficient removal in aqueous phase was shown by the relatively stable dissolved phase concentrations throughout the WRP, the apparent decrease in combined concentrations in effluents was attributed to the concurrent removal of adsorbed PFCs with elimination of suspended solids in sludge disposal. This emphasizes that sorption on sludge is the major removal mechanism for the PFCs with high sorption tendency in WWTPs (Rayne & Forest, 2009; Kunacheva et al., 2011; Ratola et al., 2012). As a result, monitoring of post-treatment of sludge is essential to evaluate the fate of PFCs in terms of contaminants control and management.

The behavior of PFBA and PFHxS were similar with reference to Figure 4.7 (a) and (e). The relatively low and stable solid to liquid ratios which seemed less dependent on MLSS concentrations, showed that PFBA and PFHxS may be less likely to sorb to suspended solids compared to the other PFC compounds discussed earlier. The literature also showed similar trends where sorption capacity increased with increasing C-F chain length and was higher for PFASs compared to PFCAs for the same number of carbons (Guo et al., 2010; Zhou et al., 2010). This is consistent with the results in the subsequent sorption study which are illustrated below. As such, the fractions

of PFBA and PFHxS are relatively larger in the aqueous phase with 65%-83% and 32%-67% correspondingly (excluding effluent samples). The total removal efficiencies were -9.1%/-41.5% and 48.9%/51.4% (A3/B3) respectively throughout the WRP, assuming no TSS in the effluents. This reflects the inefficiency of the WRP for PFBA and PFHxS removal.

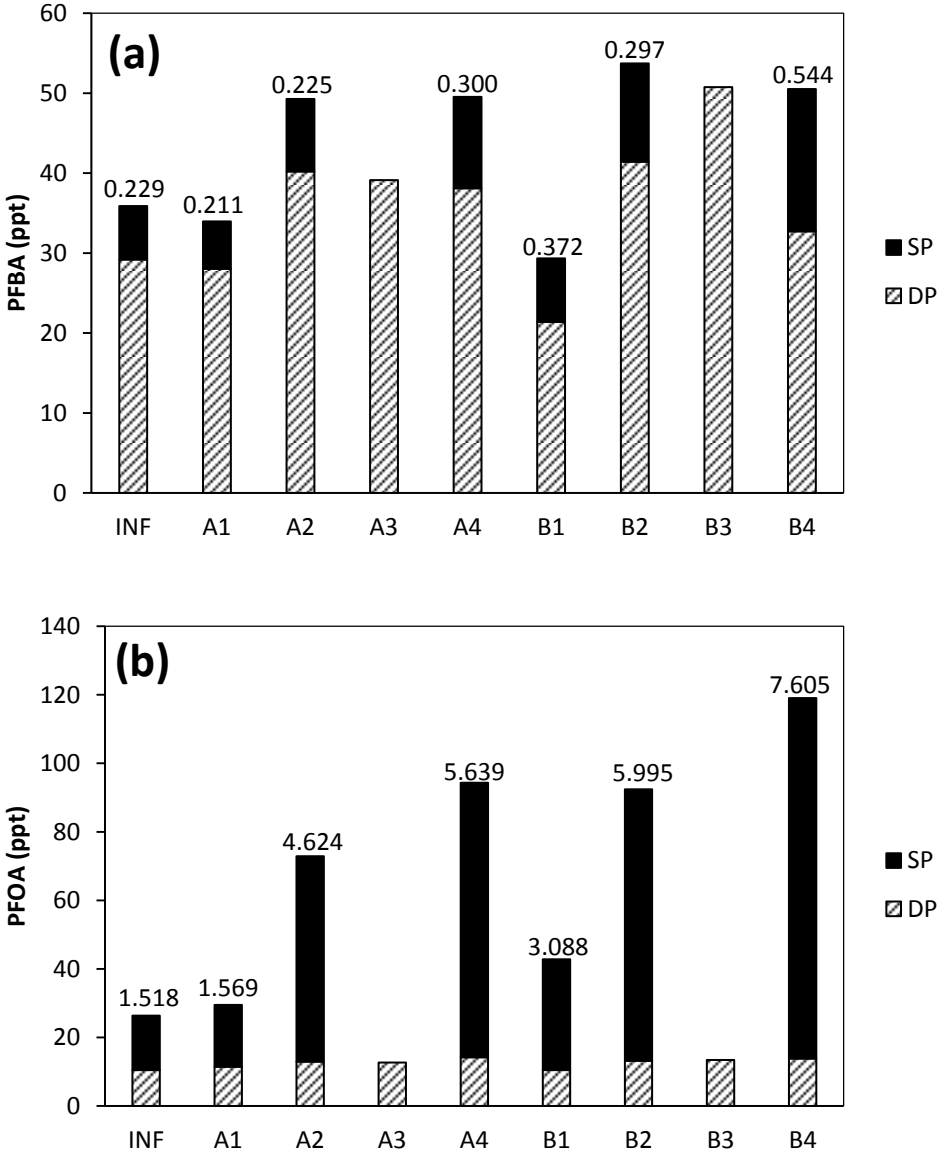
On the one hand, with steady aqueous concentrations in various treatment units throughout the WRP, the total concentration of PFHxS was relatively stable except for the reduction in effluents. This may indicate that there was no precursor for PFHxS in the influents. The slight increase in suspended solid phase concentrations (and solid to liquid ratios) in RAS (A4/B4) and MBR reactors (A2) could be caused by the continuous accumulation of PFHxS on the MLSS, with much higher TSS concentrations in these units (Table A) for a long SRT. The reduction in effluents further verifies that sludge is a significant sink for PFCs in WWTPs, as mentioned earlier. On the other hand, PFBA showed higher total concentrations in the biological tanks and effluents with increasing concentrations in both dissolved phase and suspended solid phase. This was probably contributed by the degradation of precursors in biological tanks. For instance, PFBA was detected as one of the stable transformation products of 6:2 FTS salt by activated sludge in a lab-scale biodegradation study by Wang et al. (2011). Most of the results in this study depict that degradation of precursors can produce significant loads of selected PFCs (except PFHxS) in biological tanks and the different amounts can depend on the availability of precursors in influent sources and the relative transformation rates under the operating conditions for the biological processes. (Rhoads et al., 2008). Despite the variability in TSS concentration,

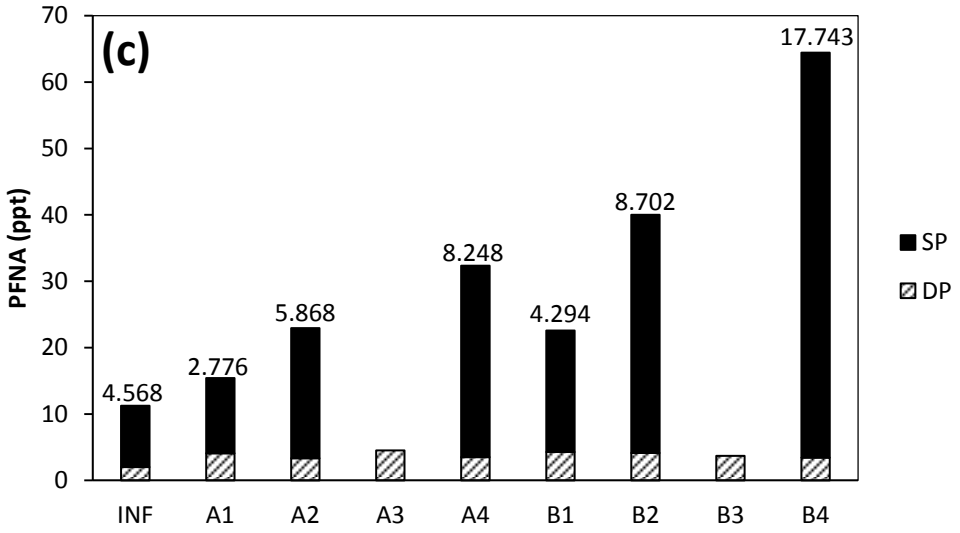
the fairly stable solid to liquid ratios of PFBA throughout the WRP confirms its limited sorption capacity, which we also observed in the bench-scale sorption study (Section 4.2.2.2).

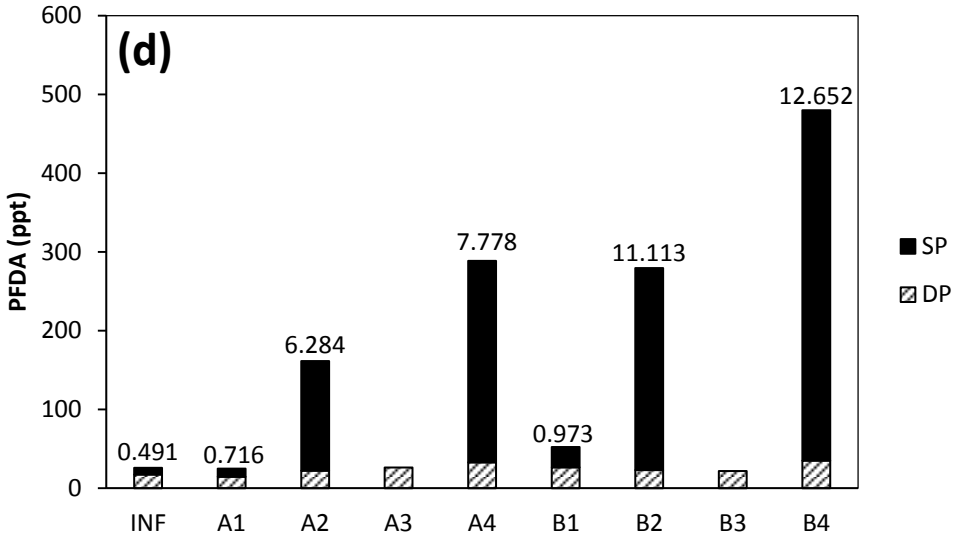
Figure 4.7 (i) illustrates the average concentrations of the total PFCs in both dissolved and suspended solid phases in wastewater samples for various treatment processes. The influent carried approximately 197.6 ppt total PFCs and the removal efficiencies were approximately 43% and 34% in the Southworks and Northworks of the WRP, which resulted in around 113 and 130 ppt total PFCs in the dissolved phase of the effluent discharge, respectively. The results showed that the WRP was unable to remove the selected PFCs completely from the wastewater. In addition, degradation of potential precursors probably produced significant loadings of the selected PFCs during the biological processes. Due to long SRT and high sorption capacities of most PFCs on activated sludge, sludge was shown to be the most important sink for PFCs. As such, sludge disposal was the major removal mechanism of PFCs due to their non-biodegradability.

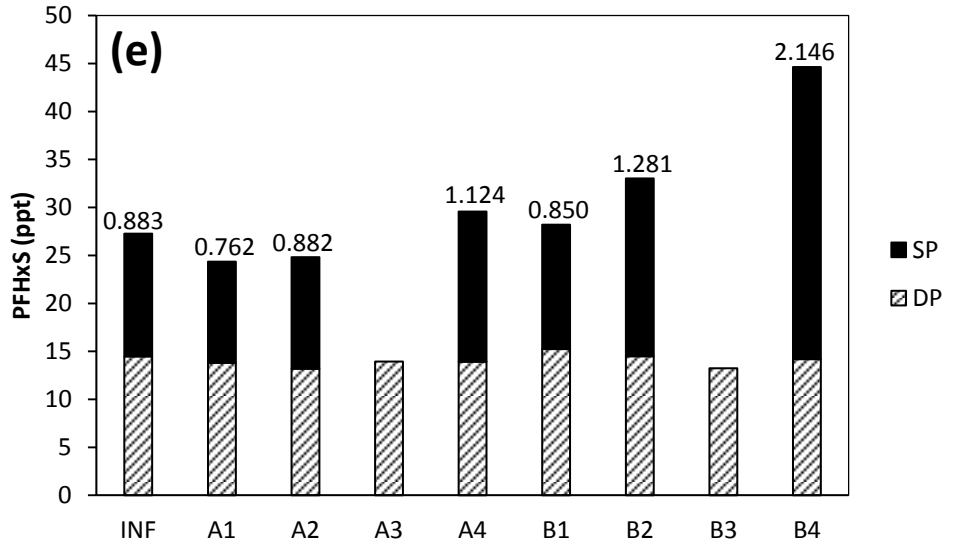
In conclusion, both the wastewater effluent (i.e. mainly dissolved phase) and the sludge (i.e. settleable suspended solids) are significant contamination sources/sinks for PFCs to enter the environment, with higher loadings in the sludge in this WRP. As a result, in addition to monitoring of PFCs in wastewater effluent discharge, it is also essential to examine sludge disposal and provide treatment where necessary for contaminant control as well. In Singapore, incineration is mainly applied to post-treat sludge from WWTPs. However, as a solid waste treatment method, although incineration was shown to reduce the PFC concentration in sludge by 2-10 folds (Loganathan et al.,

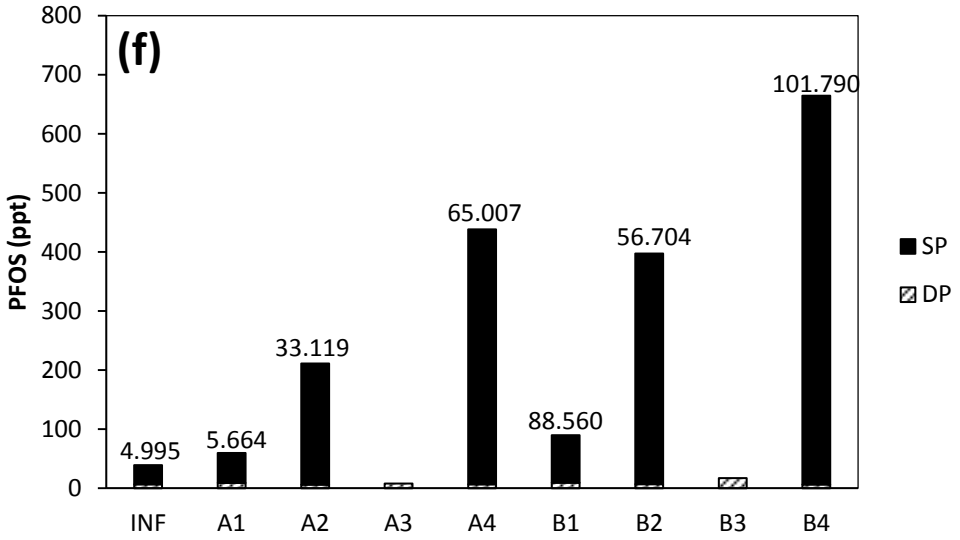
2007), the concentration of PFCs in the ashes after incineration would still be of significant concern. Therefore, leachate from landfills where the ashes will finally reside in should be studied to understand the pathways of PFCs in this environment.

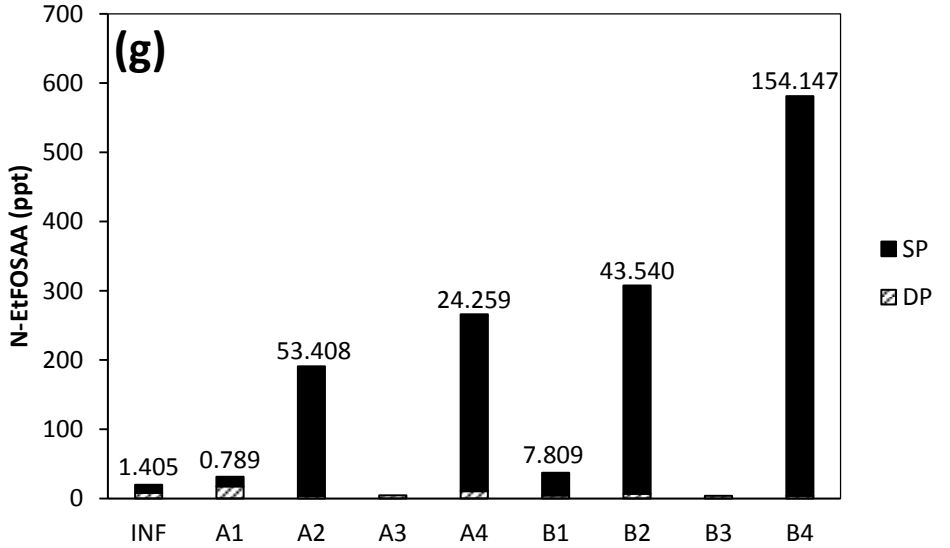


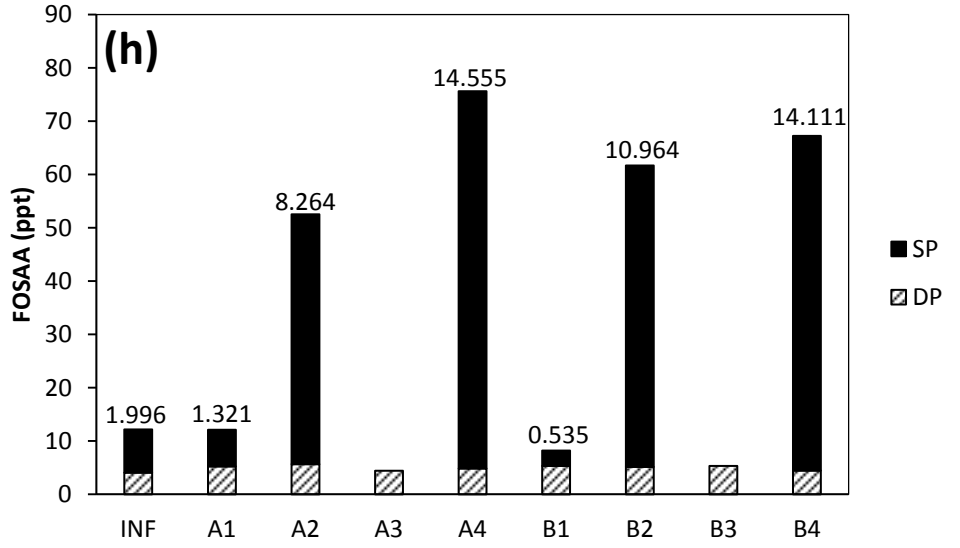

(c)
PFNA (ppt)
70
60
50
40
30
20
10
0
4.568
2.776
5.868
8.248
4.294
8.702
17.743
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(d)
PFDA (ppt)
600
500
400
300
200
100
0
0.491
0.716
6.284
7.778
0.973
11.113
12.652
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(e)
PFHxS (ppt)
50
45
40
35
30
25
20
15
10
5
0
0.883
0.762
0.882
1.124
0.850
1.281
2.146
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(f)
PFOS (ppt)
800
700
600
500
400
300
200
100
0
4.995
5.664
33.119
65.007
88.560
56.704
101.790
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(g)
N-EtFOSAA (ppt)
700
600
500
400
300
200
100
0
1.405
0.789
53.408
24.259
7.809
43.540
154.147
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP


(h)
FOSAA (ppt)
90
80
70
60
50
40
30
20
10
0
1.996
1.321
8.264
14.555
0.535
10.964
14.111
INF
A1
A2
A3
A4
B1
B2
B3
B4
SP
DP

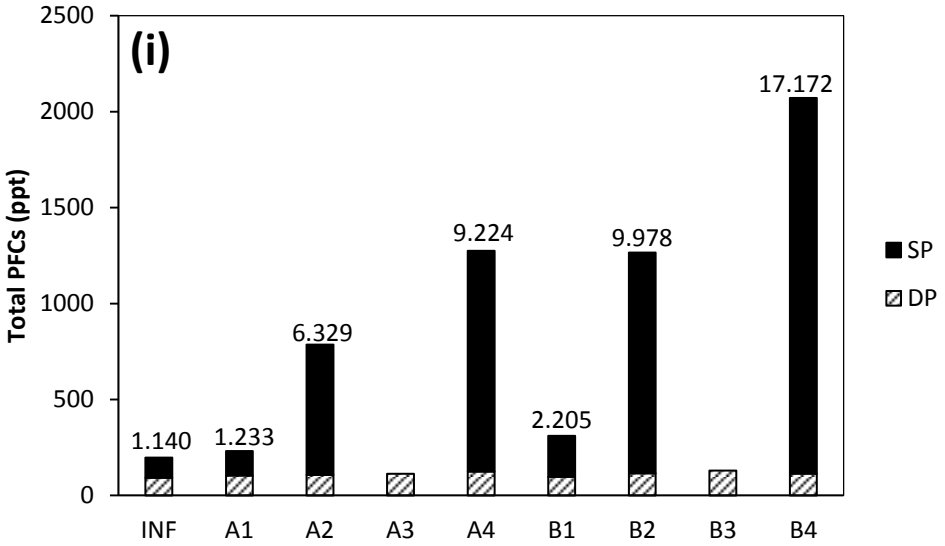


Figure 4.7. Mean concentrations (ppt) of (a) PFBA, (b) PFOA, (c) PFNA, (d) PFDA, (e) PFHxS, (f) PFOS, (g) N-EtFOSAA, (h) FOSAA and (i) total PFCs in the dissolved phase and the suspended solid phase (volume unit) of the wastewater samples collected for each treatment process in the WRP in June and July 2013.

Table 4.6 tabulates the ratios (L/g) of the suspended solid phase concentrations (ng/g dw) to the corresponding dissolved phase concentrations (ppt) of the selected PFCs of various treatment stages. As mentioned earlier in Section 4.1.1.3, the solid to liquid ratio is more appropriate for use in this study instead of the distribution coefficient, since the equilibrium condition cannot be confirmed in the WRP. PFOS and PFOA showed higher ratios compared to the values reported for primary and secondary sludge by Yu et al. (2009), where PFOS showed values more than 3 times larger than PFOA in both studies. In general, PFOS showed the highest ratios followed by PFNA, PFOA, PFHxS and PFBA. This result is consistent with findings in the subsequent bench-scale sorption study we conducted. The trend also matches the literature that sorption capacity is increased with increasing C-F chain length and is higher for PFASs compared to PFCAs for the same number of carbons (Guo et al., 2010; Zhou et al., 2010). However, the exception was

PFDA which had the longest C-F chain length but showed low solid to liquid ratios. This could be because the release of PFDA was mainly in the dissolved phase in wastewaters, which resulted in high aqueous concentration in influents and subsequently low solid to liquid ratios.

Table 4.6. Average solid (ng/g dw) to liquid (ppt) ratios with the respective standard deviation of each PFC. Unit: L/g.

	Influent	A1	A2	A4	B1	B2	B4
PFBA	0.61 ±0.13	1.76 ±0.50	0.13 ±0.01	0.09 ±0.00	1.32 ±0.51	0.07 ±0.00	0.07 ±0.01
PFOA	4.15 ±0.40	12.84 ±0.47	2.82 ±0.48	1.81 ±0.15	10.27 ±0.29	1.43 ±0.03	0.95 ±0.12
PFNA	13.33 ±3.85	22.80 ±0.22	3.50 ±0.50	2.70 ±0.42	13.51 ±1.28	2.53 ±1.07	2.38 ±0.67
PFDA	1.39 ±0.34	5.96 ±1.36	6.74 ±4.27	2.47 ±0.13	3.33 ±1.06	3.27 ±1.28	1.65 ±0.27
PFHxS	2.50 ±0.85	6.24 ±0.83	0.52 ±0.01	0.35 ±0.05	2.91 ±0.91	0.31 ±0.02	0.28 ±0.09
PFOS	13.63 ±1.11	46.01 ±16.34	18.19 ±4.70	21.68 ±10.09	27.98 ±4.61	13.92 ±2.55	13.50 ±3.28
N-EtFOSAA	4.01 ±1.51	6.74 ±2.90	41.57 ±23.63	8.14 ±2.20	32.17 ±17.95	10.67 ±2.46	21.39 ±8.22
FOSAA	5.50 ±0.64	10.70 ±0.47	4.99 ±0.89	4.62 ±0.63	1.75 ±0.34	2.65 ±0.12	1.78 ±0.19

4.2. Bench-scale sorption studies of ASs and PFCs

4.2.1. Artificial sweeteners

Wet fresh sludge solids, with and without inhibition by sodium azide were tested. Inhibited biomass is assumed to have minimum bioactivity (Lin et al., 2010). As such, difference in aqueous mass under the two test conditions could indicate biodegradation. The initial concentration was determined to be around 70 ppb which is typical of wastewaters containing ACE and SUC.

4.2.1.1. Blank tests and control tests of SUC and ACE

The results in blank tests (i.e. solution controls) showed negligible abiotic reduction of analytes in solutions within the experimental duration of 17 days.

As such, few analytes was adsorbed onto test vessels. Since the test solution was distilled water only without addition of any buffer, potential interaction with analytes was minimized. Although artificial sweeteners may be subject to photodegradation due to their conjugated ring structures, test bottles covered by aluminum foil were shown to prevent photodegradation efficiently based on the relatively constant concentrations in the blank tests. The average concentrations of SUC and ACE were 67.9 (±2.7) ppb and 69.7 (±2.0) ppb in blank tests respectively.

All aqueous concentrations in control tests (i.e. adsorbent controls) were below the detection limit, which confirmed insignificant contamination and matrix interferences.

4.2.1.2. Sorption tests of SUC

In sorption tests, it is almost impossible to eliminate biodegradation (Lin et al., 2010). The addition of sodium azide was targeted to restrict bioactivity of wet biomass. To illustrate, both sorption and loss of parent compound via biodegradation were expected when wet biomass was directly used; while sorption was supposed to govern the removal mechanism when sodium azide was added in the test solution with wet biomass. As such, the relative importance of sorption and biodegradation may be demonstrated by comparing results from these two tests.

With reference to Figure 4.8, the same trend in SUC aqueous concentration was observed in both sorption tests with and without inhibitor. Compared to sorption, the small difference implied negligible biodegradation of SUC by the wet biomass without inhibitor during the test period. The persistence was consistent with the findings in wastewater treatment plants summarized in

Chapter 2 and the conclusion of nonbiodegradability of SUC in the aerobic and anaerobic reactors for 42-62 days by Torres et al. (2011) (Torres et al., 2011; Lange et al., 2012). As such, the removal mechanism was mainly attributed to sorption onto biomass.

However, the kinetic behavior was different for the two tests. The inhibited wet biomass showed a consistent and relatively steady reduction rate of aqueous concentration over the experimental duration; while the original wet biomass displayed quicker loss in the initial period and then nearly leveled off afterwards. This may indicate two different mechanisms behind the sorption behavior. Inhibited biomass seemed to have unlimited sorption sites available for continuous adsorption while a limited number of sorption sites on wet biomass tended to be saturated. These possible mechanisms cannot be confirmed unless further studies such as desorption tests are done. Furthermore, this test emphasized the impact of chemical addition, i.e. NaN_3, in this sorption experiment which was supposed to be the major reason that resulted in the two sorption behaviors. This will be further discussed shortly.

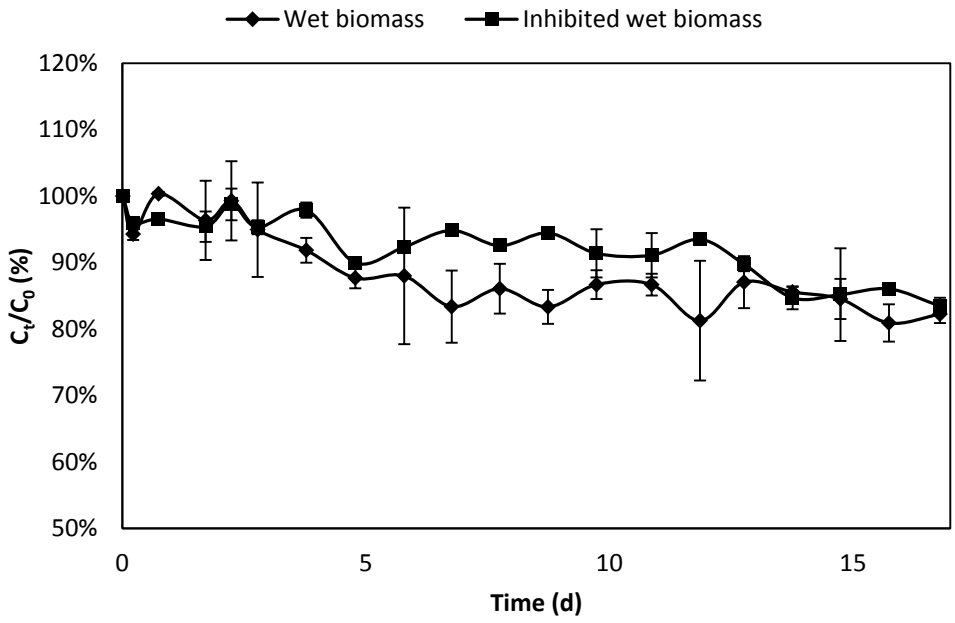


Figure 4.8. The sorption test of SUC. The graph shows the average relative concentrations normalized to the initial concentration with standard deviation over an experimental duration of 17 days.

The result for the sorption test without inhibitor is illustrated in Figure 4.9. Although the SUC aqueous concentration tended to stabilize, the sorption did not reach equilibrium even after 17 days. To be conservative, at least 3-4 weeks is recommended for the slow uptake of SUC by activated biomass until equilibrium is reached. The total removal was 18%, with fast adsorption of 12% within the first 5 days. The slow uptake rate and small adsorption capacity are possible due to the hydrophilic property of the compound (Tran et al., 2014).

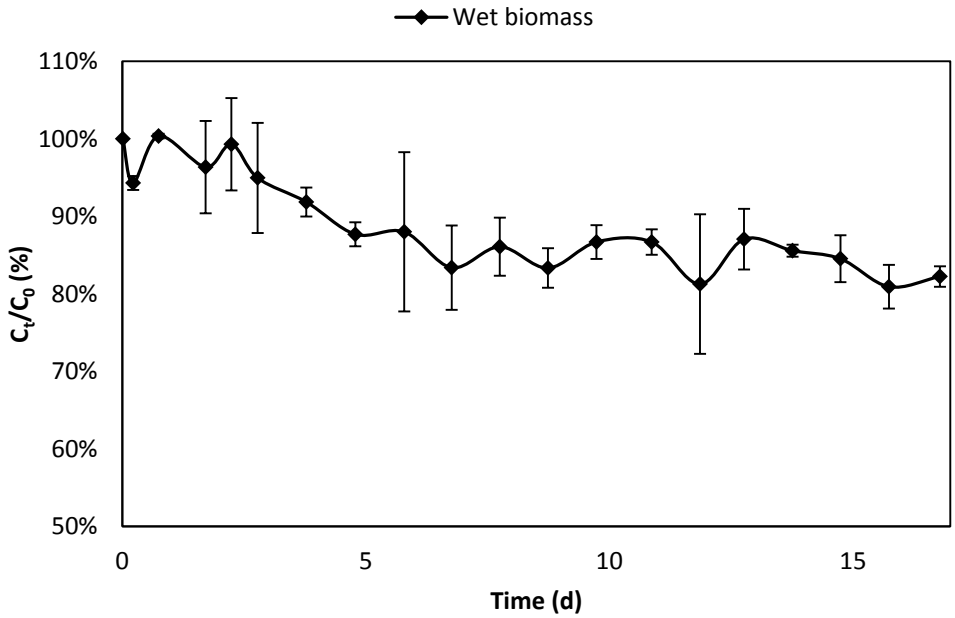


Figure 4.9. The sorption test of SUC using wet activated biomass without inhibitor. The graph shows the average relative concentrations normalized to the initial concentration with standard deviation over an experimental duration of 17 days.

4.2.1.3. Sorption tests of ACE

With reference to Figure 4.10, the sorption test using inhibited wet biomass showed negligible reduction in dissolved phase concentration, which indicated insignificant sorption of ACE by activated biomass within the experimental duration of 17 days. This observation was consistent with its properties of high water solubility and hydrophilicity.

In comparison, the sorption capacity for SUC by inhibited activated biomass was higher than that for ACE, which is consistent with the field monitoring of the WRP. One of the possible reasons could be due to the ionic nature of ACE under test conditions. At a pH of around 6, the major form of SUC present in solution would be the undissociated form as its pK_a value is 11.8 (Lange et al., 2012). In contrast, ACE would be mainly in dissociated form with negative surface charges based on its pK_a value of 2, which may be more likely to repel the negatively charged surface of activated biomass

(Lange et al., 2012). This would result in negligible and less sorption of ACE onto biomass compared to SUC. The relative affinities onto activated biomass in our study were inconsistent with the observation of soil sorption isotherms, where ACE-K showed higher sorption affinity than SUC (Soh et al., 2011). This could be due to different surface characteristics of activated biomass in a WWTP and laboratory soils and different test conditions.

Surprisingly, however, results of the wet biomass test showed significant mass reduction in aqueous phase of ACE, with approximately 70% reduction within 17 days. The degradation of ACE continued slowly even after 17 days. This is in contrast to the general conclusion of the non-biodegradability of ACE in wastewater treatment plants from the literature. These discrepancies could be due to different microbes and test conditions. Compared to an actual operating WWTP with a dynamic source of analytes, the batch experiment generally provided higher amounts and longer contact time of analytes with activated sludge, which may trigger and enhance the production of relevant enzymes for degradation. Furthermore, the sludge was incubated in a closed system without oxygen supply over the complete experimental duration, which may alter the initial aerobic environment to anaerobic conditions and result in transformation of diverse microbial enzymes. In fact, literature has shown the biodegradation potential of ACE: the half life of ACE-K in a soil incubation test was found to be 6.1 days by Buerge et al. (2011). In addition, a recent study by Tran et al. (2014) showed 16-21% deduction in aqueous concentrations of ACE and SUC after 7 days of incubation in nitrifying activated sludge which was supplemented with primary substrates. They proposed the significant roles of co-metabolism and the presence of

autotrophic ammonia oxidizers in the biodegradation (Tran et al., 2014). In comparison, our study used lower initial concentrations of ACE with higher concentrations of MLSS which may help in removing a higher percentage of ACE, in addition to the different microbes and test conditions. Furthermore, their sorption control experiment using heat-inactivated nitrifying activated sludge showed negligible sorption of ACE and SUC over an incubation time of 7 days, which was partially consistent to our findings (Tran et al., 2014). In our study, there was also minor sorption of SUC over 17 days. The difference could be due to different pre-treatment methods of biomass. Heat treatment of biomass may dry the solids completely with disappearance of volatile organic compounds attached on biomass; while addition of chemicals such as NaN_3 (as in this study) were demonstrated to potentially enhance or reduce sorption rates (Patel & Suresh, 2008; Lin et al., 2010). The mechanism remains unknown. This emphasizes the importance of choosing the right pre-treatment method of biomass for sorption studies and suggests the need for further studies to clarify various effects on biomass and sorption potential using the different pre-treatment methods, e.g. using microscopy, desorption test, etc.

Last but not least, the kinetics of wet biomass showed an initial quick loss in aqueous concentration, followed by a flattening of the curve (Figure 4.10). The stabilization in concentration may be attributed to nutrient depletion and resulting anoxic/anaerobic conditions. A respike experiment and full scale biodegradation incubation test are recommended to confirm the biodegradation potential of ACE and the functional enzymes. This could be a potential research topic for further investigation.

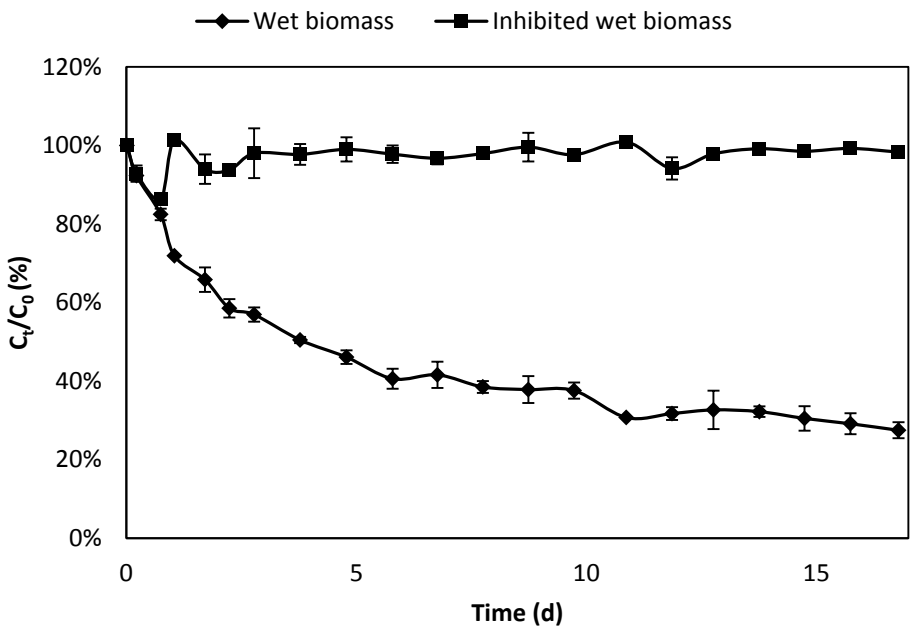


Figure 4.10. The sorption tests of ACE using wet activated biomass with or without sodium azide as inhibitor. The graph shows the average relative concentrations normalized to the corresponding initial concentration with standard deviation over an experimental duration of 17 days.

4.2.1.4. Implications in WRPs

Sorption onto activated biomass could be a small sink for SUC in WWTPs. The accumulation may be underestimated because equilibrium may not be reached within normal HRT (~7.1 hours). However, higher SRT (~6.6 days) resulting from recycling of MLSS in WWTPs could increase the contact time of activated sludge with continuous incoming flows. Thus, the biosorption of SUC on activated biomass could be higher than expected based on the HRT. The current high consumption of artificial sweeteners may increase the inflow concentration and enhance the sorption rate, so the effect of initial concentration on sorption uptake rates could be an important factor to consider. However, due to the small sorption capacity of biomass and partial sorption of SUC, the majority of SUC is likely to be discharged as effluent to the aquatic environments and as such, could be of more concern.

Although ACE is unlikely to adsorb onto activated biomass, it was shown to have high biodegradation potential. Research on the dominant active enzymes that contribute to the biodegradation of ACE could help to optimize plant operating parameters for better bio-removal or the design of specific treatment units.

4.2.2. Perfluorinated compounds

There are various ways of preparing activated biomass for a sorption study. They include dried biomass which is oven dried under 60 ^{o}C (Aksu, 2001; Gulnaz et al., 2004), wet sludge (Arican et al., 2002; Zhou et al., 2010), autoclaved sludge (Zhao et al., 2008; Lin et al., 2010) and NaN_3-inhibited sludge (Yu & Hu, 2011). USEPA also has a standardized method for sorption onto activated sludge - USEPA OPPTS 835.1110 (The Office of

Prevention,, Pesticides and Toxic Substances, United States Environmental Protection Agency1998). In this study, results of sorption tests using the USEPA lyophilization-heat pre-treatment was compared with normal temperature oven dryness- NaN_3 inhibition treatment which was considered to have better weight control with less change on biomass characteristics. The lyophilization and dry-heat inactivation technique in the USEPA method was demonstrated to selectively inhibit microbial activity for a period of approximately 24 hours which has the advantage of non-chemical aqueous matrix effect with insignificant alteration of activated sludge solids (Stevens-Garmon et al., 2011). Since PFCs are very refractory compounds, the USEPA method was considered to be suitable for the sorption study.

4.2.2.1. Blank tests and control tests of PFCs

The results in blank tests (i.e. solution controls) showed no abiotic reduction of analytes in both test solutions within the experimental duration of 5 days. As such, few analytes were adsorbed onto test vessels. No interaction of analytes with buffer compounds and NaN_3 and no photodegradation and hydrolysis were expected. The average concentrations of PFCs with their corresponding standard deviations are summarized in Table 4.7. The fluctuation in data could be attributed to experimental errors such as instability of instrument and faulty sample preparation. The result of PFOS was excluded in the discussion since it was found that there was significant loss of PFOS during the filtration step through nylon filter tips, without correction of surrogates in the sampling events.

Table 4.7. Average concentrations of PFCs (PFBA, PFHxA, PFOA, PFNA, PFDA, PFBS and PFHxS) with respective standard deviation in blank tests over a test period of 5 days for both inhibited dried biomass and USEPA deactivated biomass.

Analytes	Inhibited dried biomass	USEPA deactivated biomass
	Concentration (± SD)(ppb)	Concentration (± SD)(ppb)
PFBA	50.5 (±3.8)	51.8 (±3.3)
PFHxA	42.4 (±4.7)	44.3 (±4.6)
PFOA	33.7 (±3.4)	34.3 (±3.7)
PFNA	59.2 (±6.6)	64.5 (±6.1)
PFDA	25.1 (±6)	29.2 (±7.7)
PFBS	43.0 (±4.7)	45.7 (±4.5)
PFHxS	37.6 (±4.0)	39.0 (±5.8)

All aqueous concentrations in control tests (i.e. adsorbent controls) were below detection limit which confirmed insignificant contamination and matrix interferences.

4.2.2.2. Sorption tests of PFCs

With reference to Figure 4.11 and Figure 4.12 graph (a), for the inhibited dried biomass, all the PFCs reached equilibrium around 10 hours which was comparable to 11 hours in literature using wet activated sludge from WWTP as adsorbents (Zhou et al., 2010). The sorption was very fast in the initial hours. It was also observed that the concentrations of all the PFCs were consistently high around 6 hours. This may have been caused by experimental error such as faulty injection of internal standards.

However, for the USEPA deactivated sludge, it took longer time (more than 10 hours but less than 1 day) to reach equilibrium (Figure 4.11 and Figure 4.12 (b)). Results showed slow sorption uptake after rapid sorption in the initial hours followed by stabilization with higher sorption capacities for PFCs. The rapid sorption kinetics in the initial hours was similar to the observations for inhibited dried biomass. This may be because lyophilization dried the

biomass into porous fine particles with large surface area for sorption reaction. Subsequently, the slow uptake may be attributed to intraparticle diffusion processes. On the other hand, inhibited dried biomass solids are generally coarser particles and are likely more compacted during the crushing process, reducing the surface area for reactions. In addition, another possible reason for the slow uptake by lyophilization-dry heat-treated biomass could be the cell-membrane diffusion limiting step via intact cell membrane, as demonstrated in literature or rehydration of biomass-solids (Stevens-Garmon et al., 2011). Different drying temperatures may result in significant changes in surface characteristics as well as contents of volatile organic compounds and coatings of water films. Further clarification is needed to better understand the mechanisms. Selection of more uniform time points in repeated tests over the equilibration time could enhance the understanding of sorption kinetics, while desorption studies could help to improve understanding of the mechanisms involved.

The importance of standardizing sorption experiments when using activated sludge is emphasized. This will also facilitate comparison between different studies. Microscopic imaging such as Scanning Electron Microscopy (SEM) is recommended in the investigation to analyze changes in surface characteristics by different pre-treatment processes, especially the heating process.

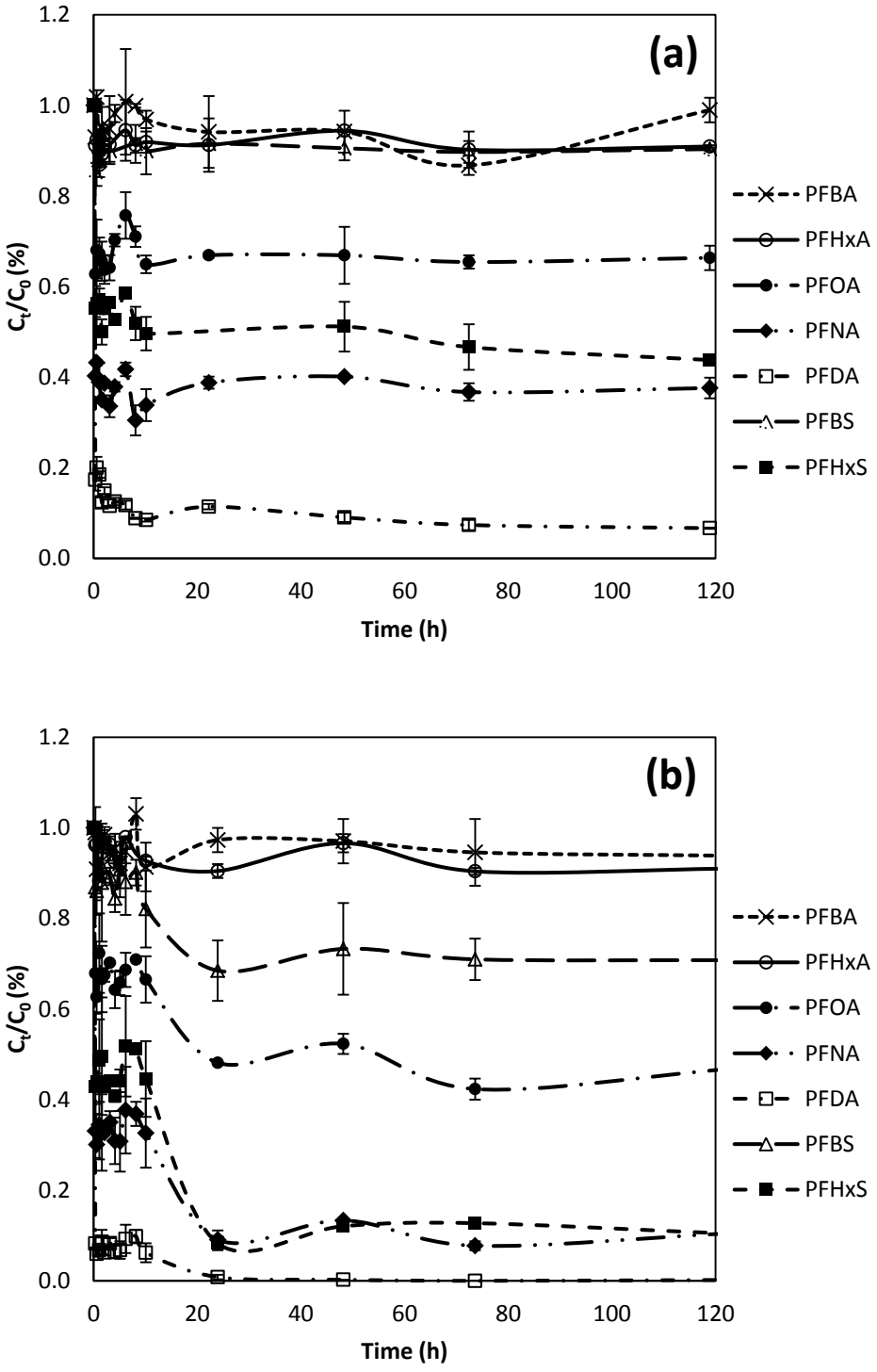


Figure 4.11. Sorption tests of PFCs using (a) inhibited dried sludge and (b) USEPA-deactivated biomass. The graphs show the average relative concentrations normalized to the corresponding initial concentration with standard deviation over an experimental duration of 5 days.

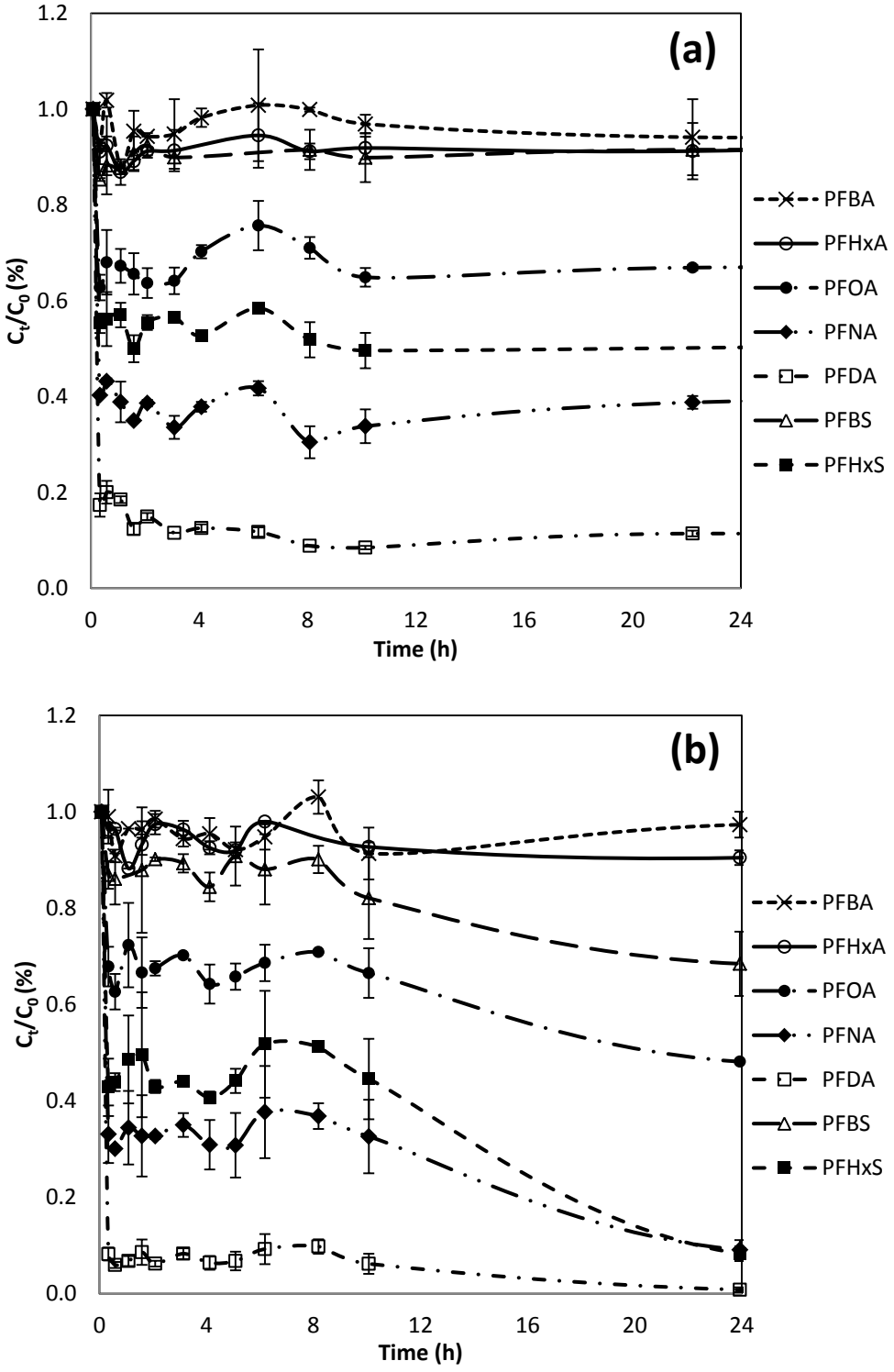


Figure 4.12. Sorption tests of PFCs using (a) inhibited dried sludge and (b) USEPA-deactivated biomass. The graphs show the average relative concentrations normalized to the corresponding initial concentration with standard deviation over an experimental duration of 1 day.

Despite the difference in biomass treatment methods, the relative sorption affinities onto biomass between compounds were the same (Figure 4.12 (b)).

From the results, compounds with longer C-F length possessed higher affinity to biomass, with larger percentage removal than that of shorter C-F-chain-length compounds at equilibrium within the same family (carboxylates and sulfonates). To illustrate, the sorption affinities of perfluorinated carboxylic acids decreased in the order of PFDA (9 C-F units), PFNA (8 C-F units) and PFOA (7 C-F units); while affinities of perfluorinated sulfonates decreased in the order of PFHxS (6 C-F units) and PFBS (4 C-F units). This emphasizes the importance of hydrophobic interaction (Higgins & Luthy, 2006; Zhou et al., 2010). Sorption from aqueous phase towards organic and mineral surfaces was proposed to be entropy driven (Zareitalabad et al., 2013). As such, an increase in C-F unit in the C-chain elevates the hydrophobicity of the compound, and so does the sorption affinity.

Secondly, perfluorinated sulfonates were observed to have better sorption affinities onto activated biomass compared to corresponding perfluorinated carboxylic compounds with the same number of carbons. This was attributed to the more hydrophobic property of sulfonates because they have one more C-F tail compared to their respective carboxylates (Zhou et al., 2010).

Furthermore, it can be seen that there was little sorption of PFBA, PFHxA and PFBS by activated biomass within the experimental duration. It is possible that the total hydrophobic effects of C-F units in the short perfluorocarbon chains are counteracted by the hydrophilic carboxylic functional group ($–COO^-$) or sulfonic functional group ($-SOO^-$). This further emphasizes the importance of hydrophobic interaction in the sorption of PFCs.

As a result, care is needed in handling the short perfluorocarbon-chain compounds. Since they are not easily biodegradable and they are not readily

adsorbed to biomass, it is highly possible that they will escape from conventional wastewater treatment plants and keep accumulating in aquatic environments. Recent trends show that these compounds have been replacing PFOA and PFOS in more and more industries, mainly to reduce potential chemical risks of PFOA and PFOS to human beings (Betts, 2007; Eriksen et al., 2010; Ochoa-Herrera & Sierra-Alvarez, 2008). However, there is limited research on the toxicity and ecological impacts of these short chain PFCs.

4.2.2.3. Implications in WRPs

Comparing equilibration time with normal HRT which is around 7.1 hours, the majority of sorption capacity can be achieved in the WRP. Although equilibrium may not be reached within the HRT which results in partial adsorption of PFCs, the accumulation of PFCs on activated biomass is still of concern because the SRT (~6.6 days) of activated biomass is far longer than wastewater HRT with recycling stream. As such, both pollution of PFCs in the aqueous solution and on activated biomass are important factors to consider for contaminant management. Dynamic sorption tests using bench-scale or pilot-scale reactors may help to predict the sorption behavior and fate of PFCs in a wastewater reclamation plant.

High sorption affinities of long-chain perfluorocarbon-chain PFCs verify that activated biomass is an important sink for PFCs in biological treatment units. This means that aqueous concentrations in effluent and mobility can be reduced to a significant extent. However, since PFCs are very resistant to biodegradation, they are still present in particulates; the sorption process only transforms the problem of PFCs from the aqueous effluent discharge to sludge handling problem. In other words, post-treatment or application of sludge

should also be controlled. For example, care should be taken to apply the sludge as fertilizer in case of further accumulation of PFCs in vegetation. A post-treatment method should be selected to prevent production of more toxic by-products.

It is also noted that there is limited capacity of activated biomass in adsorbing short perfluorocarbon-chain PFCs (e.g. PFBA,PFHxA and PFBS). These short chain PFCs pose a new challenge for public health and ecological impacts since they are likely to escape from the WRP into natural environments. Toxicity tests are recommended for investigation.

Chapter 5. Conclusion and recommendation

5.1. Findings

This research has studied the occurrence and fate of artificial sweeteners and perfluorinated compounds in a water reclamation plant of Singapore. The abundance and behavior of these compounds were discussed over various treatment units throughout the WRP. In addition, relevant bench-scale sorption studies have been conducted to verify the sorption capacity for ASs and PFCs on activated biomass solids, using different pre-treatment method to prepare the sorbents.

5.1.1. Occurrences and fates of ASs and PFCs

a. All the four selected ASs, including ACE, SUC, CYC and SAC, were detected in the wastewater samples, where the detected ranges of concentrations were 5.63-10.91 ppb, 1.30-6.50 ppb, n.d.-41.88 ppb and n.d.-18.80 ppb in the dissolved phase, and n.d.-8709 ng/g dw, 33.6-5702.6 ng/g dw, n.d.- 18951.5 ng/g dw and n.d.-18818.2 ng/g dw in the suspended solid phase respectively throughout the whole WRP.

b. Due to high solubility, ASs were likely to stay in the dissolved phase of wastewater samples with proportions ranging from 84% to 95%. Among them, CYC and SAC were dominant species in the influents but ACE and SUC were dominant in the effluents.

c. Overall, the anoxic and aerobic biological treatment processes adopted in this WRP resulted in 84% removal of total ASs, with total concentrations of around 10.5 ppb in the effluent discharge. The effluent consisted of persistent ACE and SUC only, since CYC and SAC were almost completely biodegraded and removed at >99.9% and ~99.7% respectively.

d. Sorption and sedimentation of suspended solids are assumed to be the only important removal mechanism for ACE and SUC in the WRP, although the efficiency was very limited.
e. SUC showed higher solid to liquid ratios followed by SAC, ACE and CYC in wastewater samples, which may indicate their relative sorption capacity on suspended solids. However, since equilibrium conditions could not be confirmed in the real WWTP, the interpretation may need to be qualified.
f. The 8 selected PFCs, including carboxylic acids (PFBA, PFOA, PFNA and PFDA), sulfonates (PFHxS and PFOS) and derivatives (N-EtFOSAA and FOSAA), were detected in the wastewater samples, where the detected ranges of total concentrations were 82.4 ppt - 148.8 ppt in the dissolved phase, and 226.2 ng/g dw - 1390.3 ng/g dw in the suspended solid phase respectively throughout the whole WRP.
g. Due to high sorption capacity, PFCs (except PFBA) were more likely to stay in the suspended solid phase of wastewater samples. Among them, PFOS and PFBA were the dominant species in the suspended solid phase and the dissolved phase respectively.
h. The biological processes in the WRP removed the PFCs by less than 43%, due to the non-biodegradability of PFCs.
i. The concentrations of PFCs were exceptionally higher in aeration tanks and RAS, which may be due to the biodegradation of precursors, bioaccumulation on the activated sludge and high MLSS concentrations with more protein and organic contents, etc.
j. Both effluent and sludge were considered as significant sinks for PFCs, especially MLSS which contained much higher PFCs contents.

k. Generally, the solid to liquid ratios of fully fluorinated PFCs in the WRP followed the trend in accordance with their relative hydrophobicity.

5.1.2. Bench-scale sorption studies of ASs and PFCs

a. Both sorption tests using wet biomass with and without chemical inhibition consistently showed ~18% removal of SUC in aqueous solution in 17 days, which indicated persistence of SUC under the experimental conditions. Equilibrium was not reached within the experimental duration. The slow uptake rate and small adsorption capacity are in accordance with the hydrophilic property of the compound. In addition, the impact of the chemical addition, i.e. NaN_3 which was demonstrated to potentially enhance or reduce sorption rates , was assumed to be the possible cause for the two different kinetic behaviors of SUC between the two tests (Patel & Suresh, 2008; Lin et al., 2010). Overall, the results imply that sorption onto activated biomass could be a small sink for SUC in WWTPs.

b. No sorption of ACE was observed in the test using inhibited wet biomass within 17 days, which demonstrated lower sorption capacity for ACE than SUC, in accordance with the relative solid to liquid ratios in the above field study. This could be due to the negative ionic nature of ACE under test conditions indicated by its low pK_a value, which may be more likely to repel the sludge solids. In contrast, significant aqueous mass reduction of 70% within 17 days was observed in the original wet biomass test without chemical inhibition, which may indicate high biodegradation potential of ACE, although this observation was inconsistent with the general conclusion in the WRP field study and the field literature.

c. For PFCs, sorption equilibrium was reached in both tests using either USEPA-lyophilization-heat pre-treated biomass (<1 day) or inhibited oven-dried biomass (~10 hours), and most PFCs (PFDA, PFNA, PFOA, PFOS and PFHxS) showed significant sorption affinity onto activated biomass. This indicates that sludge is an important sink for PFCs in WWTPs. However, the USEPA deactivated sludge showed longer equilibration time and higher sorption capacity compared to the inhibited oven-dried biomass. This emphasizes the importance of standardizing the pre-treatment method for activated sludge in sorption studies.

d. PFBA, PFHxA and PFBS showed little/limited sorption under the specific test conditions in 5 days due to their relatively low hydrophobicity related to their short C-F chain length. Therefore, these compounds are likely to escape from WWTPs in effluents, with limited immobilization onto MLSS.

e. Sorption was preferential for compounds with longer C-F chain length within the same family, which strongly suggests hydrophobic reactions. In addition, perfluorinated sulfonates showed higher sorption capacity than perfluorinated carboxylic acids with the same number of carbons. The results are in accordance with the relative solid to liquid ratios in the above WRP field study.

5.2. Recommendations for future work

5.2.1. Occurrence and fate of ASs and PFCs in WRPs

The monitoring data could be better interpreted with some supplemental information and improvements. First, more frequent sampling incorporated with the relevant operational parameters of the WRP, such as flow data, could

help consolidate the trend and fluctuation of data. Characterization of wastewater (including both dissolved and solid phases) combined with scientific studies on the physicochemical properties of the analytes could enhance the understanding of their behaviors. In addition, composite sampling in replacement of grab sampling, can obtain more representative concentration data which is supposed to be more independent of storm events and slug loadings, etc. Furthermore, concentrations on disposed sludge could be obtained to develop a mass balance of the analytes and subsequently, estimate the removal efficiency over the whole WRP. With sufficient flow data, the mass balance and distribution in each treatment unit can further help to determine the most efficient removal mechanisms.

In terms of biotransformation, more common precursors, intermediates and metabolites should be investigated and monitored. As such, the source characterization of influents would be significant since its variation directly determines the availability of various precursors. For PFCs, there is lack of studies on the metabolic transformation and pathways of potential precursors other than PFOA and PFOS. In addition, for biodegradable CYC and SAC in WWTPs, little information is available about their metabolic pathways, the corresponding metabolites and the associated risks. These are all knowledge gaps to be filled.

Since effluent discharge is a potential point source for receiving water bodies or poses challenges to subsequent industrial water recycling, diverse EOCs should be included in the monitoring program and different treatment processes applied in different WWTPs should be assessed. For instance, although most PFC studies focus on linear PFCs, there is a knowledge gap in

the understanding of the behavior of branched PFC isomers. Furthermore, other treatment processes, such as photodegradation, chemical oxidation and activated carbon adsorption are often applied in tertiary treatment in WWTPs. As such, these processes would require further studies for a comprehensive understanding and better prediction of the fate of the contaminants in a general WWTP.

Due to increasing consumption of ASs and PFCs, bioaccumulation and toxicity studies are necessary in risk assessment, including those for their corresponding metabolites. For instance, the short perfluorocarbon-chain PFCs, such as PFBA, indicate new challenges in risk assessment with limited knowledge on its toxicity, when industries are shifting from PFOA/PFOS to these compounds. Epidemiology studies could be conducted to confirm the impacts of PFCs and ASs on human health. Furthermore, cumulative impacts should be assessed for multiple stressors and also long-term multigenerational effects (Stahl et al., 2011). These toxicological studies could provide the scientific basis to develop environmental thresholds for PFCs and ASs, most of which are non-regulated and which are of concern to ecological and human health.

5.2.2. Bench-scale sorption studies of ASs and PFCs

The different kinetic behaviors using different pre-treated biomass solids in this study suggest the importance of standardizing the preparation protocols for activated sludge with proper inactivation in sorption studies, which can facilitate comparisons between different literature studies. Ideally, the method should not alter the characteristics of biomass solids and represent similar behavior in real environments but with sufficient suppression of microbial

activity. The protocols may be reasonably different for fast-adsorbing compounds and slow-uptake compounds since a stronger suppression method may be needed for microbial activity for slow-uptake compounds in longer duration sorption studies.

Furthermore, regardless of the apparent behaviors of selected PFCs and ASs in the sorption study for activated sludge, the mechanisms behind the observations are still unknown which are potential research opportunities. Firstly, a standard biodegradation study under various test conditions is required to confirm the biodegradability of ACE. It is significant to identify dominant active enzymes and bacteria for ACE biodegradation, which could be applied in the field as an economic and efficient treatment method. The relevant metabolites and their fates are a knowledge gap as well in the risk assessment. Secondly, the sorption mechanisms for PFCs and SUC should be explored to better predict their behaviors when faced with different kinds of solids and wastewater matrix. Desorption tests may be conducted to know the reversibility of the adsorbed analytes, with regards to kinetic behavior. Different combination of test conditions should be tested in accordance with real cases. For example, the impacts of initial concentration on sorption behaviors should be investigated since with increasing consumption, the concentration of ASs in wastewater influents has been increasing.

Last but not least, care must be taken to extrapolate bench-scale data in real WWTPs since the test conditions are controlled and the test solutions are much simpler in the lab. For example, in our bench-scale sorption studies, there was sufficient time for PFCs to reach equilibrium. However, in dynamic WWTPs, the low HRT may prevent all PFCs in dissolved phase of wastewater

from reaching equilibrium with the adsorbed analytes on suspended solids. Another example could be the complex matrix in real wastewater compared to simple test solutions in lab studies. As an improvement, bench-scale reactors and pilot studies with continuous flows of real wastewater matrix could help obtain better kinetic parameters, which would be more useful for plant optimization.

Chapter 6. Bibliography

Abou-Donia, M. B., El-Masry, E. M., Abdel-Rahman, A. A., McLendon, R. E., & Schiffman, S. S. (2008). Splenda alters gut microflora and increases intestinal P-glycoprotein and cytochrome P-450 in male rats. *Journal of Toxicology and Environmental Health - Part A: Current Issues, 71*(21), 1415-1429.

Aksu, Z. (2001). Biosorption of reactive dyes by dried activated sludge: Equilibrium and kinetic modelling. *Biochemical Engineering Journal, 7*(1), 79-84.

American Diabetes Association. (2014). Low-Calorie Sweeteners. http://www.diabetes.org/food-and-fitness/food/what-can-i-eat/understanding-carbohydrates/artificial-sweeteners/

American Public Health Association, American Water Works Association, & Water Pollution Control Federation. (1989). 2540-D. Total Suspended Solids Dried at 103-105℃. In L. S. Clesceri, A. E. Greenberg & R. R. Trussell (Eds.), *Standard methods for the examination of water and wastewater* (17th ed., pp. 2-55 - 52-59). Washington, D.C.: American Public Health Associaton.

Arican, B., Gokcay, C. F., & Yetis, U. (2002). Mechanistics of nickel sorption by activated sludge. *Process Biochemistry, 37*(11), 1307-1315.

Arvaniti, O. S., Ventouri, E. I., Stasinakis, A. S., & Thomaidis, N. S. (2012). Occurrence of different classes of perfluorinated compounds in Greek wastewater treatment plants and determination of their solid-water distribution coefficients. *J Hazard Mater, 239-240*, 24-31. doi: 10.1016/j.jhazmat.2012.02.015

Betts, K. S. (2007). PERFLUOROALKYL ACIDS: What Is the Evidence Telling Us? *Environ Health Perspect, 115*(5), A250–A256.

Boulanger, B., Vargo, J. D., Schnoor, J. L., & Hornbuckle, K. C. (2005). Evaluation of perfluorooctane surfactants in a wastewater treatment system and in a commercial surface protection product. *Environmental Science and Technology, 39*(15), 5524-5530.

Brorström-Lundén, E., Svenson, A., Viktor, T., Woldegiogis, A. , Remberger, M., Kaj, L., Dye, C., Bjerke, A., & Schlabach, M. (2008). Measurements of sucralose in the Swedish screening program 2007 - Part I; Sucralose in surface and STP samples. (Vol. IVL Report B1769): IVL Swedish Environmental Research Institute Ltd. .

Buerge, I. J., Buser, H. R., Kahle, M., Müller, M. D., & Poiger, T. (2009). Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: An ideal chemical marker of domestic wastewater in groundwater. *Environmental Science and Technology, 43*(12), 4381-4385.

Buerge, I. J., Keller, M., Buser, H. R., Müller, M. D., & Poiger, T. (2011). Saccharin and other artificial sweeteners in soils: Estimated inputs from agriculture and households, degradation, and leaching to groundwater. *Environmental Science and Technology, 45*(2), 615-621.

Butenhoff, J. L., Kennedy Jr, G. L., Frame, S. R., O'Connor, J. C., & York, R. G. (2004a). The reproductive toxicology of ammonium perfluorooctanoate

(APFO) in the rat. *Toxicology, 196*(1-2), 95-116.

Butenhoff, J. L., Kennedy Jr, G. L., Hinderliter, P. M., Lieder, P. H., Jung, R., Hansen, K. J., Gorman, G. S., Noker, P. E., & Thomford, P. J. (2004b). Pharmacokinetics of perfluorooctanoate in cynomolgus monkeys. *Toxicological Sciences, 82*(2), 394-406.

Case, M. T., York, R. G., & Christian, M. S. (2001). Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds. *International Journal of Toxicology, 20*(2), 101-109.

ChemSpider. (2014a). Acesulfame. London, UK: Royal Society of Chemistry.

ChemSpider. (2014b). Aspartame. London, UK: Royal Society of Chemistry.

ChemSpider. (2014c). Cyclamic acid. London, UK: Royal Society of Chemistry.

ChemSpider. (2014d). Oxybenzone. London, UK: Royal Society of Chemistry.

ChemSpider. (2014e). Saccharin. London, UK: Royal Society of Chemistry.

ChemSpider. (2014f). Sodium azide. London, UK: Royal Society of Chemistry.

D'Eon, J. C., Hurley, M. D., Wallington, T. J., & Mabury, S. A. (2006). Atmospheric Chemistry of N-methyl Perfluorobutane Sulfonamidoethanol, C4F9SO2N(CH3)CH2CH2OH: Kinetics and Mechanism of Reaction with OH. *Environmental Science & Technology, 40*(6), 1862-1868.

Dol, W., & Verhoog, D. (2010). Common Agricultural Policy Regionalized Impact – The Rural Development Dimension: D 2.3.5: Methodology for data quality management: LEI The Hague.

Drinking Water Inspectorate. (2009). *Guidance on the Water Supply (Water Quality) Regulations 2000 specific to PFOS (perfluorooctane sulphonate) and PFOA (perfluorooctanoic acid) concentrations in drinking water*. London, UK: DWI Retrieved from http://dwi.defra.gov.uk/stakeholders/information-letters/2009/10_2009annex.pdf.

Duerr, R. I. (2013). Water scarcity in Singapore pushes 'toilet to tap' concept. Retrieved from GLOBAL IDEAS website: http://www.dw.de/water-scarcity-in-singapore-pushes-toilet-to-tap-concept/a-16904636

Eriksen, K. T., Raaschou-Nielsen, O., Sørensen, M., Roursgaard, M., Loft, S., & Møller, P. (2010). Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 700*(1–2), 39-43.

European Commission. (2004). Directive 2006/52/EC of European Parliament and of the Council of 5 July 2006 amending Directive 95/2/EC on food additives other than colors and sweeteners and Directive 94/35/EC on sweeteners for use in foodstuffs. *Official Journal of the European Union, L024*, 10.

European Commission Scientific Committee on Food. (2000). Opinion of the Scientific Committee on Food on sucralose *Scientific Opinions* (pp. 25). Brussels: European Commission.

European Food Safety Authority. (2008). Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific opinion of the panel on contaminants in the food chain. *EFSA Journal, 653*, 1-131.

Farre, M., Kantiani, L., Petrovic, M., Perez, S., & Barcelo, D. (2012). Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques. *J Chromatogr A, 1259*, 86-99.

Fromel, T., & Knepper, T. P. (2010). Biodegradation of fluorinated alkyl substances. *Rev Environ Contam Toxicol, 208*, 161-177.

Fromme, H., Tittlemier, S. A., Völkel, W., Wilhelm, M., & Twardella, D. (2009). Perfluorinated compounds - Exposure assessment for the general population in western countries. *International Journal of Hygiene and Environmental Health, 212*(3), 239-270.

Gan, Z., Sun, H., Feng, B., Wang, R., & Zhang, Y. (2013). Occurrence of seven artificial sweeteners in the aquatic environment and precipitation of Tianjin, China. *Water Research, 47*(14), 4928-4937.

Giesy, J. P., & Kannan, K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environmental Science and Technology, 35*(7), 1339-1342.

Gulnaz, O., Kaya, A., Matyar, F., & Arikan, B. (2004). Sorption of basic dyes from aqueous solution by activated sludge. *J Hazard Mater, 108*(3), 183-188.

Guo, R., Sim, W. J., Lee, E. S., Lee, J. H., & Oh, J. E. (2010). Evaluation of the fate of perfluoroalkyl compounds in wastewater treatment plants. *Water Res, 44*(11), 3476-3486.

Haley, S. (2013). Sugar and Sweeteners Outlook: NAFTA and World Sugar June 2013 (pp. 24). Washington, D.C., U.S.: The United States Department of Agriculture.

Hansen, K. J., Clemen, L. A., Ellefson, M. E., & Johnson, H. O. (2001). Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environmental Science and Technology, 35*(4), 766-770.

Higgins, C. P., & Luthy, R. G. (2006). Sorption of perfluorinated surfactants on sediments. *Environmental Science and Technology, 40*(23), 7251-7256.

Hu, W. Y., Jones, P. D., DeCoen, W., King, L., Fraker, P., Newsted, J., & Giesy, J. P. (2003). Alterations in cell membrane properties caused by perfluorinated compounds. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, 135*(1), 77-88.

Hu, X. Z., & Hu, D. C. (2009). Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma Hep G2 cells. *Archives of Toxicology, 83*(9), 851-861.

Jahnke, A., Berger, U., Ebinghaus, R., & Temme, C. (2007). Latitudinal Gradient of Airborne Polyfluorinated Alkyl Substances in the Marine Atmosphere between Germany and South Africa (53° N−33° S). *Environmental Science & Technology, 41*(9), 3055-3061.

Kim, M. H., Wang, N., McDonald, T., & Chu, K. H. (2012). Biodefluorination and biotransformation of fluorotelomer alcohols by two alkane-degrading Pseudomonas strains. *Biotechnology and Bioengineering, 109*(12), 3041-3048.

Koelemay, J. (2014). Low- and No-Calorie Sweetener Safety and Estimated Intakes. http://beverageinstitute.org/us/article/understanding-low-and-no-calorie-sweeteners-safety-guidelines-adi-and-estimated-intakes/

Kokotou, M. G., & Thomaidis, N. S. (2013). Determination of eight artificial sweeteners in wastewater by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Analytical Methods, 5*(16), 3825.

Kroger, M., Meister, K., & Kava, R. (2006). *Comprehensive Review in Food Science and Food Safety, 5*, 35.

Kunacheva, C., Tanaka, S., Fujii, S., Boontanon, S. K., Musirat, C., Wongwattana, T., & Shivakoti, B. R. (2011). Mass flows of perfluorinated compounds (PFCs) in central wastewater treatment plants of industrial zones in Thailand. *Chemosphere, 83*(6), 737-744.

Labadie, P., & Chevreuil, M. (2011). Partitioning behaviour of perfluorinated alkyl contaminants between water, sediment and fish in the Orge River (nearby Paris, France). *Environmental Pollution, 159*(2), 391-397.

Lange, C. C. (2000). The aerobic biodegradation of N-EtFOSE alcohol by the microbial activity present in municipal wastewater treatment sludge. St. Paul, MN: 3M Company.

Lange, F. T., Scheurer, M., & Brauch, H. J. (2012). Artificial sweeteners--a recently recognized class of emerging environmental contaminants: a review. *Anal Bioanal Chem, 403*(9), 2503-2518.

Lau, C., Thibodeaux, J. R., Hanson, R. G., Narotsky, M. G., Rogers, J. M., Lindstrom, A. B., & Strynar, M. J. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. *Toxicological Sciences, 90*(2), 510-518.

Lau, C., Thibodeaux, J. R., Hanson, R. G., Rogers, J. M., Grey, B. E., Stanton, M. E., Butenhoff, J. L., & Stevenson, L. A. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: Postnatal evaluation. *Toxicological Sciences, 74*(2), 382-392.

Li, J., Langford, C. H., & Gamble, D. S. (1996). Atrazine Sorption by a Mineral Soil: Processes of Labile and Nonlabile Uptake. *Journal of Agricultural and Food Chemistry, 44*(11), 3672-3679.

Lin, A. Y. C., Lin, C. A., Tung, H. H., & Chary, N. S. (2010). Potential for biodegradation and sorption of acetaminophen, caffeine, propranolol and acebutolol in lab-scale aqueous environments. *Journal of Hazardous Materials, 183*(1-3), 242-250.

Liu, C., Gin, K. Y. H., Chang, V. W. C., Goh, B. P. L., & Reinhard, M. (2011). Novel Perspectives on the Bioaccumulation of PFCs – the Concentration Dependency. *Environmental Science & Technology, 45*(22), 9758-9764.

Liu, J., & Mejia Avendaño, S. (2013). Microbial degradation of polyfluoroalkyl chemicals in the environment: A review. *Environment International, 61*(0), 98-114.

Loganathan, B. G., Sajwan, K. S., Sinclair, E., Senthil Kumar, K., & Kannan, K. (2007). Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. *Water Res, 41*(20), 4611-4620.

Loos, R., Carvalho, R., Antonio, D. C., Comero, S., Locoro, G., Tavazzi, S., . . . Gawlik, B. M. (2013). EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res, 47*(17), 6475-6487.

Ma, R., & Shih, K. (2010). Perfluorochemicals in wastewater treatment plants and sediments in Hong Kong. *Environmental Pollution, 158*(5), 1354-1362.

Martin, J. W., Ellis, D. A., Mabury, S. A., Hurley, M. D., & Wallington, T. J. (2005). Atmospheric Chemistry of Perfluoroalkanesulfonamides: Kinetic and Product Studies of the OH Radical and Cl Atom Initiated Oxidation of N-Ethyl Perfluorobutanesulfonamide. *Environmental Science & Technology, 40*(3), 864-872.

Mead, R. N., Morgan, J. B., Avery, G. B., Kieber, R. J., Kirk, A. M., Skrabal, S. A., & Willey, J. D. (2009). Occurrence of the artificial sweetener sucralose in coastal and marine waters of the United States. *Marine Chemistry, 116*(1-4), 13-17.

National Institute of Environmental Health Sciences. (2012). *Perfluorinated Chemicals (PFCs)*. North Carolina, U.S.: National Institute of Environmental Health Sciences Retrieved from http://www.niehs.nih.gov/health/materials/perflourinated_chemicals_508.pdf.

Neset, T. S. S., Singer, H., Longrée, P., Bader, H. P., Scheidegger, R., Wittmer, A., & Andersson, J. C. M. (2010). Understanding consumption-related sucralose emissions - A conceptual approach combining substance-flow analysis with sampling analysis. *Science of the Total Environment, 408*(16), 3261-3269.

Nguyen, Viet Tung. (2011). *OCCURRENCE AND PHOTOCHEMICAL FATE OF PERFLUOROCHEMICALS IN A TROPICAL URBAN WATERSHED*. Thesis. School of Civil and Environmental Engineering. Nanyang Technological University. Singapore. Retrieved from http://dr.ntu.edu.sg/handle/10356/46533

Ochoa-Herrera, V., & Sierra-Alvarez, R. (2008). Removal of perfluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge. *Chemosphere, 72*(10), 1588-1593.

Oppenheimer, J., Eaton, A., Badruzzaman, M., Haghani, A. W., & Jacangelo, J. G. (2011). Occurrence and suitability of sucralose as an indicator compound of wastewater loading to surface waters in urbanized regions. *Water Research,*

45(13), 4019-4027.

Ordóñez, E. Y., Quintana, J. B., Rodil, R., & Cela, R. (2012). Determination of artificial sweeteners in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 1256*(0), 197-205.

Ordóñez, E. Y., Quintana, J. B., Rodil, R., & Cela, R. (2013). Determination of artificial sweeteners in sewage sludge samples using pressurised liquid extraction and liquid chromatography-tandem mass spectrometry. *J Chromatogr A, 1320*, 10-16.

Pan, Y., Shi, Y., Wang, J., & Cai, Y. (2011). Evaluation of perfluorinated compounds in seven wastewater treatment plants in Beijing urban areas. *Science China Chemistry, 54*(3), 552-558.

Patel, R., & Suresh, S. (2008). Kinetic and equilibrium studies on the biosorption of reactive black 5 dye by Aspergillus foetidus. *Bioresource Technology, 99*(1), 51-58.

Pescod, M. B. (1992). *Wastewater treatment and use in agriculture / by M.B. Pescod.* (T0551). Rome, Italy: The Food and Agriculture Organization of the United Nations Retrieved from http://www.fao.org/docrep/T0551E/T0551E00.htm.

Qazi, M. R., Bogdanska, J., Butenhoff, J. L., Nelson, B. D., DePierre, J. W., & Abedi-Valugerdi, M. (2009). High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. *Toxicology, 262*(3), 207-214.

Qin, X. F. (2002). Impaired inactivation of digestive proteases by deconjugated bilirubin: The possible mechanism for inflammatory bowel disease. *Medical Hypotheses, 59*(2), 159-163.

Qin, X. (2011). What caused the recent worldwide increase of inflammatory bowel disease: Should sucralose be added as a suspect? *Inflammatory Bowel Diseases, 17*(10), E139-E139.

Ratola, N., Cincinelli, A., Alves, A., & Katsoyiannis, A. (2012). Occurrence of organic microcontaminants in the wastewater treatment process. A mini review. *J Hazard Mater, 239-240*, 1-18.

Rayne, S., & Forest, K. (2009). Perfluoroalkyl sulfonic and carboxylic acids: A critical review of physicochemical properties, levels and patterns in waters and wastewaters, and treatment methods. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering, 44*(12), 1145-1199.

Reig, P., Maddocks, A., & Gassert, F. (2013). World's 36 Most Water-Stressed Countries. Retrieved from http://www.wri.org/blog/world%E2%80%99s-36-most-water-stressed-countries

Reinders, A., Sivitz, A. B., Hsi, A., Grof, C. P. L., Perroux, J. M., & Ward, J. M.

(2006). Sugarcane ShSUT1: Analysis of sucrose transport activity and inhibition by sucralose. *Plant, Cell and Environment, 29*(10), 1871-1880.

Rhoads, K. R., Janssen, E. M. L., Luthy, R. G., & Criddle, C. S. (2008). Aerobic biotransformation and fate of N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE) in activated sludge. *Environmental Science and Technology, 42*(8), 2873-2878.

Sáez, M., De Voogt, P., & Parsons, J. R. (2008). Persistence of perfluoroalkylated substances in closed bottle tests with municipal sewage sludge. *Environmental Science and Pollution Research, 15*(6), 472-477.

Sardesai, V. M., & Waldshan, T. H. (1991). Natural and synthetic intense sweeteners. *The Journal of Nutritional Biochemistry, 2*(5), 236-244.

Scheurer, M., Brauch, H. J., & Lange, F. T. (2009). Analysis and occurrence of seven artificial sweeteners in German waste water and surface water and in soil aquifer treatment (SAT). *Analytical and Bioanalytical Chemistry, 394*(6), 1585-1594.

Scheurer, M., Storck, F. R., Brauch, H. J., & Lange, F. T. (2010). Performance of conventional multi-barrier drinking water treatment plants for the removal of four artificial sweeteners. *Water Res, 44*(12), 3573-3584.

Scheurer, M., Storck, F. R., Graf, C., Brauch, H. J., Ruck, W., Lev, O., & Lange, F. T. (2011). Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer treatment. *Journal of Environmental Monitoring, 13*(4), 966-973.

Schröder, H. F. (2003). Determination of fluorinated surfactants and their metabolites in sewage sludge samples by liquid chromatography with mass spectrometry and tandem mass spectrometry after pressurised liquid extraction and separation on fluorine-modified reversed-phase sorbents. *Journal of Chromatography A, 1020*(1), 131-151.

Schultz, M. M., Barofsky, D. F., & Field, J. A. (2003). Fluorinated alkyl surfactants. *Environmental Engineering Science, 20*(5), 487-501.

Shivakoti, B. R., Tanaka, S., Fujii, S., Kunacheva, C., Boontanon, S. K., Musirat, C., Seneviratne, S. T., & Tanaka, H. (2010). Occurrences and behavior of perfluorinated compounds (PFCs) in several wastewater treatment plants (WWTPs) in Japan and Thailand. *Journal of Environmental Monitoring, 12*(6), 1255-1264.

Sibinski, L J. (1987). Final report of a two-year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats 1987 (Vol. 1-4): 3 M Company/RIKER.

Sinclair, E., & Kannan, K. (2006). Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants. *Environmental Science and Technology, 40*(5), 1408-1414.

Singapore Department of Statistics. (2014). Statistics Latest Data. Retrieved Feb. 11, 2014, from Singapore Department of Statistics http://www.singstat.gov.sg/statistics/latest_data.html#14

Soh, L., Connors, K. A., Brooks, B. W., & Zimmerman, J. (2011). Fate of sucralose through environmental and water treatment processes and impact on plant indicator species. *Environ Sci Technol, 45*(4), 1363-1369.

Stahl, T., Mattern, D., & Brunn, H. (2011). Toxicology of perfluorinated compounds. *Environmental Sciences Europe, 23*(1).

Stasinakis, A. S. (2012). Review on the fate of emerging contaminants during sludge anaerobic digestion. *Bioresour Technol, 121*, 432-440.

Stevens-Garmon, J., Drewes, J. E., Khan, S. J., McDonald, J. A., & Dickenson, E. R. (2011). Sorption of emerging trace organic compounds onto wastewater sludge solids. *Water Res, 45*(11), 3417-3426.

Subedi, B., Lee, S., Moon, H. B., & Kannan, K. (2014). Emission of artificial sweeteners, select pharmaceuticals, and personal care products through sewage sludge from wastewater treatment plants in Korea. *Environ Int, 68C*, 33-40.

Sun, H., Zhang, X., Wang, L., Zhang, T., Li, F., He, N., & Alder, A. C. (2012). Perfluoroalkyl compounds in municipal WWTPs in Tianjin, China--concentrations, distribution and mass flow. *Environ Sci Pollut Res Int, 19*(5), 1405-1415.

The European Parliament, & The European Council. (2006). Directive of the European Parliament and of the Council: Relating to Restrictions on the Marketing and Use of Perfluorooctane Sulfonates (Amendment of Council DIRECTIVE 2006/122/ECOF). *Official Journal of the European Union*.

The Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency. (1998). *Fate, Transport and Transformation Test Guidelines: OPPTS 835.1110 Activated Sludge Sorption Isotherm*. (EPA 712–C–98–298). Washington, DC: The U.S. Government Printing Office Retrieved from http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0152-0003.

The Organisation for Economic Co-operation and Development. (2007). Environmental Outlook to 2030. Paris: The Organisation for Economic Co-operation and Development.

The Organisation for Economic Co-operation and Development. (2009). Managing Water for All: An OECD Perspective on Pricing and Financing. Paris: The Organisation for Economic Co-operation and Development.

The Public Utilities Board. (2010). Water Supply. http://www.pub.gov.sg/about/historyfuture/Pages/WaterSupply.aspx

The Public Utilities Board. (2013a). Overview: Four National Taps Provide Water for All. http://www.pub.gov.sg/water/Pages/default.aspx

The Public Utilities Board. (2013b). The Singapore Water Story. http://www.pub.gov.sg/water/Pages/singaporewaterstory.aspx

The United States Environmental Protection Agency. (2000). *Perfluorooctyl*

sulfonates: Proposed significant new use rule. (202). Washington, DC, U.S.: Federal Register Retrieved from http://www.epa.gov/oppt/chemtest/pubs/snur49.pdf.

The United States Environmental Protection Agency. (2013). Perfluorooctanoic acid (PFOA) and fluorinated telomers: 2010/2015 PFOA Stewardship Program. Retrieved Feb. 23, 2014, from http://www.epa.gov/oppt/pfoa/pubs/stewardship/

The United States Geological Survey. (2014a). Investigations - Emerging Contaminants: Emerging Contaminants In the Environment. *Toxic Substances Hydrology Program*. http://toxics.usgs.gov/regional/emc/index.html

The United States Geological Survey. (2014b). Research Projects - Emerging Contaminants: Sources and Source Pathways. *Toxic Substances Hydrology Program*. http://toxics.usgs.gov/regional/emc/sources_pathways.html

Thomford, P J. (2002). 104-week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. Madison, Wisconsin, U.S.: Covance Laboratories Inc,.

Torres, C. I., Ramakrishna, S., Chiu, C. A., Nelson, K. G., Westerhoff, P., & Krajmalnik-Brown, R. (2011). Fate of sucralose during wastewater treatment. *Environmental Engineering Science, 28*(5), 325-331.

Tran, N. H., Nguyen, V. T., Urase, T., & Ngo, H. H. (2014). Role of nitrification in the biodegradation of selected artificial sweetening agents in biological wastewater treatment process. *Bioresource Technology, 161*(0), 40-46.

Tran, N. H., Hu, J., & Ong, S. L. (2013). Simultaneous determination of PPCPs, EDCs, and artificial sweeteners in environmental water samples using a single-step SPE coupled with HPLC–MS/MS and isotope dilution. *Talanta, 113*(0), 82-92.

Tremblay, L. A, Stewart, M., Peake, B. M., Gadd, J. B., & Northcott, G. (2011). Review of the Risks of Emerging Organic Contaminants and Potential Impacts to Hawke's Bay. Nelson, New Zealand: Cawthron Institute.

Wang, N., Liu, J., Buck, R. C., Korzeniowski, S. H., Wolstenholme, B. W., Folsom, P. W., & Sulecki, L. M. (2011). 6:2 Fluorotelomer sulfonate aerobic biotransformation in activated sludge of waste water treatment plants. *Chemosphere, 82*(6), 853-858.

Wang, N., Szostek, B., Buck, R. C., Folsom, P. W., Sulecki, L. M., & Gannon, J. T. (2009). 8-2 Fluorotelomer alcohol aerobic soil biodegradation: Pathways, metabolites, and metabolite yields. *Chemosphere, 75*(8), 1089-1096.

Wang, N., Szostek, B., Folsom, P. W., Sulecki, L. M., Capka, V., Buck, R. C., Berti, W. R., & Gannon, J. T. (2005a). Aerobic biotransformation of 14C-labeled 8-2 telomer B alcohol by activated sludge from a domestic sewage treatment plant. *Environmental Science and Technology, 39*(2), 531-538.

Wang, N., Szostek, B., Buck, R. C., Folsom, P. W., Sulecki, L. M., Capka, V., Berti, W. R., & Gannon, J. T. (2005b). Fluorotelomer Alcohol BiodegradationDirect Evidence that Perfluorinated Carbon Chains Breakdown. *Environmental*

Science & Technology, 39(19), 7516-7528.

Water Resources Institute (Cartographer). (2013). Aqueduct Country and River Basin Rankings - Average exposure to Aqueduct water risk indicators. Retrieved from http://www.wri.org/applications/maps/aqueduct-country-river-basin-rankings/#x=109.23&y=3.82&l=5&v=home&d=bws&f=0&o=144&init=y

Yang, Q., Xie, Y., & Depierre, J. W. (2000). Effects of peroxisome proliferators on the thymus and spleen of mice. *Clinical and Experimental Immunology, 122*(2), 219-226.

Yu, J., Hu, J., Tanaka, S., & Fujii, S. (2009). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in sewage treatment plants. *Water Res, 43*(9), 2399-2408.

Yu, J., & Hu, J. (2011). Adsorption of Perfluorinated Compounds onto Activated Carbon and Activated Sludge. *Journal of Environmental Engineering, 137*(10), 945-951.

Zareitalabad, P., Siemens, J., Hamer, M., & Amelung, W. (2013). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater - A review on concentrations and distribution coefficients. *Chemosphere, 91*(6), 725-732.

Zhao, J., Li, Y., Zhang, C., Zeng, Q., & Zhou, Q. (2008). Sorption and degradation of bisphenol A by aerobic activated sludge. *Journal of Hazardous Materials, 155*(1–2), 305-311.

Zhou, Q., Deng, S., Zhang, Q., Fan, Q., Huang, J., & Yu, G. (2010). Sorption of perfluorooctane sulfonate and perfluorooctanoate on activated sludge. *Chemosphere, 81*(4), 453-458.

Zushi, Y., Hogarh, J. N., & Masunaga, S. (2012). Progress and perspective of perfluorinated compound risk assessment and management in various countries and institutes. *Clean Technologies and Environmental Policy, 14*(1), 9-20.

Zygler, Agata, Wasik, Andrzej, & Namieśnik, Jacek. (2009). Analytical methodologies for determination of artificial sweeteners in foodstuffs. *TrAC Trends in Analytical Chemistry, 28*(9), 1082-1102.

Appendix A : Monthly concentrations of total suspended solids (TSS) in wastewater samples.

Table A. Monthly concentrations of total suspended solids of the collected wastewater samples in the local WRP from February 2013 to July 2013. Unit: ppm.

Sample label	Feb		Mar		Apr		May		Jun		Jul	
	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD
Inf	400.0	22.2	492.9	29.3	287.5	9.0	353.8	10.9	391.7	31.7	345.4	20.2
A1	151.3	5.8	137.7	2.3	99.3	5.8	135.0	6.9	110.0	3.5	137.7	4.2
A2	1806.7	137.8	1994.4	44.4	1893.3	28.3	1427.8	40.7	1897.8	19.2	1516.7	37.1
A3	23.2	1.3	11.2	0.9	5.5	0.7	9.1	0.8	17.0	1.4	9.1	1.0
A4	3982.2	100.3	4685.0	160.9	3700.0	14.1	2348.3	104.1	3260.0	43.6	3121.7	11.5
B1	224.0	5.7	258.3	22.5	177.0	4.2	257.7	5.0	216.7	4.2	432.6	5.1
B2	4655.6	110.0	5861.7	75.1	3585.0	304.1	6708.3	146.4	4373.3	83.3	3935.0	870.0
B3	5.5	0.5	0.9	0.8	0.0	4.6	1.1	0.6	3.0	0.0	0.0	2.9
B4	9243.3	66.6	11205.0	334.2	6403.3	80.8	15035.0	931.5	7736.7	90.7	8261.7	90.7

Appendix B : Monthly concentrations of ASs in the dissolved phase and the suspended solid phase of the collected wastewater samples in the local WRP in Singapore from February 2013 to July 2013

Table B. Monthly concentrations of ASs in the dissolved phase of the collected wastewater samples in the local WRP from February 2013 to July 2013. Unit: ppb.

Date	Sample label[a]	ACE		SUC		CYC		SAC		Total AS
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Feb	Inf	7.09	0.06	6.50	1.18	35.17	4.81	13.20	0.00	62.0
	A1[a]	6.20	0.24	5.33	1.36	25.00	1.00	11.53	0.81	48.1
	A2	5.82	0.21	4.56	0.64	<LOD	0.00	<LOD	0.00	10.4
	A3	5.84	0.10	4.71	0.71	<LOD	0.00	<LOD	0.00	10.6
	A4	5.63	0.22	4.68	0.24	<LOD	0.00	<LOD	0.00	10.3
	B1[b]	5.83	0.39	4.47	0.28	19.73	0.25	10.73	1.15	40.8
	B2	5.68	0.13	4.56	0.56	<LOD	0.00	<LOD	0.00	10.3
	B3	6.13	0.71	4.19	0.21	0.07	0.11	<LOD	0.00	10.4
	B4	6.28	0.70	4.75	1.03	<LOD	0.00	<LOD	0.00	11.1
Mar	Inf	6.32	0.66	2.42	0.72	29.57	3.46	9.31	0.81	47.6
	A1	6.74	0.38	1.88	0.64	26.73	0.67	11.43	0.75	46.8
	A2	6.68	0.82	1.68	0.26	<LOD	0.00	<LOD	0.00	8.4
	A3	6.27	0.76	1.66	0.29	<LOD	0.00	<LOD	0.00	8.0
	A4	6.53	1.36	1.48	0.11	<LOD	0.00	<LOD	0.00	8.0
	B1	6.49	0.42	1.48	0.09	12.23	0.25	6.78	0.55	27.0
	B2	6.31	0.84	1.32	0.15	<LOD	0.00	<LOD	0.00	7.7
	B3	6.00	0.88	1.30	0.24	<LOD	0.00	<LOD	0.00	7.3
	B4	6.40	0.69	1.65	0.20	<LOD	0.00	<LOD	0.00	8.1
Apr	Inf	6.47	0.20	2.11	0.05	37.77	1.48	15.33	0.15	61.7
	A1	6.56	0.23	1.95	0.60	23.96	1.06	10.29	0.42	42.8
	A2	6.26	0.37	1.85	0.32	<LOD	0.00	0.03	<LOD	0.00
	A3	6.31	0.82	2.00	0.30	<LOD	0.00	0.03	<LOD	0.00
	A4	6.10	0.64	1.74	0.34	<LOD	0.00	0.03	<LOD	0.00

	B1	6.50	0.43	1.65	0.26	11.04	0.27	6.05	0.59	25.2
	B2	6.18	0.74	1.67	0.19	<LOD	0.00	0.03	<LOD	0.00
	B3	6.66	0.52	1.60	0.48	<LOD	0.00	0.03	<LOD	0.00
	B4	6.62	0.26	1.84	0.25	<LOD	0.00	0.03	<LOD	0.00
	Inf	10.51	1.31	6.29	0.37	41.34	5.89	15.88	3.31	74.0
	A1	9.80	0.46	5.63	0.25	30.21	0.80	13.00	0.43	58.6
	A2	8.71	0.25	5.68	0.27	<LOD	0.00	<LOD	0.00	14.4
	A3	8.63	0.41	5.26	0.38	<LOD	0.00	<LOD	0.00	13.9
May	A4	8.89	0.27	4.09	0.50	<LOD	0.00	<LOD	0.00	13.0
	B1	10.91	0.91	5.74	0.58	15.17	1.34	8.93	1.30	40.7
	B2	8.54	0.33	5.33	0.37	<LOD	0.00	<LOD	0.00	13.9
	B3	8.09	0.25	4.94	0.40	<LOD	0.00	<LOD	0.00	13.1
	B4	8.78	0.24	5.56	0.59	<LOD	0.00	<LOD	0.00	14.4
	Inf	9.01	0.22	2.37	0.60	33.89	14.28	17.04	0.57	62.3
	A1	9.72	0.27	2.06	0.17	28.17	1.19	14.38	1.78	54.3
	A2	8.30	0.39	3.07	0.62	<LOD	0.00	<LOD	0.00	11.4
	A3	8.27	0.23	2.35	0.71	<LOD	0.00	<LOD	0.00	10.7
Jun	A4	8.29	0.33	2.49	0.72	<LOD	0.00	<LOD	0.00	10.8
	B1	8.27	0.55	2.52	0.48	14.09	1.46	8.75	0.54	33.6
	B2	7.78	0.06	2.58	0.22	0.02	0.02	<LOD	0.00	10.4
	B3	8.84	0.94	2.97	0.38	<LOD	0.00	<LOD	0.00	11.8
	B4	8.02	0.10	2.24	0.51	0.02	0.01	<LOD	0.00	10.3
	Inf	9.75	1.01	4.07	0.58	41.88	6.02	18.80	2.70	74.5
	A1	9.68	0.65	3.63	0.91	32.67	1.88	17.04	1.46	63.0
	A2	9.02	0.21	2.80	0.15	0.02	0.01	0.23	0.11	12.1
Jul	A3	9.00	0.56	2.66	0.64	0.05	0.02	<LOD	0.00	11.7
	A4	8.71	0.88	3.56	1.02	0.03	0.02	<LOD	0.00	12.3
	B1	10.00	0.67	2.02	0.20	13.83	0.29	9.89	0.23	35.8
	B2	8.69	0.63	4.84	0.75	0.25	0.04	0.21	0.06	14.0

	B3	9.15	0.39	3.44	1.25	0.16	0.03	0.32	0.08	13.1
	B4	9.26	1.01	2.85	0.22	0.03	0.01	<LOD	0.00	12.2

[a] Label A refers to samples collected in the Southworks while label B refers to samples collected in the Northworks.

Table C. Monthly concentrations of ASs in the suspended solid phase of the collected wastewater samples in the local WRP from February 2013 to July 2013. Unit: ng/g dw.

Date	Sample label[a]	ACE		SUC		CYC		SAC		Total AS
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Feb	Inf	<LOD	409.7	1367.5	571.8	2915.0	1660.7	1521.7	836.5	6216.7
	A1[a]	<LOD	648.8	5702.6	2210.5	9118.9	2106.0	6011.0	1965.3	21922.8
	A2	<LOD	171.0	535.0	83.8	<LOD	0.0	197.0	0.0	851.0
	A4	<LOD	5.8	256.6	35.4	<LOD	0.0	62.0	9.7	372.6
	B1[b]	<LOD	99.8	3223.2	449.0	4273.8	184.4	3821.4	223.6	12055.0
	B2	<LOD	43.0	278.2	68.7	<LOD	0.0	69.6	48.3	394.0
	B4	59.2	122.4	96.8	6.1	<LOD	0.0	34.8	6.9	196.2
Mar	Inf	<LOD	51.4	540.3	43.7	842.6	141.7	536.3	52.6	2253.9
	A1	<LOD	169.0	1260.3	242.3	3581.1	854.8	4267.6	121.0	10307.5
	A2	288.8	18.6	114.0	16.5	<LOD	0.0	<LOD	6.3	465.5
	A4	173.1	39.9	66.2	5.0	<LOD	0.0	<LOD	5.0	266.0
	B1	<LOD	213.8	651.6	71.1	956.1	157.0	1672.3	194.4	3918.7
	B2	140.8	4.7	47.4	2.7	<LOD	0.0	<LOD	0.9	209.5
	B4	90.5	10.9	36.0	2.9	<LOD	0.0	13.7	0.8	144.7
Apr	Inf	<LOD	94.1	674.8	87.9	3506.1	516.7	2918.3	265.8	7673.1
	A1	3329.6	284.2	1953.0	271.7	6674.5	631.6	6634.2	536.1	18591.3
	A2	305.2	34.7	164.4	20.6	<LOD	0.0	<LOD	5.2	535.6
	A4	192.5	18.0	118.6	6.7	<LOD	0.0	46.2	5.0	370.8
	B1	1987.9	226.2	969.9	176.6	1798.5	208.7	2570.6	150.6	7326.9
	B2	198.0	29.9	84.0	9.4	<LOD	0.0	<LOD	6.6	316.9

	B4	112.3	14.2	55.1	15.2	<LOD	0.0	<LOD	2.3	186.9
May	Inf	1149.6	48.9	916.8	74.6	3335.7	165.3	2569.6	125.0	7971.7
	A1	4182.7	869.0	2121.0	141.6	9086.4	1073.3	8987.7	1228.6	24377.8
	A2	578.3	28.8	408.1	27.4	<LOD	0.0	<LOD	28.5	1073.9
	A4	365.5	39.5	182.3	18.0	<LOD	0.0	67.7	56.7	636.8
	B1	1912.0	147.7	1135.8	177.9	1702.5	128.4	2809.8	109.0	7560.1
	B2	144.0	17.0	95.1	14.1	<LOD	0.0	40.5	7.6	287.1
	B4	84.0	7.3	57.6	3.9	<LOD	0.0	<LOD	1.5	149.9
Jun	Inf	2141.3	387.3	916.8	74.6	7370.2	1478.6	6757.4	1545.2	17185.7
	A1	8709.1	1158.0	2121.0	141.6	18951.5	3713.9	18818.2	1704.4	48599.8
	A2	902.1	31.7	408.1	27.4	<LOD	0.0	<LOD	5.3	1376.1
	A4	458.7	48.7	182.3	18.0	<LOD	0.0	<LOD	2.8	679.3
	B1	4495.4	427.1	1135.8	177.9	4021.5	248.4	7476.9	918.9	17129.6
	B2	385.8	8.2	95.1	14.1	<LOD	0.0	<LOD	3.2	509.5
	B4	293.0	17.2	57.6	3.9	<LOD	0.0	20.9	0.8	378.0
Jul	Inf	1328.8	109.1	598.3	143.9	4429.4	456.9	3831.1	240.9	10187.6
	A1	3537.5	200.8	1399.5	132.2	7866.8	733.6	9031.5	373.7	21835.3
	A2	653.6	38.1	185.9	9.0	<LOD	0.0	184.6	33.8	1057.1
	A4	297.4	32.2	118.8	10.2	<LOD	0.0	120.1	8.7	552.3
	B1	1354.5	107.8	462.3	45.0	1013.2	77.6	2369.3	243.8	5199.3
	B2	235.2	56.6	82.2	21.7	<LOD	0.0	74.7	19.3	404.8
	B4	140.0	9.8	33.6	5.4	<LOD	0.0	58.1	2.6	237.8

[a] Label A refers to samples collected in the South works while label B refers to samples collected in the North works.

Appendix C : Summaries of AS concentrations in the dissolved phase and the suspended solid phase of the collected wastewater samples over the monitoring period.

Table D. Summary of AS concentrations in the dissolved phase of the collected wastewater samples over the monitoring period. Unit: ppb.

Analyte	Sample label[a]	Minimum	Maximum	Mean	STDEV	Median
ACE	Inf	6.32	10.51	8.19	1.80	8.05
	A1	6.20	9.80	8.12	1.78	8.21
	A2	5.82	9.02	7.46	1.37	7.49
	A3	5.84	9.00	7.39	1.40	7.29
	A4	5.63	8.89	7.36	1.43	7.41
	B1	5.83	10.91	8.00	2.09	7.38
	B2	5.68	8.69	7.20	1.31	7.04
	B3	6.00	9.15	7.48	1.39	7.38
	B4	6.28	9.26	7.56	1.30	7.32
SUC	Inf	2.11	6.50	3.96	2.01	3.24
	A1	1.88	5.63	3.41	1.73	2.84
	A2	1.68	5.68	3.27	1.57	2.93
	A3	1.66	5.26	3.11	1.50	2.51
	A4	1.48	4.68	3.01	1.30	3.03
	B1	1.48	5.74	2.98	1.73	2.27
	B2	1.32	5.33	3.38	1.74	3.57
	B3	1.30	4.94	3.07	1.43	3.21
	B4	1.65	5.56	3.15	1.63	2.54
CYC	Inf	29.57	41.88	36.60	4.70	36.47
	A1	23.96	32.67	27.79	3.27	27.45
	A2	<LOD	0.02	0.012[b]	0.01	0.010[b]
	A3	<LOD	0.05	0.017[b]	0.02	0.010[b]
	A4	<LOD	0.03	0.014[b]	0.01	0.010[b]
	B1	11.04	19.73	14.35	3.02	13.96
	B2	<LOD	0.25	0.053	0.10	0.01[b]
	B3	<LOD	0.16	0.046	0.06	0.01[b]
	B4	<LOD	0.03	0.014[b]	0.01	0.01[b]
SAC	Inf	9.31	18.80	14.93	3.32	15.61
	A1	10.29	17.04	12.95	2.46	12.27
	A2	<LOD	0.23	0.06	0.08	0.03[b]
	A3	<LOD	<LOD	0.03[b]	0.00	0.03[b]
	A4	<LOD	<LOD	0.03[b]	0.00	0.03[b]
	B1	6.05	10.73	8.52	1.80	8.84
	B2	<LOD	0.21	0.06	0.07	0.03[b]
	B3	<LOD	0.32	0.07	0.12	0.03[b]
	B4	<LOD	<LOD	0.03[b]	0.00	0.03[b]

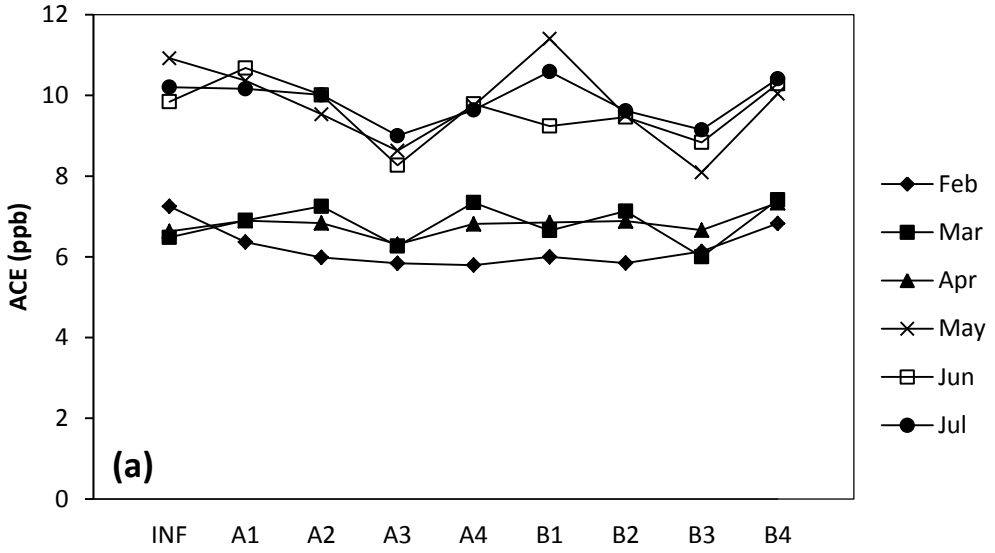
[a] Label A refers to samples collected in the South works while label B refers to samples collected in the North works.
[b] Values are below the corresponding MDLs.

Table E. Summary of AS concentrations in the suspended solid phase of the collected wastewater samples over the monitoring period. Unit: ng/g dw.

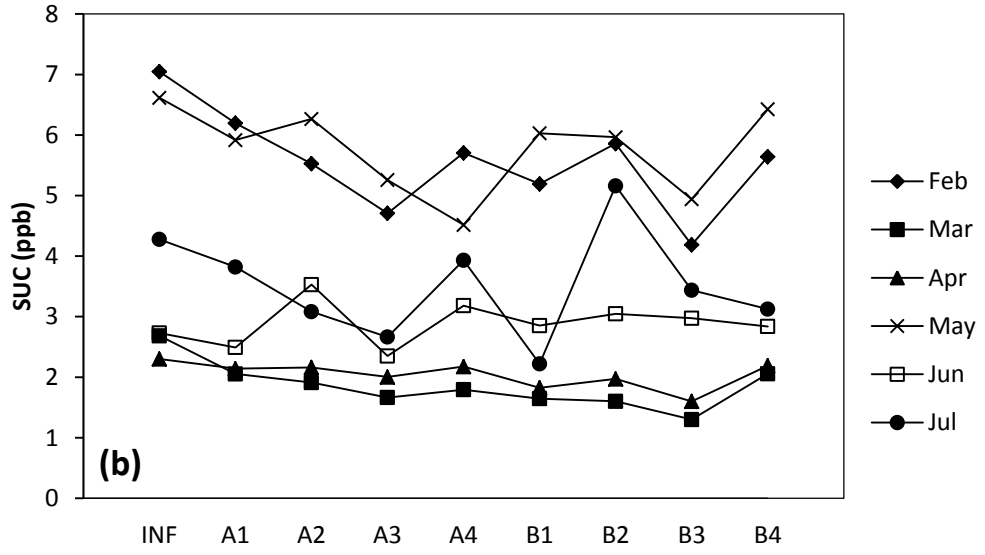
Analyte	Sample label[a]	Minimum	Maximum	Mean	STDEV	Median
ACE	Inf	<LOD	2141.3	990.1	693.1	861.8
	A1	<LOD	8709.1	3674.6	2776.7	3433.6
	A2	<LOD	902.1	469.9	295.1	441.8
	A4	<LOD	458.7	254.8	149.5	245.0
	B1	<LOD	4495.4	1854.2	1412.3	1633.3
	B2	<LOD	385.8	189.9	117.4	171.0
	B4	59.2	293.0	129.8	84.5	101.4
SUC	Inf	540.3	1367.5	835.6	305.1	795.3
	A1	1260.3	5702.6	2731.3	1745.5	2037.0
	A2	114.0	535.0	275.1	162.7	214.7
	A4	66.2	256.6	159.1	70.4	150.6
	B1	462.3	3223.2	1331.8	1001.2	1052.9
	B2	47.4	278.2	115.7	82.1	89.6
	B4	33.6	96.8	59.5	24.4	56.4
CYC	Inf	842.6	7370.2	3733.2	2142.3	3420.9
	A1	3581.1	18951.5	9213.2	5193.0	8476.6
	A2	<LOD	<LOD	28.9[b]	4.1	27.0[b]
	A4	<LOD	<LOD	14.9[b]	3.7	14.4[b]
	B1	956.1	4273.8	2294.3	1478.5	1750.5
	B2	<LOD	<LOD	10.8[b]	2.5	11.1[b]
	B4	<LOD	<LOD	5.6[b]	1.6	5.7[b]
SAC	Inf	536.3	6757.4	3022.4	2156.5	2744.0
	A1	4267.6	18818.2	8958.4	5163.6	7811.0
	A2	<LOD	197.0	91.8	77.0	46.1[b]
	A4	<LOD	120.1	55.8	37.6	54.1
	B1	1672.3	7476.9	3453.4	2091.2	2690.2
	B2	<LOD	74.7	39.3	27.2	30.7[b]
	B4	<LOD	58.1	24.0	19.5	17.3

[a] Label A refers to samples collected in the South works while label B refers to samples collected in the North works.
[b] Values are below the corresponding MDLs.

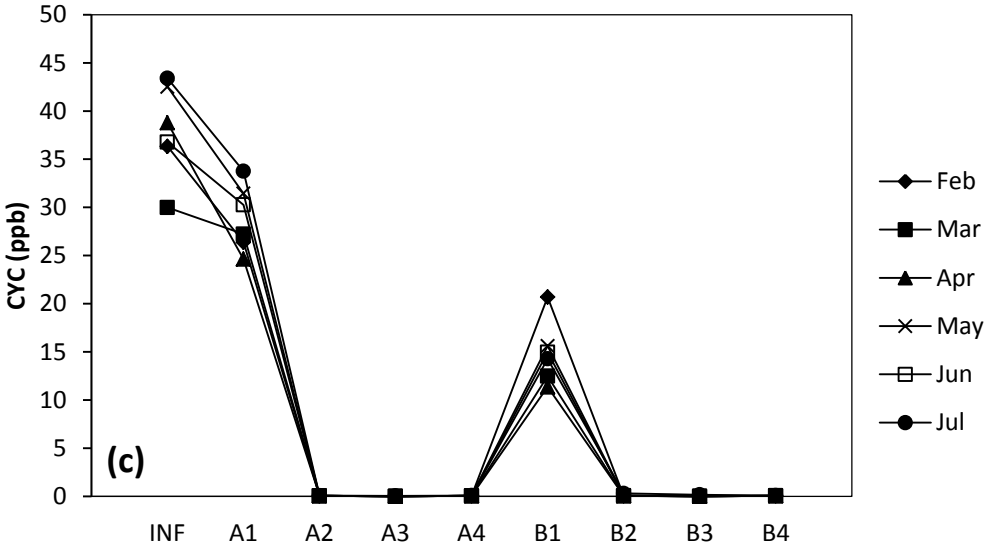
Appendix D: The trends in monthly combined concentration of ASs throughout the treatment trains in the local WRP in Singapore during the sampling period.



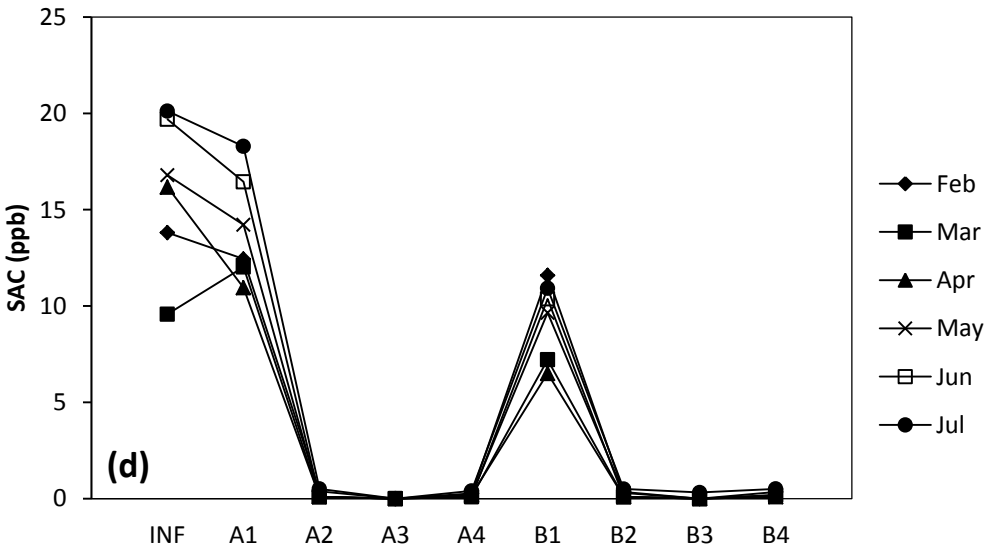
(a)



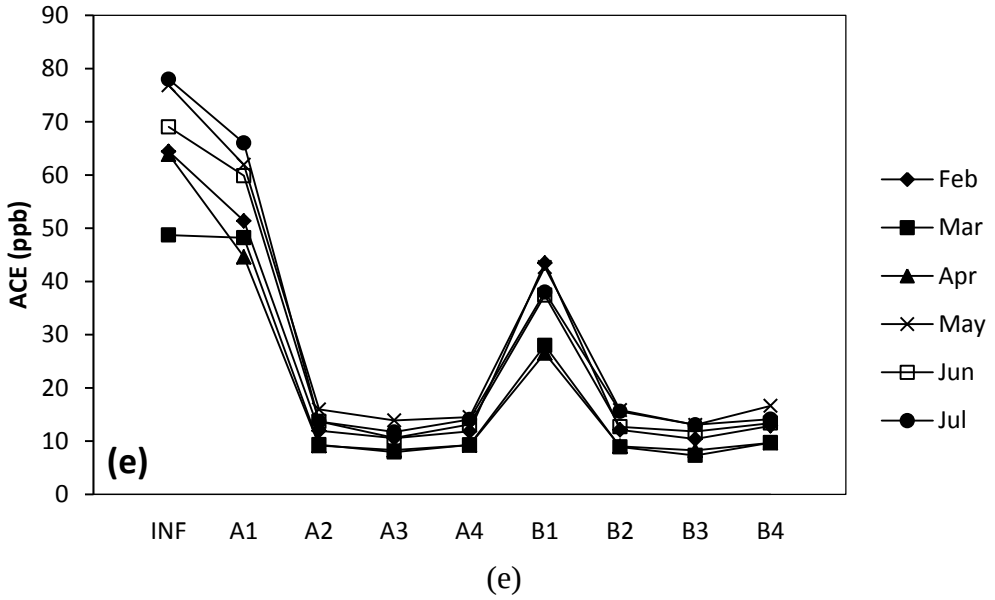
(b)


50
45
40
35
30
25
20
15
10
5
0
CYC (ppb)
(c)
INF
A1
A2
A3
A4
B1
B2
B3
B4
Feb
Mar
Apr
May
Jun
Jul

(c)


25
20
15
10
5
0
SAC (ppb)
(d)
INF
A1
A2
A3
A4
B1
B2
B3
B4
Feb
Mar
Apr
May
Jun
Jul

(d)



(e)

Figure A. The trends in monthly combined (dissolved phase and suspended solid phase) concentration of (a) ACE, (b) SUC, (c) CYC, (d) SAC and (e) total ASs throughout the treatment trains in the WRP during the sampling period from February 2013 to July 2013.

Appendix E : The results of ANOVA one-way statistical test for comparing the significance between AS concentrations in different treatment units of the WRP.

Table F. Statistical test results for ASs in the dissolved phase of wastewater samples collected for different treatment units by ANOVA one-way.

Dunn's Multiple Comparison Test	ACE Significant P < 0.05	SUC Significant P < 0.05	CYC Significant P < 0.05	SAC Significant P < 0.05
Inf vs A1	No	No	No	No
Inf vs B1	No	No	No	No
A1 vs A2	No	No	***Yes	***Yes
A1 vs B1	No	No	No	No
A2 vs A3	No	No	No	No
A2 vs B2	No	No	No	No
A3 vs A4	No	No	No	No
A3 vs B3	No	No	No	No
A4 vs B4	No	No	No	Yes
B1 vs B2	No	No	**Yes	**No
B2 vs B3	No	No	No	No
B3 vs B4	No	No	No	No

Appendix F : Monthly concentrations of PFCs in the dissolved phase and the suspended solid phase of the collected wastewater samples in the local WRP in Singapore in June and July 2013.

Table G. Monthly concentrations of PFCs in the dissolved phase (ppt) of the collected wastewater samples in the local WRP in June and July 2013.

Date	Sample label[a]	PFCAs								PFSAs				Derivatives				Total PFCs
		PFBA		PFOA		PFNA		PFDA		PFHxS		PFOS		FOSAA		N-EtFOSAA		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Jun	Inf	28.9	20.9	10.9	7.8	2.5	1.2	20.0	12.0	15.6	0.7	6.6	3.3	4.7	0.8	6.8	3.0	96.1
	A1	28.0	5.6	12.8	2.4	4.7	3.2	13.9	5.7	14.2	1.2	10.2	1.2	5.1	0.7	17.2	7.4	106.1
	A2	39.9	2.3	17.8	3.3	3.7	1.9	38.4	4.6	13.2	1.9	6.8	0.9	6.5	1.5	4.9	2.4	131.2
	A3	41.7	6.0	15.9	5.4	6.4	1.4	47.4	6.6	13.6	0.5	8.8	1.6	4.6	0.5	5.8	3.9	144.3
	A4	41.0	3.7	19.6	9.4	4.5	1.8	44.7	5.2	13.9	1.1	5.7	0.2	4.4	0.6	7.9	2.9	141.8
	B1	18.5	18.4	12.9	2.1	4.8	2.7	15.7	6.6	14.0	3.2	9.6	1.0	5.1	1.0	6.3	2.8	86.8
	B2	40.9	4.1	15.1	2.2	5.8	0.5	34.1	5.9	14.6	1.1	6.0	0.7	5.2	2.5	6.2	2.9	127.8
	B3	44.2	5.5	15.5	3.6	5.3	1.5	37.6	19.1	13.8	0.9	20.3	3.1	5.2	1.6	7.0	4.0	148.8
	B4	31.5	12.0	14.8	7.0	4.3	4.1	43.2	11.7	13.4	0.3	4.9	0.2	4.2	2.6	2.8	2.7	119.1
Jul	Inf	29.5	16.4	10.0	6.7	1.6	0.6	14.8	3.4	13.4	1.0	6.4	1.0	3.4	0.9	9.5	4.2	88.6
	A1	28.0	1.2	10.2	4.0	3.5	3.7	15.0	5.0	13.4	0.7	7.7	0.7	5.3	1.7	17.9	15.0	101.1
	A2	40.5	5.2	8.1	2.7	3.0	0.2	5.9	1.5	13.2	1.0	5.6	0.7	4.8	0.5	2.1	2.6	83.2
	A3	36.5	5.1	9.5	1.5	2.6	1.1	5.1	0.8	14.3	2.0	7.0	0.8	4.2	0.6	3.1	2.6	82.4
	A4	35.1	4.3	8.8	2.3	2.5	0.8	21.1	6.8	14.0	1.1	7.6	1.1	5.3	1.3	13.1	5.7	107.5
	B1	24.3	3.9	8.0	2.7	3.7	1.3	37.3	9.8	16.5	2.3	9.2	1.0	5.6	2.0	2.1	1.2	106.8
	B2	42.0	5.4	11.3	0.7	2.5	0.8	12.1	7.0	14.4	1.6	7.7	0.3	5.1	0.8	7.6	9.8	102.7
	B3	57.3	7.3	11.3	1.0	2.1	1.6	6.2	0.7	12.7	0.9	14.3	3.2	5.5	1.6	<LOD	0.9	110.2
	B4	33.9	6.2	12.9	2.1	2.6	0.9	27.1	5.0	14.9	0.7	8.0	1.9	4.7	0.4	4.7	5.4	108.8

[a] Label A refers to samples collected in the South works while label B refers to samples collected in the North works.

Table H. Monthly concentrations of PFCs in the suspended solid phase (ng/g dw) of the collected wastewater samples in the local WRP in June and July 2013.

Date	Sample label[a]	PFCAs								PFSAs				Derivatives				Total PFCs
		PFBA		PFOA		PFNA		PFDA		PFHxS		PFOS		FOSAA		N-EtFOSAA		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Jun	Inf	21.5	8.1	41.0	6.4	23.6	2.1	21.0	7.4	25.8	4.3	83.1	8.5	22.8	4.0	37.8	13.3	276.5
	A1	63.3	30.4	158.3	17.1	105.6	8.8	101.7	18.0	100.5	19.0	638.0	78.8	57.3	6.8	165.6	89.8	1390.3
	A2	5.5	0.4	41.7	2.1	11.2	0.5	94.8	5.6	6.9	1.5	156.0	14.4	26.7	5.9	88.2	19.6	431.1
	A4	3.9	1.0	32.6	4.0	10.3	1.1	104.5	22.4	5.6	0.3	180.6	123.0	23.0	1.0	81.9	39.2	442.5
	B1	33.8	15.2	128.8	8.5	58.5	3.3	69.2	4.6	53.4	2.3	311.4	16.8	10.7	1.7	89.6	33.0	755.3
	B2	3.0	0.1	22.0	0.9	8.4	0.5	67.8	2.2	4.8	0.7	99.4	3.4	13.3	4.0	81.1	11.9	299.7
	B4	2.5	0.4	15.8	0.9	7.3	0.5	59.3	4.2	4.9	0.6	82.9	2.5	8.2	0.3	83.6	5.5	264.4
Jul	Inf	14.3	1.4	45.5	10.5	26.7	3.5	25.6	5.8	44.7	29.4	94.1	9.6	21.1	5.3	23.9	4.1	296.0
	A1	35.4	7.6	135.2	5.0	80.4	22.4	69.2	9.4	72.6	5.4	229.3	7.5	54.4	6.6	68.6	22.2	745.3
	A2	5.1	0.3	26.9	1.8	11.8	2.8	65.1	19.5	6.7	0.4	75.1	32.7	28.4	25.2	136.5	43.8	355.5
	A4	3.2	0.5	17.3	0.9	7.7	0.0	54.8	4.7	4.2	0.8	88.0	14.3	21.3	8.3	78.2	15.7	274.6
	B1	19.8	17.8	85.0	77.2	55.4	55.0	84.7	73.7	33.1	28.9	215.4	188.4	7.9	2.5	107.6	102.3	608.9
	B2	2.9	1.0	15.8	3.7	8.9	2.2	55.0	12.4	4.1	1.2	88.0	19.5	14.0	2.2	62.7	15.3	251.4
	B4	2.0	0.3	10.7	0.3	8.0	0.1	52.1	2.1	2.7	0.1	81.7	1.5	7.5	1.2	61.5	3.1	226.2

[a] Label A refers to samples collected in the South works while label B refers to samples collected in the North works.